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Population structure, genetic diversity, and colonization history of the eastern pondmussel, *Sagittunio nasutus*, in the Great Lakes drainage

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Abstract

1. The eastern pondmussel, *Sagittunio nasutus* (Bivalvia: Unionida), has declined in abundance and distribution in eastern North America over the last few decades. The declines are predominantly the result of infestation by invasive dreissenid mussels and changes in habitat. The species is now considered imperilled across large portions of its distribution, especially in the Laurentian Great Lakes region.
2. The genetic diversity and structure of the remnant populations in the Great Lakes region were assessed using 10 newly developed microsatellite DNA loci for *S. nasutus*. Understanding the remaining populations can inform future management projects and determine whether the remnant populations have experienced a genetic bottleneck or a founder effect. We hope that this will inform the conservation of other species in regions founded by, and isolated from, a more genetically diverse source population or with disjointed geographical distributions.
3. Focusing on the Great Lakes region, samples (n = 428) from 62 collection sites in 28 water bodies were collected. Across the locations sampled for *S. nasutus* 11 genetic populations were identified, with significant genetic differentiation among them. The genetic structure of the species was assessed, with genetic similarities among populations compared and with geographical routes of colonization and gene flow evaluated.
4. Initial colonization from the Atlantic coast into Lake Erie and Lake Ontario is evident, followed by colonization events into nearby inland rivers and lakes. Analyses found evidence of inbreeding in all but one population and evidence of past genetic bottlenecks or strong founder effects in all but four populations. This study deepens our understanding of the genetic past and present of this imperilled species, providing conservation suggestions for the future management of the species.

KEYWORDS

founder effects, genetic bottlenecks, *Ligumia nasuta*, management units, microsatellite DNA, phylogeography, population structure, rescue effect, *Sagittunio nasutus*, Unionidae

1 | INTRODUCTION

Assessing geographical patterns in the genetic diversity of imperilled freshwater mussel species (order Unionida) will further our understanding of the distribution and dispersal abilities of the species, as well as inform conservation efforts. Uninformed management projects may unintentionally repopulate an area with specimens of a different genetic make-up (Jones, Hallerman, & Neves, 2006). Adding genetically mismatched specimens via hatchery propagation efforts or relocations of adults could lead to vulnerable populations with low genetic diversity, as a result of founder effects, or to altering the genetic composition of existing unionid populations (Hoftyzer, Ackerman, Morris, & Mackie, 2008; Jones et al., 2006). Informed conservation management is key to protecting the genetic diversity within and among populations of imperilled mussel species.

Sagittunio nasutus (Say, 1817; =*Ligumia nasuta*), the eastern pondmussel, is a burrowing, filter-feeding freshwater bivalve in the family Unionidae native to eastern North America. Unionids provide many ecosystem services, such as nutrient cycling, structural habitat, and an indication of habitat degradation (Vaughn, 2017). Unionids also represent one of the most imperilled families of organisms in the world (Bogan, 2008; Lopes-Lima et al., 2017; Lydeard et al., 2004). Many imperilled unionid species require conservation and restoration efforts to increase population sizes in order to maintain their role in their native ecosystems (Jones et al., 2006).

The life cycle of a unionid, such as *S. nasutus*, includes an obligate parasitic larval stage, with the host being the primary mode of long-distance dispersal in an individual's life. *Sagittunio nasutus* has a limited number of potential host fish species, with host fish trials identifying several centrarchid and percid species as potential hosts (Corey, Dowling, & Strayer, 2006; Price, Eads, & Ralley, 2011). Gravid female *S. nasutus* use a mantle flap lure to infest potential host fish with their larvae, which metamorphose the glochidia larvae to the juvenile stage and provide a dispersal mechanism for the offspring (Barnhart, Haag, & Roston, 2008; Corey et al., 2006; Cummings & Graf, 2010; Zanatta & Murphy, 2006). The location where the offspring leave their host is dependent upon the movement and dispersal abilities of the host.

The Laurentian Great Lakes were completely covered by glacial ice sheets successively over several periods during the Pleistocene (Pielou, 1991). Following the most recent Wisconsin glaciation, which ended approximately 11 000 years ago, all unionids now found in the Great Lakes region colonized from glacial refugia, via glacial meltwaters and isostatic rebound (Graf, 2002; Mandrak & Crossman, 1992; Pielou, 1991; Rahel, 2007). Shells of *S. nasutus* were found in an archaeological dig of a late 16th to mid-17th century Native American village near the village of Fairport Harbor, Ohio (Goslin, 1943). These remains place *S. nasutus* in Lake Erie before canal construction circumvented the Niagara Falls (Stansbery, 1961). *Sagittunio nasutus* is hypothesized to have colonized the Great Lakes region through an eastern-flowing meltwater outlet of Lake Erie connecting to the Atlantic coastal Mohawk or Hudson River drainages (Figure 1;

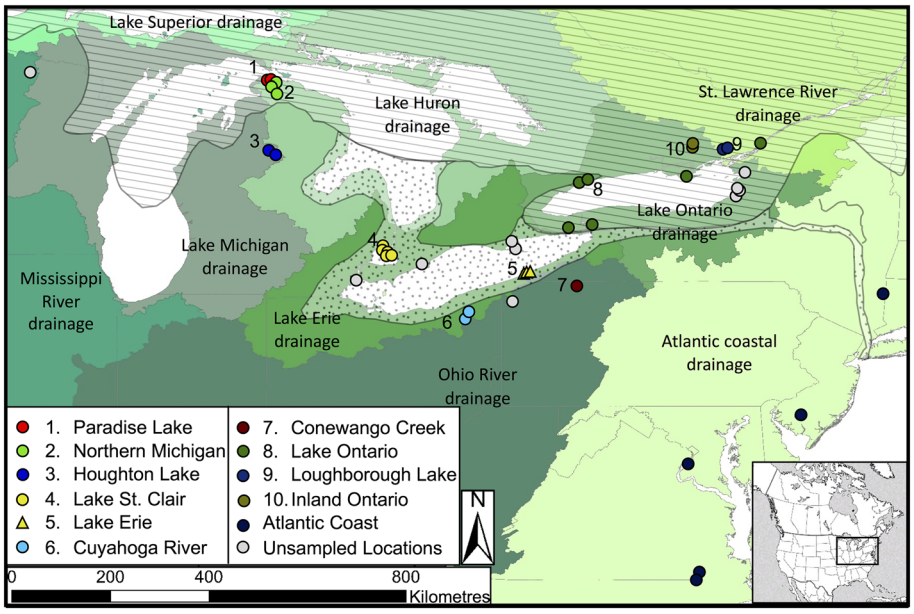

FIGURE 1 Collection locations of *Sagittunio nasutus* (coloured circles and triangles) and major drainages or regions denoted. The Atlantic coastal region locations belong to their independent coastal rivers. The colour of site locations indicates the population that they represent based on the predominant colour of their groupings in the BAPS results (Figure 2). Although Lake Erie was not differentiated from Lake St. Clair in the BAPS results, it was found to be genetically distinct overall. This is why the Lake Erie locations are marked with triangles. Conewango Creek is an outlet of Lake Chautauqua and a tributary of the Allegheny River (Ohio River drainage). The grey circles represent areas that *S. nasutus* is known to currently inhabit within and near the Great Lakes drainage, but were not sampled in this study (Wisconsin Department of Natural Resources, 2015; Zanatta et al., 2015). The dotted regions represent relevant proglacial lakes of the hypothesized route of entry for *S. nasutus* into the Great Lakes, and the glacial ice sheet (*c.* 13 000 years ago) to the north in the region indicated with diagonal lines (Farrand, 1988; Stansbery, 1961)

Schmidt, 1986; Stansbery, 1961; Underhill, 1986). Through these changing meltwaters *S. nasutus* made their way into the Great Lakes region, and thus *S. nasutus* in the Great Lakes region have been long isolated from their source population.

Within the Great Lakes region and surrounding area, natural as well as anthropogenically influenced host fish dispersal, like fish stocking, canal construction, and accidental bait fish introductions, are hypothesized to have facilitated the movement of *S. nasutus*. Canals can provide new avenues of movement for host fish carrying glochidia larvae (Dean, 1890; Hoffman, Morris, & Zanatta, 2018; Rahel, 2007). Also, fish stocking is a potential colonization route for unionids while glochidia are encysted on host fish (Popa, Baratáková, Bryja, Reichard, & Popa, 2015; Sárkány-Kiss, 1986). Goodrich (1932) hypothesized that the apparently erratic and disjunct nature of the distribution of *S. nasutus* in the Great Lakes, specifically in Northern Michigan, may be a result of anthropogenic host fish introductions and stocking. In addition, Lake Chautauqua in New York, in the headwaters of the Allegheny River (Ohio River drainage), was extensively surveyed for unionids in the early 1900s, but *S. nasutus* has only been documented since the 1980s, based on museum records (Strayer & Jirka, 1997). The species is hypothesized to have been introduced into Lake Chautauqua after these early expeditions through fish stocking (Strayer & Jirka, 1997). These hypothesized anthropogenic colonization routes should also be considered when determining the phylogeographical history of the species and when considering the conservation status and management practices, as natural waterway distance may not be the best indication of genetic similarity. This makes a genetic survey of the species even more important to inform conservation efforts.

Sagittunio nasutus has a wide geographic distribution spanning the Atlantic slope and Laurentian Great Lakes region. *Sagittunio nasutus* has historically been found along the Atlantic slope from South Carolina to Maine (Martin, 1997; Nedeau, McCollough, & Swartz, 2000; Price, 2005; Sepkoski & Rex, 1974). In the Great Lakes region, *S. nasutus* was known from the drainages of all five Great Lakes (Figure S1; Committee on the Status of Endangered Wildlife in Canada (COSEWIC), 2007; Michigan Natural Features Inventory, 2015). In the western basin of Lake Erie, *S. nasutus* was consistently found to be the second or third most common unionid species in historical surveys (Nalepa, Manny, Roth, Mozley, & Schloesser, 1991). Currently, only remnant populations are present across its previous range in the Great Lakes (COSEWIC, 2007; Michigan Natural Features Inventory, 2015; Scott, Begley, Krebs, & Zanatta, 2014; Zanatta et al., 2015). Despite the large number of historical populations and apparent ease of dispersal into new habitats, the decline and loss of *S. nasutus* populations is cause for conservation concern. Although *S. nasutus*, like other unionids in the Great Lakes region, had been historically declining as a result of pollution and habitat loss, the largest effect on *S. nasutus* populations over the past three decades is a direct result of invasion by dreissenid mussels (COSEWIC, 2007; Nalepa et al., 1991; Zanatta et al., 2015). As a result of these cumulative threats, *S. nasutus* is now considered a rare species in the Great Lakes region and is listed as an imperilled species in many jurisdictions (COSEWIC, 2017; NatureServe Explorer, 2015). Although the geographical distribution of this species is expansive, this study focuses on the remaining populations in the Great Lakes region.

A previous study on the phylogeography of *S. nasutus* using mitochondrial DNA sequences found limited genetic variation within the Great Lakes region. Scott et al. (2014) compared the within-species genetic diversity of two maternally inherited mitochondrial DNA gene regions, finding low genetic diversity within the Great Lakes region. This result suggested that the source population was a single, small founder group or a larger group with low genetic variation (Scott et al., 2014). The results of the study indicated that the entire Great Lakes region could be treated as a single population for conservation management purposes (Scott et al., 2014). The study solely analysed mitochondrial DNA, which can only effectively distinguish among management units if enough haplotypes have been identified (Moritz, 1994). Although Scott et al. (2014) were able to determine the evolutionary history of *S. nasutus* on a broader scale, additional fine-scale genetic analyses are needed to build upon those findings, to properly inform conservation and restoration efforts, to understand the phylogeography of the species, and to determine whether a genetic bottleneck or a founder effect has occurred.

New highly variable microsatellite DNA loci have been developed specifically for *S. nasutus* (Scott et al., 2016) and have been used for the fine-scale population genetic analyses described in this study. This study has three main objectives to analyse the remaining genetic diversity of *S. nasutus* in the Great Lakes region: (i) to delineate genetically distinct management units in the remaining habitats; (ii) to assess the genetic diversity of the remnant populations and make suggestions for future management projects; and (iii) to determine whether the remnant populations have experienced a recent genetic bottleneck or whether there is evidence of a founder effect. Through these objectives, this study provided a better perspective on the distribution and conservation status of this imperilled species. This study focused on the conservation of the species in the Great Lakes region, which was founded by and isolated from its more genetically diverse source population. In addition, some of the remaining inhabited water bodies were historically isolated or are currently distant through local extirpations. We hope that this will inform the conservation of other species with a similar phylogeographical history or disjunct geographical distributions.

2 | METHODS

2.1 | Specimen collection and DNA extraction

Specimens were collected from 62 sites in 28 water bodies, including rivers, bays, and inland lakes, across the range of *S. nasutus* (n = 428) for this study (Figures 1 and S1; Tables 1 and S1). Sites spanned known locations in the Great Lakes region. Additional sites were included from Conewango Creek, the outlet of Lake Chautauqua in the headwaters of the Allegheny River (Ohio River drainage). Specimens from the Atlantic coastal region, the source population for the

TABLE 1 Geographically defined collection water bodies for *Sagittunio nasutus*, with the identified genetically distinct populations shown. The number associated with the genetically distinct Great Lakes region population relates to the population numbering in Figure 1. The number of genotyped samples represents the number of individuals from each location that successfully amplified at least five loci. Water bodies are separated by shading to indicate how specimens were grouped in the Mantel tests of isolation by distance in efforts to increase distance accuracy, while maintaining at least 11 specimens. Atlantic coastal specimens were excluded from Mantel tests of isolation by distance tests

Drainage	Genetically distinct population	Water bodies	Number of genotyped samples
Lake Huron	1. Paradise Lake	Paradise Lake	12
	2. Northern Michigan	Douglas Lake	18
		Burt Lake	34
Lake Michigan	3. Houghton Lake	North Bay	16
		East Bay	35
Lake St. Clair	4. Lake St. Clair	Lake St. Clair	8
		Goose Bay	8
		Little Muscamoot Bay	34
		Big Muscamoot Bay	30
Lake Erie	5. Lake Erie	Thompson Bay	11
	6. Cuyahoga River	Cuyahoga River	29
Ohio River	7. Conewango Creek	Conewango Creek	31
Lake Ontario	8. Lake Ontario	Coyle Creek	3
		Spicer Creek	1
		Rouge River	4
		Lynde Creek Marsh	1
		East Lake	10
		Lyn Creek	6
	9. Loughborough Lake	Loughborough Lake	19
	10. Inland Ontario	White Lake	26
		Beaver Lake	18
Atlantic Coast	-	Webatuck Creek	3
		Willow Grove Lake	3
		Potomac River	4
		Nottoway River	33
		Blackwater River	2

Great Lakes, were included to provide a genetic diversity baseline for comparison. Specimens were collected during extensive surveys in 2011 and 2012 (Scott et al., 2014; Zanatta et al., 2015), with additional specimens collected in 2015 and 2016. Sampling in 2015 and 2016 was performed to increase sample sizes and to add water bodies where *S. nasutus* was previously believed to have been extirpated.

A sample of genetic material was collected from each specimen, either by gathering mucus on the foot with a sterile swab (Henley, Grobler, & Neves, 2006), where *S. nasutus* was of conservation concern, or by a small mantle biopsy cut from the ventral margin (Table S1; Berg, Haag, Guttman, & Sickel, 1995). Each mussel was then returned to the substrate. Swab or tissue samples were frozen at −80°C. DNA was extracted from the individual specimens through an overnight digestion with proteinase K, using the alcohol extraction method of Sambrook, Fritsch, and Maniatis (1989). Genomic DNA was stained with SYBR® Green and electrophoresed in a 1.5% agarose gel to confirm the quality of the extraction.

2.2 | Amplification of microsatellite loci and quality assessment

A selection of 10 microsatellite loci developed specifically for *S. nasutus* were used in this study (Table 2; Scott et al., 2016). The loci chosen were those that did not deviate from Hardy–Weinberg equilibria (HWE) during the development and characterization study (Scott et al., 2016), and that performed the best (i.e. gave the most consistent amplifications) after optimization. Full descriptions of the polymerase chain reaction (PCR) conditions, microsatellite genotyping, and allele scoring are described in Appendix S1. A total of 399 specimens, from 26 water bodies and 58 of the collection sites, successfully amplified at least five loci, where 93% of those had at least nine loci amplified (Table 1).

The compiled dataset was analysed for quality, using the method developed by Brookfield (1996). Each microsatellite locus–drainage combination was assessed for the likelihood of null alleles and

TABLE 2 Microsatellite loci, developed and characterized by Scott et al. (2016), used to genotype *Sagittunio nasutus*, listing locus names of Lina (L) primers, forward (F) and reverse (R) primer sequences, GenBank accession numbers, unique volume of 25 mM $MgCl_2$ used in polymerase chain reaction (PCR) assays, repeat motif, as well as the size range and number of alleles (*A*) found in this study (see Appendix S1 for annealing temperature and complete procedure)

Locus	Primer sequence 5′→3′	GenBank accession	$MgCl_2$ (μL)	Repeat motif	Size range (bp)	*A*
L17	**F: CCTATCTTCCTACCCGCCG**	KU561840	0.5	ATAC	305–365	15
	R: TTTCCATTAGCAGATTTCATTGC					
L19	**F: TGGGAAGAGAAGGTAGTTCAGG**	KU561842	1	ATAC	217–381	34
	R: TCGATGCACTACGAGAGTTCG					
L21	**F: AAATATGTGACTATGTCCTTTCAAGC**	KU561844	2	ATATT	299–374	15
	R: GCATTGGTATTAAGGACGTTAGG					
L22	**F: AAGACTGCGTCTTGAAAGTTGG**	KU561845	1.5	ATAC	188–268	20
	R: AAATGTGGGTCTTCATTTCACG					
L23	**F: AGTTTGAATCTGTGCCCACG**	KU561846	1.5	ATAC	315–391	21
	R: TCTTTCCCAGTTATATGTTATACCGC					
L25	**F: ATGTGAATAAGCCGGCAAGG**	KU561848	1	ATAC	224–364	27
	R: TGTACGCACTCACACACCTCC					
L26	**F: TGTATTCTTGCACACATCCATGC**	KU561849	1.5	ATAC	313–417	22
	R: TTTGCCTGAGACAATAAGAAGGG					
L28	**F: AACAGTATATTAGCAAACTTCTGTGCC**	KU561850	1	ATCT	209–401	36
	R: CACAAAGAACAGTTTGAAATCATCG					
L45	**F: CCTGAATGTATTAAAGAACCAGAACG**	KU561860	1.5	ATAC	205–277	16
	R: TTGCTCATTAGACAAGTAGGCG					
L46	**F: GACCTTCCGCATCCCAGG**	KU561861	1	ATAC	215–315	17
	R: AAACCGCGGAATTGTTTGG					

Fluorescently labelled primers (with 6-FAM or HEX fluorescent label) are in bold.

genotyping errors using the program MICROCHECKER 2.2.3 (Table 1; van Oosterhout et al., 2004). If allele size-difference frequencies deviated from the simulated expectations of 95% confidence, the test was deemed positive for null alleles; however, estimates of null-allele frequencies below 0.2 were considered acceptable, as these levels have been shown to have very little impact on population delineation and fixation index (F_{ST}) estimates (Carlsson, 2008; Dakin & Avise, 2004). Moreover, molluscs are often found to have a high frequency of null alleles (Galbraith, Zanatta, & Wilson, 2015; Kelly & Rhymer, 2005; Rowe & Zanatta, 2015; Zanatta & Murphy, 2007). GENEPOP 4.2 (Rousset, 2008) was used to calculate linkage disequilibria, using the log-likelihood ratio statistic with a dememorization number of 1000, with 100 batches and 1000 iterations per batch as a single dataset and within each drainage (Table 1). GENEPOP 4.2 calculated deviations from HWE at each locus for each drainage (Table 1), with a probability test and a dememorization number of 1000, with 100 batches and 1000 iterations per batch.

2.3 | Statistical analyses

2.3.1 | Genetic structure

Genetic structure within and among collection sites was determined using multiple individual-based Bayesian assignment. For these individuals, STRUCTURE 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 59 (i.e. the number of collection sites plus one) were analysed with 10 iterations to assess their consistency, likelihood, and robustness (Pritchard et al., 2000). The parameters given to STRUCTURE were 200 000 burn-in iterations and 400 000 Markov chain Monte Carlo repeats, and assumed potential admixture among populations (gene flow) and correlated allele frequencies. The analyses were run without *a priori* population information. STRUCTURE HARVESTER 0.6.94 (Earl & vonHoldt, 2012) was then used to evaluate the most likely *K* value using the Evanno, Regnaut, and Goudet (2005) method, which looks for the highest ΔK between sequential *K* values, and to determine the highest mean estimate of the natural log of the probability of the data [ln*P*(*K*)] (Earl & vonHoldt, 2012). If there was a discrepancy in the most likely *K* value, the *K* value with the highest ln*P*(*K*) and a local increase in ΔK was used. This method was chosen to maximize the number of informative groupings. In addition, the model-based clustering method BAPS 6.0 (Corander, Sirén, & Arjas, 2008) was used to delineate genetic structure. BAPS uses Bayesian algorithms to pool populations with non-significant allele-frequency differences. Bayesian clustering techniques, such as STRUCTURE and BAPS, can produce incorrect inferences when used to analyse subpopulations that were

unevenly sampled (Puechmaille, 2016), such as the specimens in this study. To support the results of STRUCTURE and BAPS with a method that is better at characterizing population subdivision and revealing complex genetic patterns, the multivariate discriminant analysis of principal components (DAPC) approach was used (Jombart, Devillard, & Balloux, 2010). DAPC also does not use *a priori* geographical assumptions on sample origins (Jombart et al., 2010). The process first reduces the number of variables to a value lower than the sample size while at the same time creating uncorrelated variables that still retain the original variation, using principal component analysis (PCA) (Jombart et al., 2010). Then discriminant analysis (DA) was used to maximize the separation between groups, while minimizing the variation within groups (Jombart et al., 2010). To help visualize the genetic information in a geographical context, a scatter plot of the first two discriminant functions was made with the DAPC results. The DAPC analyses were run in the R package ADEGENET (the R code used is available at https://github.com/MariahWScott/Scott_et_al._2019; Jombart, 2008; Jombart et al., 2010) for R 2.12 (R Development Core Team, 2011). If at least one method, among the analyses of all specimens in STRUCTURE, BAPS, and DAPC, suggested a distinct grouping of ≥10 specimens, it was used in subsequent analyses as a defined group. This was done in an effort to make full use of the specimens gathered.

The conventional method for additional analyses among locations is to analyse comparisons among collection sites with ≥10 specimens, in order to avoid bias from small samples sizes (e.g. Galbraith et al., 2015); however, this method was not used in this study, as only 15 of the 26 water bodies had ≥10 specimens. To confirm that small sample sizes at the water-body level (Table 1) did not qualitatively affect the STRUCTURE, BAPS, and DAPC results, the analyses were rerun excluding water bodies with <10 specimens. Any qualitative differences were reported.

The genetic differentiation among groups identified by molecular analyses were calculated to determine whether these groups were genetically distinct populations, and the coefficient of inbreeding within groups and for the whole dataset were calculated. GENALEX 6.502 was used to perform an analysis of molecular variance (AMOVA; Peakall & Smouse, 2012) to test the significance of genetic differentiation and coefficient of inbreeding among groups. As no single population genetics differentiation metric can capture all of the properties of population partitioning, we elected to calculate the fixation index (F_{ST}) and an index of genetic differentiation (Jost's D, D_{est}), which is designed to differentiate groups and describe diversity with high polymorphism and mean heterozygosity (Bird, Karl, Smouse, & Toonen, 2011; Jost, 2008). GENALEX 6.502 determined the hierarchical genetic divergence values among groups with 9999 permutations and 999 bootstrap replicates (F_{ST} and D_{est}; Jost, 2008, Peakall & Smouse, 2012), with a Bonferroni-corrected alpha. Both calculations compared group pairings to assess their statistical degrees of separation and to provide a better understanding of the degree of connectivity between the then defined populations. GENEPOP 4.2 (Rousset, 2008) calculated deviations from HWE at each locus for each group, with a probability test and a dememorization number of 1000, with 100 batches and 1000 iterations per batch.

Collection locations were then tested for genetic isolation by distance. Populations identified using the methods above were separated into their discrete collection locations using only those locations with >10 specimens (e.g. Little Muscamoot Bay from the larger Lake St. Clair), in order to increase distance accuracy (Table 1). The locations of the separated water bodies were calculated based on the centroids for these water bodies. Atlantic coastal specimens were excluded from these analyses to focus on the Great Lakes region's genetic isolation by distance and because the waterway distances could not be accurately estimated (Figure 1). Correlations between genetic distances compared with waterway distances were calculated using Mantel tests (Mantel, 1967), performed in GENALEX 6.502 (Peakall & Smouse, 2012). The genetic distances were calculated as linearized, pairwise F_{ST} values $[F_{ST}/(1 - F_{ST})]$ in GENALEX. The waterway distance was calculated from the US Geological Survey National Hydrography medium resolution and the Lakes and Rivers Shapefile datasets (US Geological Survey, 2017a; US Geological Survey, 2017b) flowline shapefiles in ARCMAP 10.2.2 (ESRI, Redlands, CA). Two canal systems were included, the Pennsylvania and Ohio (P&O) canal, connecting the Cuyahoga River to the Ohio River drainage (and thus Conewango Creek), and the historic canals of Pennsylvania, connecting the Ohio River drainage (and thus Conewango Creek) to Lake Erie (Shank, 1986; Trevorrow, 1967).

2.3.2 | Assessment of genetic diversity

The genetic diversity of all locus–population combinations were analysed. GENEPOP 4.2 (Rousset, 2008) was used to calculate observed and expected heterozygosity at each locus for each population. HP RARE June-6-2006 (Kalinowski, 2005) was used to determine the number of alleles, mean allelic richness, and mean number of private alleles for each locus–population combination. A non-parametric Kruskall–Wallis test with pairwise multiple comparison post-hoc tests among populations were conducted in MINITAB® 17.2.1 (Minitab 17 Statistical Software, 2010), to assess the significance of differences per locus for the number of alleles, observed heterozygosity, private allelic richness, and allelic richness.

The dataset as a whole and as individual populations were analysed to compare genetic diversity within, between, and among populations, as an indication of the potential loss of genetic diversity or inbreeding. To detect recent genetic bottlenecks, caused by declines in the effective population (N_e), BOTTLENECK 1.2.02 (Cornuet & Luikart, 1996; Piry, Luikart, & Cornuet, 1999) tested for excess heterozygosity using sign tests as well as allelic mode shifts in each population. The heterozygosity excess tests used the infinite allele model (IAM), the stepwise mutation model (SMM), and the two-phase model (TPM), with a fixed proportion of 95% SMM and 12% variance of geometric distribution (Piry et al., 1999). As TPM is meant to function as an intermediary test between SMM and IAM assumptions, the presence of TPM P values outside the range of their corresponding SMM and IAM values prompted the dataset to be subjected to 10 sensitivity analyses (with fixed proportions of 40–95% SMM and 12–50%

variance of geometric distribution). In addition, FSTAT 2.9.3.2 February 2002 (Goudet, 2001) was used to calculate within-population inbreeding coefficients (F_{IS}), with significance tested using two one-tailed tests and 1000 iterations.

3 | RESULTS

3.1 | Quality assessment of genetic data

MICROCHECKER was used to assess the likelihood of null alleles and scoring errors for each locus, based on drainages, using the Brookfield (1996) method. When the data were analysed for each locus–drainage combination, the mean ± standard error estimate of null alleles was 0.040 ± 0.007, with a maximum of 0.260 (at locus L28). Most calculations had null-allele estimates below 0.200, which was considered within the acceptable range (Carlsson, 2008; Dakin & Avise, 2004). Only three pairings had estimations of null-allele frequencies ≥0.200 (Lake Michigan and Lake Ontario for locus L25 and the Atlantic coast for locus L28; Table S2). No loci showed any signs of allelic dropout. There were four instances of a potential stuttering error in the locus–drainage combinations (L25 and L19 for Lake Huron and L25 and L26 for Lake Ontario). L25 and L21 showed potential stuttering errors when all specimens were considered. L28 also had potential homozygous excess in Lake Huron. Specimens with estimations of null-allele frequencies of ≥0.200 or potential stutter errors were re-analysed in GENEMARKER to confirm allelic scoring. From these analyses, L25 had the most consistent issues revealed, calling into question the inclusion of locus L25 in further analyses. The most accurate way to correct for the presence of null alleles in population differentiation analyses is debated (Chapuis & Estoup, 2007), so analyses were run with and without the potentially problematic locus L25 to look for qualitative differences in the patterns of genetic structure, which have been reported (Lopes-Lima et al., 2016; Rowe & Zanatta, 2015).

The microsatellite data were analysed using GENEPOP to determine whether linkage disequilibria or HWE deviations consistently occurred in the dataset in the separate drainages. When the entire dataset was analysed, 11 locus pairings deviated significantly from linkage equilibria after Bonferroni correction (α = 0.001111). However, when the loci pairings were compared in one drainage at a time, the same 11 locus pairings deviated from linkage equilibria in only one drainage each, after Bonferroni correction (α = 0.000159). There was an additional linkage pairing found in the drainage-based analyses that was not significant in the entire dataset analyses. Of the 12 significant linkage disequilibria found in the locus–drainage pairings, Lake Huron represented one instance and the rest were Lake Ontario. No evidence of loci linkages were found between loci, as all potential linkages were not consistent across the dataset. Within each locus–drainage combination, significant HWE deviations were found in 16 of the 70 analyses, after Bonferroni correction (α = 0.000714; Table S3). Each locus had between one and three significant deviations. Eight of these 16 significant deviations were found in Lake Ontario.

3.2 | Genetic structure

Genetic structure among geographic locations was revealed using STRUCTURE. The most probable clusters for *S. nasutus* were eight groups, using the Δ*K* method (Figures 2 and S2; Evanno et al., 2005). A higher

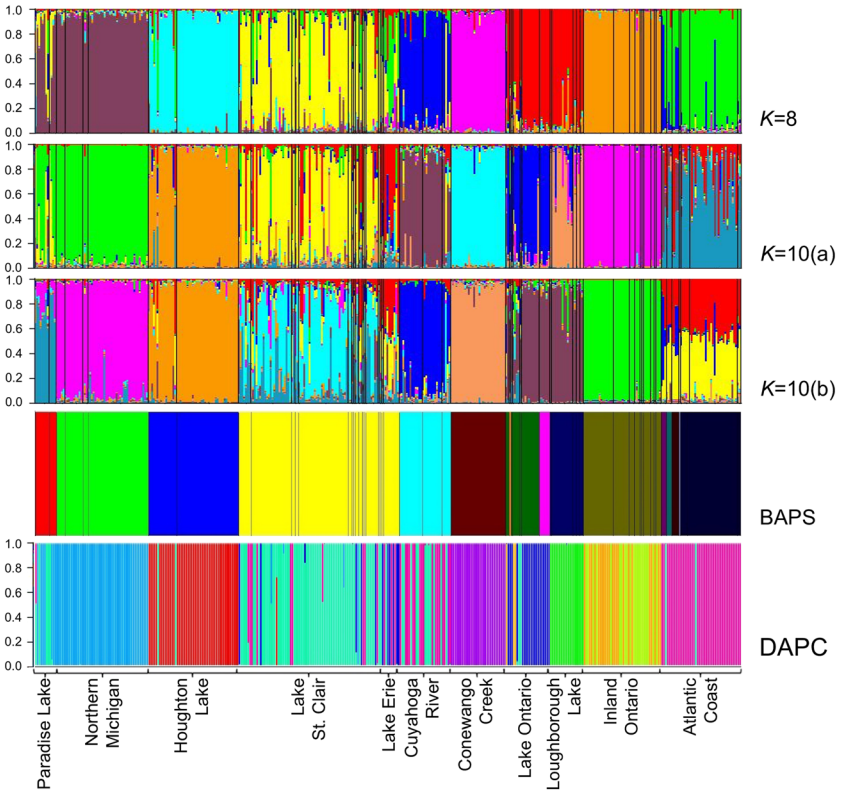

FIGURE 2 STRUCTURE, BAPS, and DAPC bar output for the 58 collection sites. STRUCTURE was run without *a priori* populations assigned (admixture and correlated alleles were assumed) for multiple genetic groups (*K*) ranging from *K* = 8 to *K* = 10. As there were two distinct STRUCTURE results for *K* = 10, both were included. Without locus L25, BAPS results suggested a distinct group was formed by Coyle Creek, Spicer Creek, Rouge River, and Lynde Creek Marsh, representing only nine specimens

resolution and higher ln*P*(*K*) suggested 10 groups (*K* = 10), although multiple iterations of *K* = 10 revealed inconsistent assignments for specimens to groups (Figures 2 and S2). At least one of the two different patterns of *K* = 10 results indicated that Paradise Lake, Lake Erie, and Loughborough Lake are distinct groupings (Figure 2). When the STRUCTURE analyses of all specimens were rerun with only water bodies of ≥10 specimens, Paradise Lake grouped with Douglas and Burt Lake (Northern Michigan), but was separate when L25 was excluded from the analyses.

The BAPS results were largely congruent with the STRUCTURE results; however, the BAPS analyses defined Paradise Lake and Loughborough Lake as distinct groupings, whereas Lake Erie and Lake St. Clair were combined (Figure 2). Additional groups were defined in Lake Ontario and the Atlantic coast by the BAPS results, but these distinctions defined groups with <10 specimens, which can introduce statistical biases as a result of the small sample sizes (e.g. Galbraith et al., 2015), so they remained combined for the purposes of this study.

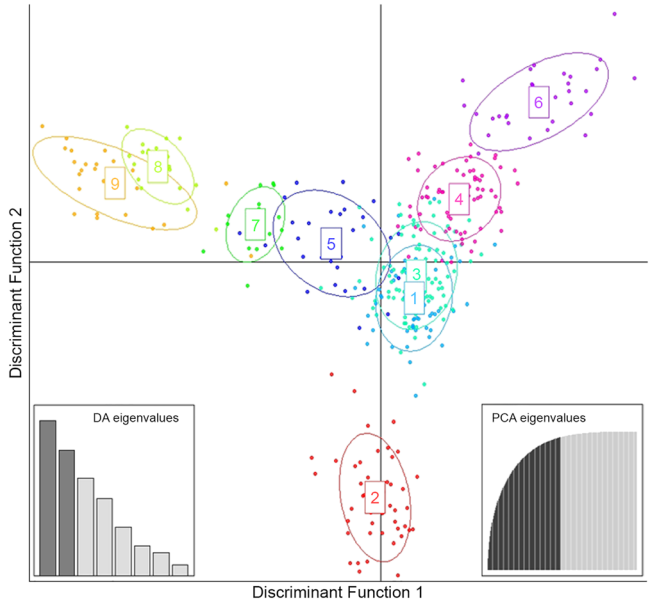

FIGURE 3 Scatter plot output for discriminant analysis of principal components (DAPC) results, showing the separation of the first and second discriminant function for the nine PC clusters. The colouration matches the DAPC assignment plot of Figure 2. The groupings of individual specimens are: 1, Paradise Lake and Northern Michigan; 2, Houghton Lake; 3, Lake St. Clair (in part) and Cuyahoga River (in part); 4, Lake St. Clair (in part), Lake Erie (in part), Cuyahoga River (in part), and Atlantic coast; 5, Lake Erie (in part) and Lake Ontario (in part); 6, Conewango Creek; 7, Loughborough Lake; 8 and 9, Inland Ontario. The final assignment of an individual to a group was based upon its predominant assignment from the DAPC results (Figure 2)

The DAPC results largely supported the findings of the other two analyses. In the DAPC analyses, 100 principal components and eight eigenvalues were retained to preserve over 90% of the variation explained in the discriminant analyses. With a maximum of 59 clusters possible, the plot of the Bayesian information criterion (BIC) values versus the potential number of principal component clusters indicated that between seven and 11 genetic populations were most probable, as they had the lowest BIC values and minimal the ΔBIC values (Figure S3). The number of principal component clusters with the lowest BIC value was nine, which is the number used in the assignment test and the scatter plot analyses (Figures 2, 3, and S3). Although the number of genetic populations was found to be nine, the regional split of these populations did not perfectly align with the delineations suggested by STRUCTURE and BAPS (Figure 2). The DAPC results suggested a moderate separation of Paradise Lake from the Northern Michigan samples. Lake Erie presented as a combination of the principal components defining Lake St. Clair, the Cuyahoga River, Lake Ontario, and the Atlantic coast. The Cuyahoga River presented as a combination of the principal components defining Lake St. Clair and the Atlantic coast. The scatter plot of the DAPC results indicated that the genetic differentiation between these groups of moderate separation had considerable overlap (Figure 3). Beaver Lake and White Lake were both represented by the same two genetic groupings, although the separations were not consistent and they had considerable overlap in the scatter plot of the DAPC results (Figures 2 and 3). When the DAPC analyses of all specimens were rerun with only water bodies of ≥10 specimens, Lake Erie and the Cuyahoga River were grouped together, but were separate when L25 was excluded from the analyses. The genetic structure analyses (STRUCTURE, BAPS, and DAPC) consistently combined Douglas and Burt Lake (Northern Michigan), as well as Beaver and White Lake (Inland Ontario). As a result of the genetic structure analyses, the 11 defined groups consisted of Paradise Lake, Northern Michigan, Houghton Lake, Lake St. Clair, Lake Erie, Cuyahoga River, Conewango Creek, Lake Ontario, Loughborough Lake, Inland Ontario, and the Atlantic coast; however, the purpose of this study was to use the Atlantic coast as a source of comparison for the Great Lakes region, not to determine the genetic structure of the Atlantic coast itself.

The AMOVA results showed a global F_{ST} = 0.150, which was significant, with genetically defined groups holding 15% of the variation (Table 3). The AMOVA results indicated that 11 and 74% of the variation was held among and within individuals, respectively (Table 3). Pairwise population differentiation for *S. nasutus*, with F_{ST} values of

TABLE 3 Analysis of molecular variance (AMOVA) results for *Sagittunio nasutus* using 10 microsatellite loci (Scott et al., 2016)

Source of variation	Degrees of freedom	Sum of squares	Percentage of variation
Among groups	10	479.8	15%
Among individuals	388	1549.8	11%
Within individuals	399	1224.0	74%

All variance components significantly differed from zero.

F_{ST} = 0.029–0.179 (with a mean of 0.100) and D_{est} values of D_{est} = 0.134–0.684 (with a mean of 0.416), showed significant differentiation among all group comparisons, with only one exception for the analyses excluding L25 (Table 4). The values ranged from little to very great genetic differentiation (Table 4). Within each locus–population combination, significant HWE deviations were found in only seven of the 110 analyses after Bonferroni correction (α = 0.000455; Table S4).

Mantel tests of isolation by distance found significant correlation between genetic distances and geographical distances. Linearized F_{ST} genetic distances were compared with waterway distances with an *R* value of 0.706, with $P < 0.001$ (Figure 4).

3.3 | Assessment of genetic diversity

The number of alleles, allelic richness, and private allelic richness varied among loci and populations, although genetic differentiation was found among populations. The locus–population combinations with significantly lower heterozygosity than expected were the same seven locus and population pairs that deviated from HWE (Table S4). The diversity of microsatellite alleles ranged considerably among the loci, with locus L28 having 36 alleles but with locus L17 and locus L21 having only 15 alleles each (Table 2). The mean number of alleles was 22.3. The average allelic richness and average private allelic richness was highest in the Atlantic coast specimens (7.4 and 2.1, respectively) and lowest in the Inland Ontario specimens (2.6 and 0.1, respectively; Table 5). Even when all the Great Lakes region specimens were combined and compared again with their source population, the Atlantic coast, the Great Lakes region collectively had a lower allelic richness and private allelic richness at every locus. For the combined Great Lakes region, the mean ± standard error of allelic richness was 5.5 ± 0.5 and the private allelic richness was 2.9 ± 0.5. The Atlantic coast had an allelic richness of 7.4 ± 0.2 and a private allelic richness of 4.8 ± 0.3. The non-parametric Kruskall–Wallis test for the number of alleles, observed heterozygosity, private allelic richness, and allelic richness all showed significant differences overall, with all four tests having $P < 0.001$. Multiple-comparison tests revealed that the Atlantic coast was the most distinct population (α = 0.000909; Table 6).

BOTTLENECK analyses revealed a significant probability of genetic bottlenecks at most locations. Seven of the 11 locations had at least one positive test for a genetic bottleneck (Tables 5 and S5). Lake St. Clair had three tests suggesting a genetic bottleneck (Tables 5 and S5). Northern Michigan, Houghton Lake, Loughborough Lake, Inland Ontario, and the Atlantic coast had two tests indicating a genetic bottleneck. The Cuyahoga River had one test suggesting a genetic bottleneck (Tables 5 and S5). The TPM tests for the Cuyahoga River, Conewango Creek, Lake Ontario, and Inland Ontario had *P* values outside the range of their SMM and IAM tests. Sensitivity analyses were unable to remove the phenomenon of TPM *P* values lying outside the ranges of their corresponding SMM and IAM *P* values. The anomalous result was considered an artefact of the data and qualitatively irrelevant. The analyses without locus L25 had similar results (Table 5).

Potential signs of inbreeding within populations were assessed by calculating the inbreeding coefficient, F_{IS}. AMOVA calculated a global F_{IS} of 0.131, which was significant, with $P = 0.001$. The range of F_{IS} scores was −0.038 to 0.216, with a mean of 0.096 (Table 5). Lake Ontario had the highest F_{IS} and Conewango Creek had the lowest and only negative F_{IS} score (Table 5). The positive F_{IS} scores in Northern Michigan, Lake Erie, the Cuyahoga River, Loughborough Lake, and Inland Ontario were not significant (Table 5).

4 | DISCUSSION

The results of this study indicated genetic structure among populations of *S. nasutus*. The pattern of genetic diversity and structure among the genetic populations of *S. nasutus* provides insight into the phylogeography and colonization history of the species in the Great Lakes region. The genetic diversity of the populations of *S. nasutus* can inform conservation efforts in the future as to the delineation of populations, as well as help prioritize the conservation efforts between populations. Despite the likely presence of null alleles and potential for some stuttering errors at a few loci, the results appear to be robust, especially because the exclusion of the potentially problematic locus L25 did not result in major changes to the interpretations of the results.

4.1 | Genetic structure and phylogeographic implications

Contrary to the findings of Scott et al. (2014), microsatellite markers identified distinct genetic populations across the distribution of *S. nasutus*. Mitochondrial analyses of two genes in *S. nasutus* found only one cytochrome *c* oxidase subunit I (*CO1*) and two NADH dehydrogenase subunit 1 (*ND1*) haplotypes within the Great Lakes region and no evidence of geographical structure, compared with four *CO1* and six *ND1* haplotypes found on the Atlantic coast (Scott et al., 2014). This increase in the number of loci used and the hypervariable nature of microsatellite markers allowed finer-scale comparisons between, within, and among sampling locations. The same overall pattern of the Great Lakes region being a subset of the genetic diversity in the Atlantic coastal region was found in both the results of this study and in those of Scott et al. (2014). It is important to note, however, that the microsatellite DNA analyses provide further detail to the overall lack of Great Lakes region structure found in the mitochondrial DNA data. A similar pattern of genetic diversity is often, but not always, found between microsatellite and mitochondrial datasets (Chong, Harris, & Roe, 2016; Zanatta & Murphy, 2007). The microsatellite results indicated that the Atlantic coastal region had higher allelic richness and private allelic richness than the Great Lakes region. Although some private alleles were found in the Great Lakes region, the specimens analysed from the Atlantic coastal region were, as a whole, more genetically diverse and unique in comparison. The Atlantic coast was the origin for the populations in and near the Great

TABLE 4 Pairwise population differentiation for *Sagittunio nasutus*, with F_{ST} values below the diagonal and D_{est} values above the diagonal

	Paradise Lake	Northern Michigan	Houghton Lake	Lake St. Clair	Lake Erie	Cuyahoga River	Conewango Creek	Lake Ontario	Loughborough Lake	Inland Ontario	Atlantic Coast
Paradise Lake	–	0.199	0.349	0.234	0.446	0.357	0.565	0.431	0.478	0.445	0.684
Northern Michigan	0.080	–	0.338	0.253	0.370	0.391	0.548	0.450	0.340	0.399	0.653
Houghton Lake	0.129	0.112	–	0.301	0.409	0.453	0.597	0.468	0.413	0.419	0.674
Lake St. Clair	0.063	0.056	0.069	–	0.138	0.205	0.439	0.309	0.413	0.418	0.418
Lake Erie	0.115	0.088	0.101	0.031	–	0.298	0.306	0.134[a]	0.356	0.443	0.339
Cuyahoga River	0.087	0.081	0.097	0.029	0.050	–	0.442	0.396	0.468	0.426	0.347
Conewango Creek	0.152	0.135	0.152	0.073	0.064	0.073	–	0.493	0.550	0.635	0.536
Lake Ontario	0.130	0.120	0.130	0.058	0.042[a]	0.071	0.108	–	0.268	0.330	0.551
Loughborough Lake	0.179	0.124	0.152	0.101	0.100	0.111	0.155	0.092	–	0.261	0.648
Inland Ontario	0.174	0.145	0.158	0.104	0.121	0.105	0.179	0.110	0.117	–	0.661
Atlantic Coast	0.115	0.098	0.107	0.037	0.041	0.032	0.064	0.071	0.116	0.122	–

No comparisons were found to be insignificant or to have zero genetic differentiation after Bonferroni correction. Grey cells indicate little genetic differentiation (F_{ST} or D_{est} < 0.05). Green cells indicate moderate genetic differentiation (F_{ST} or D_{est} ≥ 0.05–0.15). Yellow cells indicate great genetic differentiation (F_{ST} or D_{est} ≥ 0.15–0.25). Orange cells indicate very great genetic differentiation (F_{ST} or D_{est} ≥ 0.25; Hartl & Clark, 1997).

[a]The pairwise comparison was not significant when locus L25 was excluded.

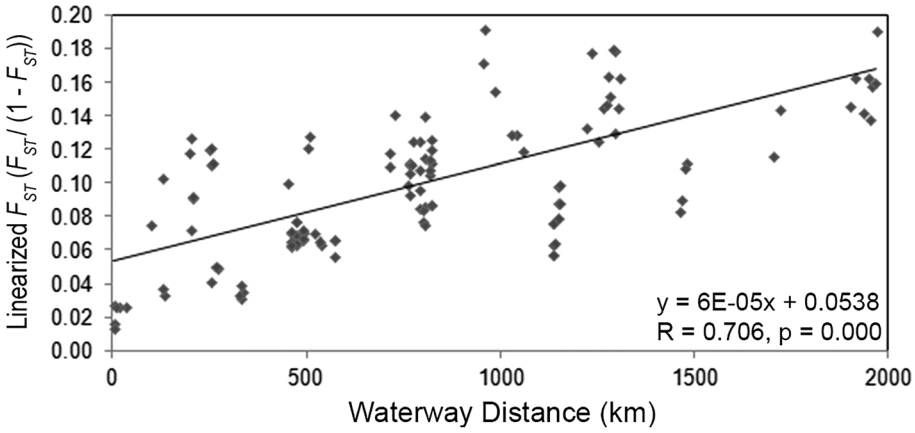

FIGURE 4 Mantel tests of isolation by distance for *Sagittunio nasutus* comparing collection site groups within the Great Lakes region, linearized F_{ST} genetic distances, and geographical waterway distances. Table 1 provides a reference for how collection sites were grouped to increase distance accuracy. The linear regression equation, *R*, and *P* values are included in the graph

TABLE 5 The number of specimens (*n*), mean allelic richness (based on minimum of *n* = 11), and mean private allelic richness for each population of *Sagittunio nasutus*. Calculation of F_{IS} for all genetic populations of *S. nasutus*, with significant results after Bonferroni correction in bold. Summary of tests for genetic bottlenecks in BOTTLENECK for *S. nasutus*. Four tests were conducted: the infinite allele model (IAM); the two-phase model (TPM), run with a fixed proportion of 95% stepwise mutation and then run with a 12% variance of geometric distribution; the stepwise mutation model (SMM), and tests for an allelic mode shift

Population	*n*	Allelic richness	Private allelic richness	F_{IS}	IAM/TPM/ SMM/mode
Paradise Lake	12	3.5	0.2	**0.199**	–/–*/–*/–
Northern Michigan	52	3.5	0.2	0.072*	–/+/+/–
Houghton Lake	51	3.3	0.3	**0.133**	–/+/+/–
Lake St. Clair	80	5.6	0.5	**0.054**	+*/+/+/–
Lake Erie	11	5.5	0.5	0.086	–/–/–/–
Cuyahoga River	29	5.6	0.7	0.045	+/–/–/–
Conewango Creek	31	3.9	0.2	–0.038	–/–/–/–
Lake Ontario	25	4.3	0.1	**0.216**	–/–/–/–
Loughborough Lake	19	3.4	0.2	0.061	–/+/+/–
Inland Ontario	44	2.6	0.1	0.094	+/–/–/+
Atlantic Coast	45	7.4	2.1	**0.132**	+/+*/–/–

+ positive test for a genetic bottleneck; –, insignificant or null result.
*Analyses excluding locus L25 found the opposite significance result in that test.

Lakes region, so the results indicate that the colonization of the new regions represented a comparative reduction from the total diversity found within the source population.

Analyses identified 10 distinct populations within the Great Lakes region. The populations were defined based on the combined results of genetic structure analyses, although additional substructure was possible within Lake Ontario in the Great Lakes region. The strongest evidence that these genetically defined groups are distinct resolved populations was the global F_{ST}, which indicated great genetic differentiation, and all pairwise D_{est} values being significant (Table 4).

4.2 | Patterns of spatial genetic structure and post-glacial colonization

Consistent with the findings using mtDNA sequence data (Scott et al., 2014), the Atlantic coast has a higher allelic richness and private allelic richness than any other population, even when compared with all other populations combined. This is consistent with the Great Lakes region being a genetic subset of the Atlantic coast. The pairwise measures of genetic differentiation were lowest or among the lowest between the Atlantic coast and populations in the Lake Erie drainage. Goslin (1943) found evidence of *S. nasutus* existing in Lake Erie before any canals were constructed. In addition, Lake Ontario was genetically similar to Lake Erie, with one analysis indicating that they were not significantly differentiated. We cannot exclude the possibility, however, that the Lake Ontario population, as well as the adjacent Loughborough Lake and Inland Ontario populations, represent a second colonization event from an as yet unsampled area of the Atlantic Coast. Other inland lake populations sampled in this study represent even less genetic diversity and appear to have been subsequently colonized by small founding populations; however, populations in this study that have lower genetic diversity may be the result of human

TABLE 6 Results of non-parametric Kruskal–Wallis multiple comparison tests for the number of alleles, observed heterozygosity, allelic richness, and private allelic richness, in that order. Statistically significant results are denoted with a '+' ($\alpha = 0.00909$)

	Paradise Lake	Northern Michigan	Houghton Lake	Lake St. Clair	Lake Erie	Cuyahoga River	Conewango Creek	Lake Ontario	Loughborough Lake	Inland Ontario	Atlantic Coast
Paradise Lake	-										
Northern Michigan	−/−/−/−	-									
Houghton Lake	−/−/−/−	−/−/−/−	-								
Lake St. Clair	+/−/−/−	−/−/−/−	−*/−/−/−	-							
Lake Erie	−/−/−/−	−/−/−/−	−/−/−/−	−/−/−/−	-						
Cuyahoga River	−/−/−/−	−/−/−/−	−/+*/−/−	−/−/−/−	−/−/−/−	-					
Conewango Creek	−/−/−/−	−/−/−/−	−/−/−/−	−/−/−/−	−/−/−/−	−/−/−/−	-				
Lake Ontario	−/−/−/−	−/−/−/−	−/−/−/−	−/−/−/−	−/−/−/−	−/−/−/−	−/−/−/−	-			
Loughborough Lake	−/−/−/−	−/−/−/−	−/−/−/−	+/−/−/−	−/−/−/−	−/−/−/−	−/−/−/−	−/−/−/−	-		
Inland Ontario	−/−/−/−	−/−/−/−	−/−/−/−	+/−/+/+*	+*/−/+/+*	+/−/+/+*	−/−/−/−	−/−/−/−	−/−/−/−	-	
Atlantic Coast	+/−/+/+	+/−/+/+	+/+*/+/+	−/−/−/−	−/−/−/−	−/−/−/−	+/−/+/+	+*/−/−/+	+/+/+/+	+/+*/+/+	-

+ significant differentiation.
*Analyses excluding locus L25 found the opposite result in that test.

impact as well as signatures of historical founding effects. Geist et al. (2018) found that Irish populations of *Margaritifera margaritifera* that had experienced a deterioration of habitat quality had lower diversity indices, some by more than a factor of two, compared with other Irish populations with fewer human impacts.

4.3 | Genetic diversity in remnant populations

Sagittunio nasutus had differing levels of genetic diversity in populations and positive tests for recent genetic bottlenecks in most of the populations. The populations of *S. nasutus*, as defined by this study, represented differing levels of genetic diversity in the form of allelic richness and private allelic richness. The cause of the detected bottlenecks was unclear, as it could be the result of founder effects in historical colonization events or recent declines in N_e, linked to habitat changes and competition with dreissenid mussels. For unionids, it is difficult to detect recent bottlenecks in historically common species because of their longevity, as the test can only detect bottlenecks within the range of 2–4 N_e generations (Galbraith et al., 2015; Keyghobadi, 2007; Rowe & Zanatta, 2015). Therefore, the detected genetic bottlenecks are more likely to be signals of founder effects linked to colonization history rather than recent declines in N_e. This means additional demographic bottlenecks may have also occurred but happened too recently to detect. Detected and as yet undetected genetic bottlenecks are cause for concern when considering the resilience of the species to a changing environment. The presence of TPM *P* values outside the range of their corresponding IAM and SMM values was considered an artefact of the data. Other microsatellite studies have found the same phenomenon (Fine, Misiewicz, Chavez, & Cuthrell, 2013; Hawley, Hanley, Dhornt, & Lovette, 2006; Hänfling, Hellemans, Volckaert, & Carvalho, 2002; Jackson, Talbot, & Farley, 2008). Pascual, Aquadro, Soto, and Serra (2001) hypothesized that TPM models may produce different results from IAM and SMM models, if the data represent the combination of mostly single-step mutations with occasional complex mutations. If the signal of a bottleneck is the result of a founding effect or a recent population decline, it may indicate that the population is at higher risk from environmental changes than a more genetically diverse population.

Positive inbreeding coefficient (F_{IS}) scores were found in all populations, except for Conewango Creek, which also showed no sign of a recent genetic bottleneck. Paradise Lake, Lake Erie, and Lake Ontario had the highest F_{IS} scores of their regions, whereas Lake St. Clair, Conewango Creek, and Loughborough Lake had the lowest F_{IS} scores of their regions. In a study on the Eurasian unionid *Anodonta anatina*, it was concluded that inbreeding was occurring, possibly through human-caused population loses, with F_{IS} scores ranging from −0.03 to 0.38 (Lopes-Lima et al., 2016), which was similar to the range found for *S. nasutus* (F_{IS} = −0.038 to 0.216; Table 5). There was evidence of a loss of genetic diversity in most of the *S. nasutus* populations studied that could

be related to the colonization history and relatively recent changes in N_e.

4.4 | Conclusions and conservation recommendations

This study was successful in defining genetic populations for *S. nasutus* through the use of more detailed microsatellite markers (Scott et al., 2016) than the mtDNA markers used by Scott et al. (2014). The Lake Erie drainage appears to be the region of initial colonization from the Atlantic coast, with subsequent colonization events throughout the Great Lakes region. There was evidence of a loss of genetic diversity in most of the populations studied; however, the cause of those losses may have been recent genetic bottlenecks caused by dreissenid invasions and habitat changes or historical founder effects as the species initially colonized new habitats.

The data from this study should better inform future conservation and management efforts for *S. nasutus* across its distribution, but also provide insight into the general conservation of freshwater species in recently glaciated regions. For the purposes of the conservation management of *S. nasutus*, we suggest that a distinct population should be defined as a management unit (e.g. Moritz, 1994). The most important distinction for this species in the Great Lakes region is that it is geographically and genetically distinct from the Atlantic coast, a finding that was not unexpected given the physical and temporal isolation of the two regions. *Sagittunio nasutus* in the Great Lakes region was founded by and isolated from its more genetically diverse source population on the Atlantic coast. For species with a similar phylogeographic history, a similar strategy of preserving the long-term isolation of the regions may be advisable when possible.

We recommend that conservation and restoration efforts should take the 10-management-unit conclusion into account for *S. nasutus* in the Great Lakes region. We believe this more detailed view of geographical structure in the Great Lakes region will be more informative when developing conservation and restoration plans for *S. nasutus*. It is possible that the genetic structure found in the microsatellite analyses, but not in the mtDNA analyses performed by Scott et al. (2014), is the result of recent anthropogenically induced population declines (Hoffman, Willoughby, Swanson, Pangle, & Zanatta, 2017). We also recommend that in larger genetically diverse populations (i.e. within the entire Great Lakes region), efforts should be made to avoid using a single population to propagate stock for another population; however, if a population has very low genetic diversity or drastic demographic declines, we recommend that managers use other populations with similarly lower levels of genetic differentiation, or the likely source population, to propagate stock for the impoverished or extirpated population. The complex geographical pattern of genetic diversity and structure found in *S. nasutus* reinforces the need for conservation practitioners to balance concerns about a population's demographic declines, genetic diversity, and potential inbreeding with attempting to preserve distinctions among populations, and ultimately their evolutionary legacy (Waples, 1995).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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