Journal of Herpetology, Vol. 54, No. 1, 78–86, 2020

Phylogeographic Analysis of Mudpuppies (*Necturus maculosus*)

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Abstract.—**The geology of the Pleistocene, and particularly the Last Glacial Maximum approximately 26.5 ka, is a critical driver of species present-day distributions and levels of genetic diversity in northern regions. Using mitochondrial DNA sequence data, we tested several predictions relating to the postglacial recolonization of the northern United States and southern Canada by Mudpuppies (*Necturus maculosus*). Our analyses revealed a significant split between western and eastern lineages, with the divide corresponding to the location of the Mississippi River. Our data support the presence of one or more Mississippian glacial refugia, with subsequent expansion and diversification of a western cluster into the upper Midwest and an eastern cluster into the eastern Great Lakes and New England. As predicted in cases of postglacial colonization, each of these clusters contains a single widespread and common haplotype along with numerous low-frequency, closely related haplotypes. Given recent conservation concerns about amphibians in general, and Mudpuppies specifically, we discuss our results in light of species conservation. Knowledge of a species' genetic diversity allows for informed management and facilitates decisions that preserve local adaptation and evolutionary potential.**

Geological events of the Pleistocene played a crucial role in structuring present-day distribution and genetic diversity of northern species. During the Last Glacial Maximum 33.0–26.5 ka (Clark et al., 2009), the Great Lakes region and much of the northeastern United States was covered with glacial ice and therefore inhospitable to species present today. Studies in both Europe and North America have shown that many species in formerly glaciated areas were able to persist in southern glacial refugia and subsequently recolonized northward following glacial retreat (Mandrak and Crossman, 1992; Hewitt, 2000).

The processes of deglaciation and recolonization generate predictable patterns in genetic diversity of populations present in these formerly glaciated regions. For example, when compared to southern counterparts, populations in northern regions tend to have reduced genetic diversity because of a series of bottleneck events as populations followed the glacial retreat northward (Bernatchez and Wilson, 1998). These relatively rapid serial founding events at the leading edge of the dispersal front also resulted in widespread common haplotypes across relatively large spatial areas (Hewitt, 2000). In formerly glaciated regions, these common haplotypes are accompanied by numerous low-frequency, but closely related, haplotypes resulting in "star-like" haplotype clusters (Mäkinen and Merilä, 2008).

We use regional geology to generate predictions relating to the genetic diversity of Mudpuppies (*Necturus maculosus*), which are fully aquatic salamanders found throughout the Great Lakes region, parts of Southern Canada, and as far east as New England. Throughout their range, Mudpuppies occupy a wide variety of aquatic habitats including lakes, reservoirs, canals, and streams. Though geographically widespread, Mudpuppies are behaviorally cryptic and tend to be most plentiful in habitats containing cover objects (e.g., flat rocks, logs, and planks) or retreats (e.g., crayfish burrows, undercut banks, and tree roots; Lannoo, 2005). Their entirely aquatic nature and long lifespans make Mudpuppies ideal study organisms for assessing water quality and overall ecosystem health (Bonin et al., 1995; Harding and Mifsud, 2017). Mudpuppies are also ecologically significant as the sole host organism to the larval life stage of the Salamander Mussel (*Simpsonais ambigua*), which is listed as threatened or endangered within most of its range (Roe, 2003; Bogan et al., 2017). Over a century ago, Eycleshymer (1906) reported that Mudpuppies were common, but current evidence suggests some populations are declining, particularly in the Midwest and Northeast (King et al., 1997; Harding and Mifsud, 2017). The major threats to population persistence are overcollection, lampricide application, and habitat degradation through siltation, pollution, and climate change (Gilderhus and Johnson, 1980; Matson, 1990; Boogaard et al., 2003; Chellman et al., 2017).

Understanding the natural history and genetic diversity of Mudpuppies could aide in more-effective management of the species. Phylogeographic analysis may identify genetically distinct populations, which could warrant separate management to avoid the effects of outbreeding depression and maximize evolutionary potential (Allendorf and Luikart, 2007; Sabatino and Routman, 2009). Based on historic population structure, it may be possible to organize populations into evolutionary significant units (ESUs) or management units (MUs) that may be used to guide future management (Palsbøll et al., 2007; Pan et al., 2014). Another fully aquatic salamander species, Hellbenders (*Cryptobranchus alleganiensis*), exhibit substantial genetic divergence among river drainages (Unger et al., 2013, 2016), warranting distinct management to maintain genetic integrity. Similarly, recent work on the congener *Necturus beyeri* identified six lineages, each associated with a unique river drainage along the Gulf Coast (Chabarria et al., 2018). This study, which included four *N. maculosus* individuals, found separation between Mississippi River ($n = 2$) and Great

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DOI: 10.1670/19-070

Lakes ($n = 2$) lineages based on the mitochondrial *ND2* gene. The authors suggest that Mudpuppies in the Great Lakes basin may warrant separate species status.

For the purposes of determining possible postglacial colonization routes, studies involving fish movement provide a model for comparison because movement of Mudpuppies is also restricted to aquatic habitat (Hecht and Walters, 1955). In a study of likely colonization routes of fish with present-day distributions in Ontario, Canada, most species were believed to have originated from a Mississippi River refugium (Mandrak and Crossman, 1992). Recent geographical analyses suggest that Mudpuppies may have persisted in a Mississippian refugium as well and subsequently migrated from west to east during the Nipissing Phase 4,000–5,000 yr ago (Mills and Hill, 2016). During this time, water levels were sufficiently high to connect the Mississippi River to Lake Michigan via the Illinois River and the Sag Channel (Hansel et al., 1985), allowing aquatic species to colonize previously glaciated regions. An alternative hypothesis suggests a Mississippian origin and subsequent northward colonization from Minnesota via a drainage reversal that caused the Red River of the North to flow into ancient Lake Agassiz and into the Hudson Bay (Hecht and Walters, 1955). While a southern Mississippian refugium appears likely for Mudpuppies, populations may also have persisted along the Mississippi in southeastern Minnesota, which was part of the unglaciated Driftless Area (Dyke and Prest, 1987; Clark et al., 2012).

We conducted phylogeographic analyses based on samples collected from nearly 350 Mudpuppies from nine US states and two Canadian provinces using mitochondrial sequence data (mtDNA) to test the following four predictions: 1) populations will include widespread common haplotypes across broad regions of postglacial colonization; 2) less common haplotypes will also be present and closely associated with the widespread common haplotypes ("star-like" haplotype network) because of recent diversification in these formerly glaciated regions; 3) haplotypes in formerly glaciated regions will be derived from haplotypes in unglaciated southern and western regions; and 4) given the large spatial scale of sampling, populations will exhibit significant isolation-by-distance relationships.

Materials and Methods

Our analyses include Mudpuppies from nine states in the United States (Iowa, Kentucky, Massachusetts, Michigan, Minnesota, Missouri, New York, Ohio, and Vermont) and two Canadian provinces (Ontario and Quebec). Because of the large number of collaborators on this project (see Acknowledgments), trapping and sampling protocols varied across sites. Collaborators caught Mudpuppies via a combination of direct capture, modified minnow trapping, as bycatch during set-line fisheries surveys, or lampricide treatments. Collaborators obtained most tissue samples from tail fins; however, liver tissue represented some Canadian samples. To collect tail tissue samples, some collaborators restrained Mudpuppies in clear plastic tubes sufficiently wide to accommodate body width and reduce discomfort while also restricting movement. Collaborators placed the tail fin on a sterilized surface and removed a small tissue sample, measuring <5 mm^2, using a razor blade or surgical scissors sterilized with ethanol. We stored samples either at room temperature or at −20°C in 95% ethanol until DNA extraction.

We used a Qiagen DNeasy® Blood and Tissue Kit to extract genomic DNA from each tissue sample following the manufacturer's protocol. We amplified a 742-base pair (bp) segment of the cytochrome b (*Cyt b*) region of the mitochondrial genome with primers MVZ15 (5′-GAACTAATGGCCCACACWWTACGNAA-3′) and MVZ16 (5′-AAATAGGAARTATCAYTCTGGTTTRAT-3′; Moritz et al., 1992). Each reaction contained 4 μL of MyTaq™ Red Mix, 1 μM of each primer, 50–100 ng of genomic DNA and distilled H_2O, for a total volume of 10 μL. The polymerase chain reaction (PCR) program consisted of an initial denaturation of 2 min at 95°C followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 50°C for 1 min, and extension at 72°C for 2 min. A final extension cycle followed for 5 min at 72°C. We confirmed successful DNA amplification using a 1% agarose gel. We sequenced successful reactions at the University of Michigan DNA Sequencing Core (Ann Arbor, Michigan). We extracted genomic DNA from samples from the Northeastern United States using a Gentra Puregene® Tissue Kit. We used additional primers described by Moritz et al. (1992) MVZ25 (5′-TAAAGAAACTTGAAAYATYGGHGT-3′) and cyt-b2 (5′-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3′) to provide better resolution at the 3′ or 5′ ends of the *Cyt b* fragment. We conducted amplifications with illustra PuReTaq Ready-To-Go™ polymerase beads (GE Healthcare). Amplified PCR products were treated with ExoSAP-IT™ (USB Corporation) to remove unincorporated primers and nucleoside triphosphates (NTPs) prior to sequencing. The PCR product was sequenced using Big Dye® Terminator v3.1 (Applied Biosystems), and cycle sequencing products were purified on G-50 fine Sephadex® columns before fractionization and visualization on a 3130 Genetic Analyzer (Applied Biosystems) at the Vermont Integrative Genomics Resource; see Chellman (2011) and Kilpatrick and Chellman (2012) for further details.

We edited forward and reverse sequences using Geneious ver. 6.1.8 (Kearse et al., 2012). We generated a minimal spanning network of haplotypes using the Templeton, Crandall, and Sing method (TCS; Templeton et al., 1992) in PopART ver. 1.7 (Leigh and Brayant, 2015). We used Geneland (Guillot et al., 2005a,b) to determine the number of populations present among the localities sampled. Geneland uses a spatially explicit Bayesian clustering program with which we conducted with 100,000 Markov chain Monte Carlo (MCMC) iterations and sampled every 100th iteration with a burn-in of the first 200 sampled iterations. We measured molecular diversity using DnaSP ver. 5 (Librado and Rozas, 2009) and population-level diversity using Arlequin ver. 3.5 (Excoffier and Lischer, 2010). For some Arlequin analyses, we grouped sampling localities by watershed. We had samples from 29 locations distributed across seven watersheds: the Great Lakes ($n = 136$), Upper Mississippi ($n = 86$), Souris-Red-Rainy ($n = 24$), Missouri ($n = 30$), Ohio ($n = 4$), Connecticut ($n = 6$), and Lake Champlain ($n = 61$), totaling 347 samples. We determined significant isolation by distance using Mantel analyses with Arlequin to evaluate the correlation between geographic distance and genetic distance. We obtained genetic distances by calculating pairwise F_{ST} values with Arlequin, and we calculated geographic distances by measuring the pairwise distances, in kilometers, between the midpoints of 29 sampling localities and between the midpoints within each watershed.

Results

The *Cyt b* sequences contained 24 unique haplotypes (Table 1; GenBank Accession Numbers KY788150–KY788155 for haplotypes V, D, I, B, W and X, respectively; and MK988094–MK988111 for haplotypes A, C, E–H and K–U, respectively).

TABLE 1. Mudpuppy (*N. maculosus)* sampling locations and mitochondrial *Cyt b* haplotypes detected (number of individuals in parentheses).

Sampling locality	Location	Watershed	*n*	Haplotype
1	Red Lake River, Minnesota	Souris-Red-Rainy	9	D (9)
2	Otter Tail River, Minnesota	Souris-Red-Rainy	8	D (7), K (1)
3	Big Cormorant Lake, Minnesota	Souris-Red-Rainy	7	D (7)
4	Lake Latoka, Minnesota	Upper Mississippi	14	D (13), F (1)
5	Minnesota River, Minnesota	Upper Mississippi	29	D (24), G (1), H (1), I (2), J (1)
6	Lower Mississippi River, Minnesota	Upper Mississippi	13	D (12), E (1)
7	Upper Mississippi River, Minnesota	Upper Mississippi	18	D (18)
8	St. Croix River, Minnesota	Upper Mississippi	12	D (11), G (1)
9	Maries, Missouri	Missouri	3	I (1), Q (1), R (1)
10	Clayton, Iowa	Missouri	27	D (23), T (4)
11	Pendleton, Kentucky	Ohio	4	B (4)
12	Kent, Michigan	Great Lakes	3	A (3)
13	St. Clair River (N), Michigan/Ontario	Great Lakes	6	B (5), M (1)
14	St. Clair River (S), Michigan/Ontario	Great Lakes	16	B (15), L (1)
15	Lake St. Clair, Michigan/Ontario	Great Lakes	6	B (5), O (1)
16	Huron River, Michigan	Great Lakes	2	B (2)
17	Detroit River, Michigan/Ontario	Great Lakes	40	B (38), C (1), P (1)
18	Lake Erie (E), Ontario	Great Lakes	3	B (3)
19	Kemptville Creek, Ontario	Great Lakes	8	B (8)
20	St Lawrence River, Ontario/Quebec	Great Lakes	22	B (17), N (4), S (1)
21	Wolfe Island, Ontario	Great Lakes	4	B (3), N (1)
22	Clinton, New York	Lake Champlain	4	B (4)
23	Niagara, New York	Great Lakes	4	X (4)
24	Lake Erie (S), Ohio	Great Lakes	5	B (5)
25	Ashtabula, Ohio	Great Lakes	17	B (16), U (1)
26	Franklin, Massachusetts	Connecticut	6	D (5), I (1)
27	Lamoille River, Vermont	Lake Champlain	21	V (21)
28	Winooski River, Vermont	Lake Champlain	6	B (2), V (3), W (1)
29	Poultney River, Vermont	Lake Champlain	30	B (28), X (2)

Haplotype B, which was found exclusively in eastern states and provinces (Kentucky, Michigan, New York, Ohio, Ontario, Quebec, and Vermont), was the most prevalent (155 individuals and 15 localities; 44.7% of samples; Table 1; Fig. 1). Haplotype D, which was found in Iowa, Minnesota, and Massachusetts, was the second-most prevalent (124 individuals and 10 localities; 35.7% of samples). Haplotype I is an intermediate haplotype linking the two most common haplotypes from our analysis (B and D; Fig. 2). The network (Fig. 2) also demonstrates that all haplotypes are only one to three mutational steps from either Haplotypes B or D and that these two haplotypes differ by only two mutational steps.

The spatially explicit Bayesian analysis of the *Cyt b* haplotypes with Geneland identified the highest likelihood for two broad genetic units being present: a western cluster including Iowa, Minnesota, and Missouri, and an eastern cluster with one sample locality, Massachusetts, which was assigned to the western cluster (Fig. 3A). Analysis of just the 201 samples assigned to the "eastern cluster" in the previous analysis showed three populations: 1) Kent, Michigan; 2) Lamoille and Winooski rivers, Vermont; and 3) a large central population cluster including samples from the Ohio River and other parts of the Great Lakes and Lake Champlain watersheds. These results suggest differentiation of samples at the margins of the eastern cluster (Fig. 3B). No substantial substructure was detected in the western cluster.

Measures of sequence diversity revealed that Mudpuppies sampled from the western cluster had a haplotype diversity (Hd) of 0.215 ± 0.047 with 11 haplotypes containing 14 polymorphic sites. The Hd in the eastern cluster was 0.383 ± 0.041 with 13 haplotypes containing 12 polymorphic sites. A nucleotide diversity (π) of 0.0006 was observed in both clusters and the mutation rates ($\theta\pi$) were similar, with 0.445 calculated for the western cluster compared with 0.460 for the eastern cluster.

Significant pairwise F_{ST} values ranged from 0.060 between samples from the Upper Mississippi and the Missouri River watersheds to 0.928 between samples from the Souris-Red-Rainy rivers and the Ohio River watersheds (Table 2). No differentiation (values not significantly different from zero) was observed between samples from the Connecticut River (Massachusetts) and samples from the Upper Mississippi, Souris-Red-Rainy, or Missouri watersheds. However, pairwise F_{ST} values between the Connecticut River and Lake Champlain, Ohio and the Great Lakes watersheds were significantly differentiated (Table 2). When the Massachusetts samples were excluded, the western and eastern clusters were highly differentiated (F_{ST} = 0.686). Significant F_{ST} values were observed between the three populations detected by Geneland within the eastern cluster, ranging from 0.817 between the large central population and Kent, and 0.786 between the Lamoille-Winooski rivers population and the large central population, including other samples from Lake Champlain. The Lamoille-Winooski rivers popula-

TABLE 2. Pairwise F_{ST} comparisons of the *Cyt b* region of the mitochondrial genome sequenced from tissue samples of Mudpuppies (*N. maculosus*) collected from seven broad watershed areas. GL = Great Lakes; MS = Upper Mississippi; SRR = Souris-Red-Rainy; OH = Ohio; MO = Missouri; CT = Connecticut; LC = Lake Champlain. Asterisks indicate values significantly different from zero.

	GL	MS	SRR	OH	MO	CT	LC
GL	—						
MS	0.776*	—					
SRR	0.781*	0	—				
OH	0	0.838*	0.928*	—			
MO	0.703*	0.060*	0.071	0.672*	—		
CT	0.733*	0	0.067	0.797*	0	—	
LC	0.260*	0.661*	0.622*	0.172	0.514*	0.510*	—

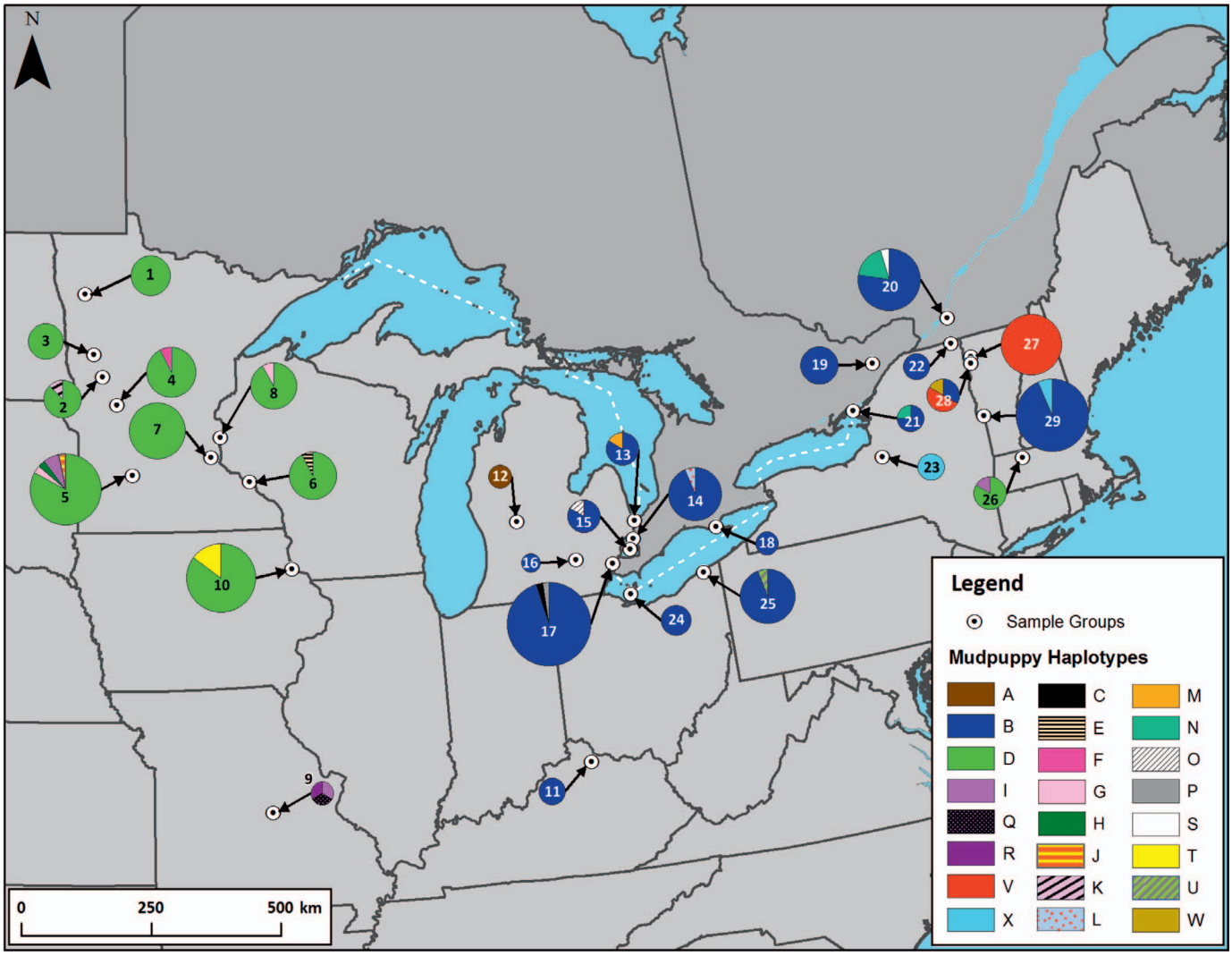

FIG. 1. *Cyt b* haplotypes documented in this study. Haplotype pie charts for each group are scaled according to sample size. Numbers associated with each pie chart indicate the sample group number, which corresponds with Table 1. The most common haplotypes were D (bright green) and B (medium blue). The intermediate haplotype (I; light purple) is found in Massachusetts, Minnesota, and Missouri.

tion was significantly differentiated ($F_{ST} = 0.815$) from the Kent population.

An analysis of molecular variance (AMOVA) revealed that 56.89% of the variation was observed among watersheds with a significant F_{CT} of 0.5689. However, significant variation occurred among sampling localities within watersheds, 21.35% ($F_{SC} = 0.4952$), and within sampling localities, 21.76% ($F_{ST} = 0.7824$).

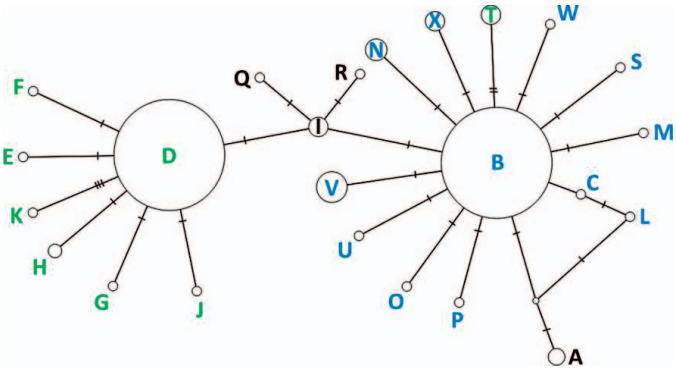

FIG. 2. Haplotype network of *Necturus maculosus Cyt b* sampled in this study. Haplotypes in the eastern cluster are indicated in blue and those in the western cluster are in green.

The Mantel test of the sampling localities recovered significant isolation by distance ($P < 0.01$) from the positive correlations between geographic distance and genetic distance (F_{ST}), either when the Massachusetts' samples were included ($R = 0.473$, $P < 0.01$; Fig. 4A) or excluded ($R = 0.578$, $P < 0.01$; Fig. 4B). However, partitioning the data by watersheds did not support isolation by distance, whether the Massachusetts' samples were included (Mantel $P = 0.235$) or excluded (Mantel $P = 0.06$), though the genetic and geographic distances were significantly correlated when the Massachusetts' samples were excluded ($R = 0.671$, $P < 0.01$; Fig. 4D). Inclusion of the Massachusetts' samples in the examination of watersheds failed to recover a significant correlation between genetic and geographic distance ($R = 0.124$, $P = 0.59$; Fig. 4C). A significant correlation was found among the samples in the western cluster ($R = 0.806$, $P = < 0.01$; Fig 4F), as was significant isolation by distance ($P = 0.018$). Genetic and geographic distances were not significantly correlated in the eastern cluster ($R = 0.116$, $P = 0.154$; Fig 4E).

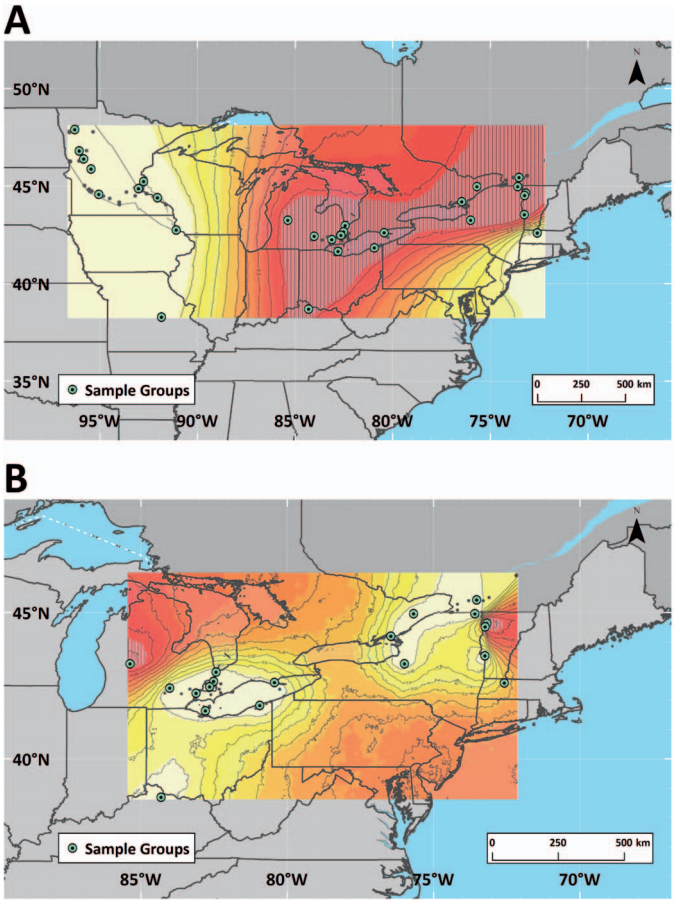

FIG. 3. (A) Geneland analysis of all samples, showing the Massachusetts location grouping with the western cluster. (B) Geneland analysis of 201 eastern cluster samples, showing three populations. No substructuring was detected in the western cluster.

DISCUSSION

The results of this study represent the most geographically widespread population genetic analysis of *N. maculosus* to date. Our study found 24 haplotypes for *Cyt b* sequences and indicated significant population structure across the region we analyzed, with distinct western and eastern lineages on either side of the Mississippi River. Most of our initial predictions relating to postglacial colonization were supported by the data. We identified large areas of genetic homogeneity across broad regions of postglacial colonization. Furthermore, these common haplotypes were associated with large numbers of closely related, low-frequency haplotypes, generating "star-like" haplotype networks.

Our data support the results of Chabarria et al. (2018) in identifying two substantially different lineages within *N. maculosus*. However, Chabarria et al. (2018) suggest a Great Lakes lineage encompassing all of the Upper Midwest whereas we found that Minnesota and Iowa sampling locations included haplotypes that were distinct from eastern Great Lakes locations. Therefore, it appears likely that the Mississippi Lineage includes the Upper and Lower Mississippi River basins while the Great Lakes basin is a distinct genetic unit. Our Kentucky samples clustered with the Great Lakes rather than with a southern Mississippi lineage, as proposed in Chabarria et al. (2018), who found that Louisiana and Ohio River sites grouped together. However, we had only four individuals from a single site in Kentucky, and Chabarria et al. (2018) had only a single individual from the Ohio River. Future work should increase sampling effort in the Ohio River basin to resolve the placement of these populations.

Our data are equivocal on specific postglacial dispersal routes but suggest a Mississippian glacial refugium, likely including one or both of the common haplotypes (B and D) or their intermediary (I). From there, founder events or other mechanisms of drift (e.g., lineage sorting) may have resulted in haplotypes I and D moving northward along the Mississippi and into Minnesota while haplotype B dominated populations moving eastward through the Great Lakes and into the Northeast. Our study only included a small number of samples from Missouri and Kentucky; it would be beneficial for future work to better sample Mudpuppies in these areas. Additionally, sampling populations in other locations in the southern and central range of Mudpuppies (e.g., Illinois, Indiana, West Virginia, and Tennessee) may be enlightening for a more complete investigation of the phylogeography of this cryptic species. It is also important to note that our results are based on a single mtDNA gene; future work that includes more loci may help clarify postglacial dispersal routes.

A clear exception to the east–west differentiation is the population from the Connecticut River, Massachusetts, composed entirely of haplotypes D and I. This population is suspected to have been anthropogenically introduced (Richmond, 1999; Chellman, 2011), and our results strongly support this conclusion. Collecting Mudpuppies for biological supply and bait has been occurring in the Great Lakes region for decades (Lannoo, 2005; Holman, 2012). Based on the evidence of matching haplotypes, the Massachusetts population may have originated from one or more introductions from Minnesota or another western population (Richmond, 1999). A second exception to the overall pattern of east–west separation is the grouping of haplotype T (from Iowa) with the eastern cluster. This may be a result of either homoplasy (independent evolution of this haplotype via four mutational steps from haplotype D) or human introduction from some unknown eastern population. We have no further evidence supporting the possibility of an introduction in this case, but it is the more parsimonious explanation.

Our population-level analyses revealed a highly significant pattern of isolation-by-distance, which was strengthened with the removal of the introduced Massachusetts sampling location. This is expected for relatively sedentary animals across such a large geographical distance (Allendorf and Luikart, 2007), particularly given the difficulty of dispersal among watersheds in these obligately aquatic organisms. Recent genomic analysis revealed similar patterns at a much finer geographic scale in Mudpuppies, with substantial population structuring among river basins (Murphy et al., 2018).

At the watershed scale, the pattern of isolation-by-distance was not significant. However, 14 out of 21 pairwise F_{ST} comparisons showed genetic differentiation between watersheds significantly different from zero ($F_{ST} = 0.060–0.928$). One of the exceptions was a comparison between the Upper Mississippi and Souris-Red-Rainy watersheds. Samples from these two locations were separated by the least distance (<400 km). Thus, gene flow may have occurred more recently between these two watersheds than in other pairwise comparisons. As expected, given the likely introduction history described above, the Connecticut River (Massachusetts) population is also not significantly different from the Upper Mississippi/Souris-Red-Rainy or Missouri watersheds.

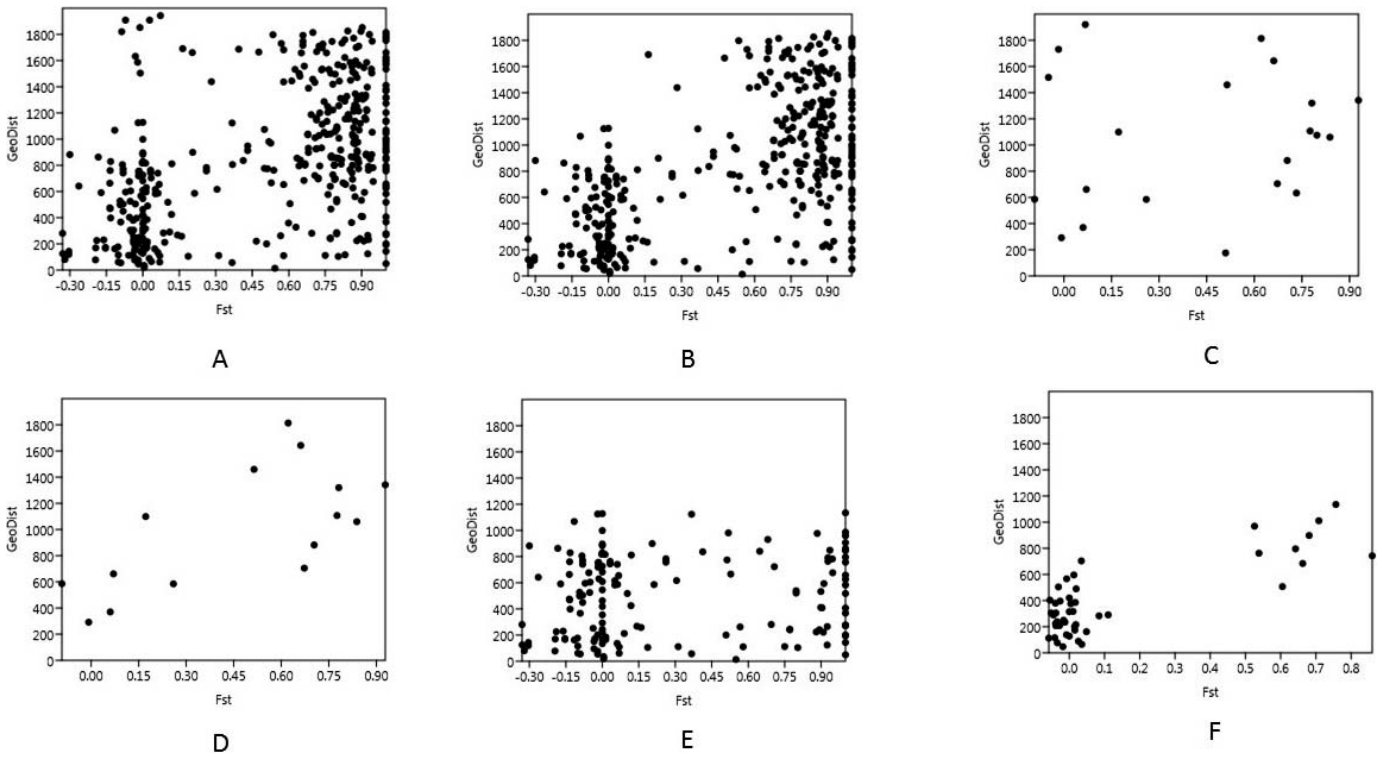

FIG. 4. Scatter plots of pairwise comparisons of genetic distance and geographic distance among (A) all 29 sampling localities, (B) Massachusetts' sampling locality excluded, (C) all seven watersheds, (D) Connecticut River (Massachusetts' sampling locality) excluded, (E) sampling localities of the eastern cluster, and (F) sampling localities of the western cluster.

Additionally, the F_{ST} comparisons between the Missouri and Souris-Red-Rainy watersheds were not significant, nor were the comparisons between the Ohio watershed and both the Great Lakes and Lake Champlain watersheds. In these cases, the geographic distance between the watersheds was greater (600–1,000 km). The lack of significant F_{ST} results may be because of the very high frequency of the D haplotype (95.8%) in the Souris-Red-Rainy watershed and the small sample size in the Ohio watershed ($n = 4$), where only the B haplotype was detected.

Management Implications.—A better understanding of species genetic structure and diversity are valuable tools for effective long-term management of imperiled populations (Schwartz et al., 2007). Our data provide insight into the genetic evolution of Mudpuppies that was previously unknown and may be used to further conservation of this declining salamander. Overall, the characterization of a species genetic diversity and evolutionary history allows for the implementation of better management practices that work to maximize evolutionary potential. The preservation of adaptive potential in animals becomes especially relevant when considering the potential effects of climate change, where few practical management interventions exist (Scoble and Lowe, 2010). Mudpuppies are identified as a species at risk of climate change effects, and managing populations based on their local adaptations may be an effective way to conserve the species range wide (Hoving et al., 2013). Furthermore, effective management of Mudpuppies will also benefit the imperiled Salamander Mussel, which relies on the presence of healthy Mudpuppy populations to survive (Bogan et al., 2017).

Results of this study may help explain patterns of migration across the United States and could be useful for comparing dispersal capabilities of similar aquatic species to gain a better understanding of potential barriers to their movement. Identification of genetically unique populations may lead to better informed management decisions (Ashley et al., 2003; Pearse and Crandall, 2004). Based on the spatially explicit Bayesian and pairwise F_{ST} analyses, our results suggest that there may be deep separation between eastern and western clusters. Our data allow for the identification of populations with unique haplotypes, or fixed rare haplotypes, such as haplotype A in Kent, Michigan, haplotype X in Niagara, New York, and haplotype V in the Lamoille River, Vermont. Although our results are based on only a single mitochondrial gene, the distinctiveness of these populations warrants further analysis. For example, future population-level analysis of Mudpuppies using additional genetic markers and contemporary techniques may reveal greater population structure or detection of genes associated with local adaptation (McCartney-Melstad and Shaffer, 2015; Hendricks et al., 2018), confirm the detected phylogeographic structure (Goncalves et al., 2009), or reveal admixture among mitochondrial clades (Waldron et al., 2019). In the meantime, separate management of the eastern and western clusters identified in this study could help preserve local adaptations needed for survival.

The genetic differentiation we observed in this study may help inform best practices for conservation actions involving translocations of Mudpuppies (Semlitsch, 2002; Germano and Bishop, 2009). Translocations are an important management tool for species conservation (IUCN/SSC 2013), but results from amphibian and reptile translocations have been mixed (Seigel and Dodd, 2002; Griffiths and Pavajeau, 2008; Germano and Bishop, 2009; Kraus et al., 2017), and publication biases complicate gaining complete understanding from previous efforts (Germano and Bishop, 2009; Miller et al., 2014). However, translocation methods are continually being refined, which has helped increase chances that properly planned

translocations may succeed (Ewan et al., 2014; Seddon et al., 2014). Investigations aimed at improving the success of translocations have identified a list of considerations including selection of source populations (Frankham et al., 2011; Zeisset and Beebee, 2013), number of individuals released (Matějů et al., 2012), and habitat quality of release sites (Cheyne, 2006), all of which have led to some general recommendations (Dodd and Seigel, 1991; Weeks et al., 2011; Moseby et al., 2014).

The International Union for Conservation of Nature (IUCN) identifies "conservation translocation" as an overarching term encompassing population restoration (including population reinforcement and reintroduction) and conservation introduction (including assisted colonization and ecological replacement), all of which are undertaken for the purpose of conservation benefit for species and ecosystems (IUCN/SSC 2013). Weeks et al. (2011) provided a discussion of genetic objectives associated with various types of conservation translocations. For reintroductions, suitable source populations in the proximity of the restoration site may be desirable to increase the likelihood of re-establishing a population with locally adapted genes. However, for augmentation (genetic rescue), source populations with a large effective population size and high genetic diversity may be more suitable (Weeks et al., 2011; Zeisset and Beebee, 2013; Frankham, 2015). While augmentation from genetically divergent populations increases risk of outbreeding depression and the loss of locally adapted genes, there may be far greater risk to recipient populations from imminent threats resulting from small population size including inbreeding depression, loss of genetic diversity, or extirpation through stochastic events (Weeks et al., 2011).

When conservation resources are limited, knowledge of population genetic structure could assist in making informed management decisions. In practice, better knowledge of population genetics could help prioritize populations that are genetically distinct. As a way of recognizing distinct evolutionary lineages, it is helpful to designate Evolutionary Significant Units (ESUs). Although the definition varies (Allendorf and Luikart, 2007), ESUs can be broadly defined as unique evolutionary lineages which have diverged from other lineages for a significant period of time. The organization of populations into ESUs can aid management decisions and facilitate the preservation of genetic diversity (Holland and Hadfield, 2002). Furthermore, if captive breeding or translocations are deemed appropriate for species conservation, ESUs could assist in deciding from which populations individuals can be selected for breeding and where captive-bred animals may be effectively relocated or reintroduced to avoid outbreeding depression (Soltis and Gitzendanner, 1999). This study provides the basis for a better understanding of Mudpuppy population genetics which may help inform these and other aspects of their conservation.

Acknowledgments.—We thank A. Briggs and the US Fish and Wildlife Service; T. Goniea, J.-M. Hessenauer, M. Thomas, and the Michigan Department of Natural Resources; J. Craig, E. Roseman, and colleagues at the US Geological Survey (USGS) Great Lakes Science Center; Herpetological Resource and Management field technicians and staff; as well as J. Briggler, K. Briggs, J.-F. Desroches, K. Hanson, R. King, G. Langlois, J. LeClere, T. Matson, M. Murphy, and S. Parren. Thanks to D. Bennion (USGS) for providing geographic information system (GIS) expertise. Funding support was provided from the US Fish and Wildlife Service through the Great Lakes Fish and Wildlife Restoration Act. We also thank L. Rodgers, J. Richer, C. Toomey, C. Francik, A. Baez, D. Farr, and N. Pradhan for their assistance with some of the lab work at the University of Vermont; C. Aylward for assistance with Geneland analyses; and the Vermont Fish and Wildlife Department for funding. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government. Collection of Lake Champlain samples was performed under the auspices of University of Vermont IACUC number 08-054. Collection of Michigan samples was performed under the auspices of Eastern Michigan University IACUC number 2014-059 and number 2017-078. The Vermont Cooperative Fish and Wildlife Research Unit is jointly supported by the USGS, Vermont Department of Fish and Wildlife, the University of Vermont, and the Wildlife Management Institute.

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Accepted: 9 November 2019.
Published online: 6 February 2020.