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RESEARCH ARTICLE

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Genetic variation and biogeography of the spotted gar *Lepisosteus oculatus* from core and peripheral populations

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Abstract

The spotted gar (*Lepisosteus oculatus*) shows a disjunct natural distribution, with a core population extending from the central Mississippi River Basin to the U.S. gulf coast and a peripheral population in the southern Great Lakes Basin. Despite significant conservation concerns for this species in the Great Lakes watersheds where it occurs, few genetic examinations and comparisons of these populations have been performed. We investigated inter- and intrapopulational variation in several mitochondrial genetic markers (cytochrome oxidase subunit I, *COI*; cytochrome oxidase subunit II, *COII*; and 16S rRNA, *16S*) from spotted gars taken from core and peripheral populations. Genetic diversity was highest in the Mississippi River Basin and lowest in the Great Lakes Basin, while the Nueces River Basin (Texas) population showed the greatest level of divergence from other populations. Average genetic distance among core and peripheral populations was over an order of magnitude less than that seen between *L. oculatus* and its sister species, the Florida gar (*L. platyrhincus*), although a significant correlation was found between genetic and geographical distance in *L. oculatus*. Genetic divergence in spotted gars is likely to be related to a combination of geographic isolation and founder effects associated with recent colonization following glacial retreat. Despite its apparent lack of significant genetic differentiation or haplotype diversity, the Great Lakes population of spotted gars has previously been shown to be a unique component of the species, and additional studies are needed to determine the genetic mechanisms underlying regional adaptations as well as potential morphological differentiation among spotted gar populations.

KEYWORDS
biogeography, conservation genetics, ecology, molecular ecology, spotted gar

1 | INTRODUCTION

Understanding the patterns of genetic diversity among populations is important to the elucidation of a species' ecology and life history, as well as informing potential conservation efforts (Johannesson & Andre, 2006). Additionally, recent advances in genomics have identified new model organisms for understanding human evolution and development (Amores, Catchen, Ferrara, Fontenot, & Postlethwait, 2011, Parichy, 2016). These non-traditional model organisms have great potential to bridge gaps in our understanding of multiple aspects of evolution, as exemplified by the recent sequencing of the genome of the spotted gar (*Lepisosteus oculatus*), which has provided key insights into evolutionary transitions among ancient lobe-finned fishes, teleosts, and tetrapods (Braasch et al., 2016).

The spotted gar is common in the southeastern United States (core population), but also has a disjunct, peripheral population in the Great Lakes basin (Page & Burr, 2011, David, Kik IV, Diana, Rutherford, & Wiley, 2015). Although fossil evidence indicates that the genus *Lepisosteus* dates back to the early Eocene (48–55 mya; Wiley, 1976; Grande, 2010), spotted gars arrived in the Great Lakes region relatively recently, approximately 8,000 years ago, following the Wisconsonian Glaciation (Hocutt & Wiley, 1986; Mandrak & Crossman, 1992). Based on previous (primarily morphologically based) phylogenetic analyses of fossil and extant species, gars are believed to have changed relatively little over time (Wiley, 1976; Inoue, Miya, Tsukamoto, & Nishida, 2003; Grande, 2010; Amores et al., 2011; Wright, David, & Near, 2012), especially when compared with teleosts. Few studies have focused on the biogeography of gars, and fewer still have investigated spatial distributions of genealogies in modern lepisosteid species (Barrientos-Villalobos and Monteros, 2008; Glass, Walter, Heath, Mandrak, & Corkum, 2015).

The ancient lineage, wide latitudinal range, and complete disjunction between core and peripheral populations of the spotted gar make it a unique species in which to explore phylogeographic patterns.

The relative young age of the Great Lakes ichthyofauna (approximately 8,000-12,000 years), including peripheral populations of the spotted gar, also presents an opportunity to compare potential genetic variation in a prehistoric lineage between geologically young (Great Lakes) and old (Mississippi River and Gulf Coast) aquatic systems (Bailey & Smith, 1981; Hocutt & Wiley, 1986; Bernatchez & Wilson, 1998; Hubbs, Lagler, & Smith, 2004). Understanding phylogeographic patterns of peripheral populations can also offer insight into a species' dispersal abilities, genetic diversity, and vulnerability to extinction, and therefore inform conservation strategies (Avise, 2009).

Previous examinations of spotted gar microsatellite data have demonstrated evidence for genetic bottlenecks and extensive population structure among peripheral populations, while also indicating that gene flow between core and peripheral populations is very limited (Glass et al., 2015). Here, we use genetic data from three mitochondrial DNA (mtDNA) loci to further explore the genetic diversity between and within populations of spotted gars from both core and peripheral populations. mtDNA has several characteristics that make it highly suitable for analyses of intra- and interspecies relationships in comparison with nuclear DNA (primarily its non-recombining nature and comparatively fast rate of evolution (see Avise et al., 1987; Avise, 2000 for full review of mtDNA in molecular analyses). Although mtDNA traces only the maternal line of inheritance, molecular phylogenetic analysis of living gars suggests that mtDNA may provide better resolution of inter- and intraspecific relationships in these species, relative to individual nuclear loci (Wright et al., 2012).

Our goal was to use concepts from phylogeography (coalescent theory; Avise, 2000) and historical biogeography (dispersal and vicariance; Mayden, 1988) to interpret current molecular genetic relationships among regional populations of spotted gars. We hypothesized that population genetic structure based on mtDNA analyses would reflect geographic position of core and peripheral populations of spotted gars. More specifically, we hypothesized that peripheral population spotted gars would exhibit comparatively low genetic diversity, influenced by both disjunction (lack of gene flow) from the core population and founder effects associated with recent colonization into a new environment (colonization of the Great Lakes region from Mississippi River refugia). Additionally, we hypothesized that genetic distance among populations would reflect geographic distance among populations, with proximal populations more similar than distal populations (isolation by distance, IBD; Wright, 1942; Jenkins et al., 2010).

2 | METHODS

2.1 | Specimen collection and study regions

Spotted gars were collected from multiple localities for genetic analysis (Table 1 and Figure 1A). Samples from peripheral population fish were taken from two Michigan inland lakes (Loon Lake, Branch County and Lake Pleasant, Hillsdale County; $N = 5$ fish) and Rondeau Bay, Lake Erie ($N = 1$ fish). Core population samples were taken from Horseshoe Lake, Illinois ($N = 5$ fish), Bayou Chevreuil, Louisiana ($N = 6$ fish), and Choke Canyon Reservoir, Texas ($N = 5$ fish). For comparison with an out-group, and in this case a sister species, Florida gar (*Lepisosteus platyrhincus*) samples were included from three localities (Lake Okeechobee, Florida, Caloosahatchee River, Florida, and Everglades Conservation Area, Florida; $N = 3$ fish). Approximately 1.0 cm^2 fin clips were taken from all fish and stored in 95% ethanol for use in DNA preparations; gars were then released or used in studies at other institutions. Spotted gar samples were coded by population as follows: MI-p, Michigan; LE-p, Lake Erie; IL-c, Illinois; LA-c, Louisiana; and TX-c, Texas. Florida gar samples from three localities (FLG1, Lake Okeechobee, FL; FLG2, Caloosahatchee River, FL; and FLG3, Everglades Conservation Area, FL) were included in analyses as a single population, FLG. Multiple sampling methods were used to collect fishes. Boat electrofishing was used to collect MI-p and TX-c fish, fyke nets for LE-p fish, experimental gill nets for LA-c fish, and dip-nets for IL-c fish and Florida gars.

The distribution of the spotted gar was divided into four major regions for this study: the Great Lakes, Mississippi River drainage, western Gulf Coast, and eastern Gulf Coast regions. Regional divisions were determined based on combinations of regions from zoogeographic studies of Hocutt and Wiley (1986) and phylogeographic studies of lepisosteids by Sipiorski (2011). Study populations were assigned to regions as follows: MI-p and LE-p to the Great Lakes region, IL-c and LA-c to the Mississippi River drainage region, TX-c to the western Gulf Coast region, and FLG to the eastern Gulf Coast region (Figure 1A).

2.2 | Genetic comparisons

Preserved tissues were used to extract DNA using Qiagen DNeasy Tissue Extraction Kits (QIAGEN, Valencia, CA), according to the manufacturer's instructions. Portions of the mitochondrial genes for cytochrome oxidase subunit I (*COI*), cytochrome oxidase subunit II (*COII*), and 16S rRNA (*16S*) were PCR amplified using previously published primer sequences and cycling conditions (Normark, McCune, & Harrison, 1991; Palumbi, 1996; Ward, Zemlak, Innes, Last, & Hebert, 2005). Amplified PCR products were prepared for sequencing by 1:5 dilution with distilled water, and all sequencing was performed at the University of Michigan DNA Sequencing Core, using the forward and reverse PCR primers. LE-p sequence data were taken from GenBank (accession #EU524699); these data were part of the "Barcode of Life Project" (BOLD; Hubert et al., 2008) and only *COI* information was available for comparisons. New sequences generated by this study were also deposited in GenBank (Table 1).

Gene sequences and chromatograms were analyzed using Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI) and were manually aligned using the program Se-Al v.2.0a11 Carbon (Rambaut, 1996), which was also used to evaluate the presence of haplotype variation in spotted gar samples. As there were no gaps in the aligned sequences and mutation levels were low, this evaluation was easily accomplished by eye. The program PAUP* 4.0b10 (Swofford, 2003) was used to generate matrices of uncorrected *p*-distances to serve as a measure of genetic differentiation and variation between and within core and peripheral populations. Uncorrected *p*-values were used due to high levels of sequence similarity between individuals and the relatively short evolutionary time scales being examined, as a method

TABLE 1 Specimen Details (Including GenBank Numbers) for Spotted and Florida Gars Included in Analyses

Species	Population Code	Individual Code	Locality	*16S*	*COI*	*COII*
Lepisosteus oculatus	MI-p	SpG118	Loon Lake, Michigan	KY938531	KY934157	MF000705
Lepisosteus oculatus	MI-p	SpG120	Loon Lake, Michigan		KY934158	MF000706
Lepisosteus oculatus	MI-p	SpG123	Loon Lake, Michigan		KY934159	MF000707
Lepisosteus oculatus	MI-p	SpG125	Lake Pleasant, Michigan		KY934160	MF000708
Lepisosteus oculatus	MI-p	SpG130	Lake Pleasant, Michigan		KY934161	MF000709
Lepisosteus oculatus	LE-p	LE SpG	Rondeau Bay, Lake Erie, Canada		EU524699	
Lepisosteus oculatus	IL-c	IL SpG1	Horseshoe Lake, Illinois	KY938533	KY934168	MF000716
Lepisosteus oculatus	IL-c	IL SpG2	Horseshoe Lake, Illinois		KY934169	MF000717
Lepisosteus oculatus	IL-c	IL SpG3	Horseshoe Lake, Illinois		KY934170	MF000718
Lepisosteus oculatus	IL-c	IL SpG4	Horseshoe Lake, Illinois		KY934171	MF000719
Lepisosteus oculatus	IL-c	IL SpG5	Horseshoe Lake, Illinois		KY934172	MF000720
Lepisosteus oculatus	LA-c	LA SpG2730	Bayou Chevruil, Louisiana	KY938532	KY934162	MF000710
Lepisosteus oculatus	LA-c	LA SpG2731	Bayou Chevruil, Louisiana		KY934163	MF000711
Lepisosteus oculatus	LA-c	LA SpG2732	Bayou Chevruil, Louisiana		KY934164	MF000712
Lepisosteus oculatus	LA-c	LA SpG2733	Bayou Chevruil, Louisiana		KY934165	MF000713
Lepisosteus oculatus	LA-c	LA SpG2734	Bayou Chevruil, Louisiana		KY934166	MF000714
Lepisosteus oculatus	LA-c	LA SpG2736	Bayou Chevruil, Louisiana		KY934167	MF000715
Lepisosteus oculatus	TX-c	Tx SpG8164	Choke Canyon Reservoir, Texas	KY938534	KY934173	MF000721
Lepisosteus oculatus	TX-c	Tx SpG8165	Choke Canyon Reservoir, Texas		KY934174	MF000722
Lepisosteus oculatus	TX-c	Tx SpG8169	Choke Canyon Reservoir, Texas		KY934175	MF000723
Lepisosteus oculatus	TX-c	Tx SpG8455	Choke Canyon Reservoir, Texas		KY934176	MF000724
Lepisosteus oculatus	TX-c	Tx SpG8456	Choke Canyon Reservoir, Texas		KY934177	MF000725
Lepisosteus platyrhincus	FLG (1)	FLG SRD 18	Lake Okeechobee, Florida	KY938535	KY934178	MF000726
Lepisosteus platyrhincus	FLG (2)	FLG SRD 19	Caloosahatchee River, Ft Meyers, Florida		KY934179	MF000727
Lepisosteus platyrhincus	FLG (3)	FLG SRD 21	Everglades Conservation Area, Florida		KY934180	MF000728

correcting for multiple substitution events produced negligibly different results (data not shown). These measures were also derived from data sets containing sequence information for *L. platyrhincus* (in which peripheral and core *L. oculatus* were treated as both a single population and individual populations), to offer an indication of these values for interspecific comparisons of closely related gar species.

Haplotype diversity (H) was calculated for all genes, populations, and combined for both species using the following formula:

$$H = \frac{N}{N-1}\left(1 - \sum_i x_i^2\right)$$

where N is the sample size and x_i is the relative haplotype frequency for each sample (Nei & Tajima, 1981). Haplotype diversity was used to compare variation among populations as well as across species. Additionally, analysis of molecular variance (AMOVA; Excoffier, Smouse, & Quattro, 1992) and F_{ST} values (a measure of population differentiation) were used to further evaluate genetic variation within and among core and peripheral populations (ARLEQUIN 3.5; Excoffier et al., 2010).

Correlations between genetic distance (F_{ST} values) and geographic distance among spotted gar populations were examined for evidence of isolation by distance (IBD) effects (Wright, 1942; Jenkins et al., 2010). Pairwise genetic and geographic distance matrices were subjected to Mantel tests (1,000 iterations) using the Isolation by Distance Web Service (IBDWS version 3.23; Jensen et al. 2005). Geographic distance (km) was estimated from Euclidean distances between population localities from GIS data (Google Earth, 2011). Correlations were also tested and visualized using all pairwise combinations of genetic distance ($F_{ST}/(1 - F_{ST})$) regressed against geographic distances among populations (Rousset, 1997).

3 | RESULTS

3.1 | Genetic comparisons

For our population genetic and phylogeographic study, we analyzed three regions of the mitochondrial genome: *16S*, *COI*, and *COII*. Although these three regions represent a single locus, we obtained different sets of sequence data for different sets of gar populations, and thus describe the results from each mtDNA region in addition to the concatenated loci in the following. The total genetic data set consisted of 1,919 base positions, with similar contributions from each of the regions sampled (*16S* = 608 bp, *COI* = 685 bp, *COII* = 626 bp). All three regions sampled showed different levels of variation among regions as well as within and between *L. oculatus* populations. A single *16S* haplotype was observed from all *L. oculatus* samples. A single *16S* haplotype

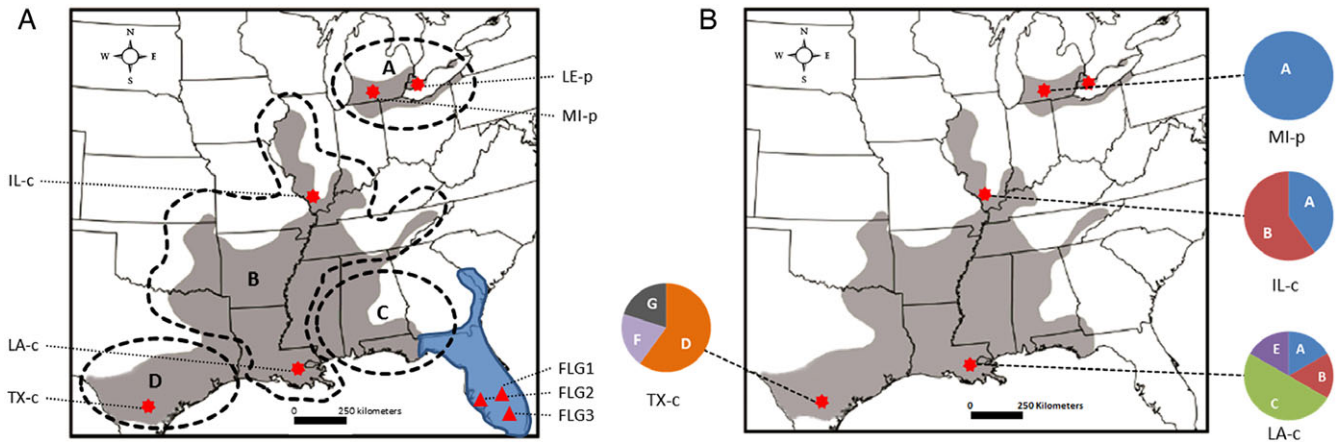

FIGURE 1 (A) Collection sites (by population code), range distribution, and geographic regions for spotted (gray) and Florida (blue) gars used in genetic analyses. Spotted gar localities are as follows: Loon and Pleasant Lakes, Michigan (MI-p), Rondeau Bay, Lake Erie (LE-p), Horseshoe Lake, Illinois (IL-c), Bayou Chevreuil, Louisiana (LA-c), and Choke Canyon Reservoir, Texas (TX-c). Florida gar localities are as follows: Lake Okeechobee, Florida (FLG1), Caloosahatchee River, Florida (FLG2), and Everglades Conservation Area, Florida (FLG3). Distribution was divided into four major regions based on zoogeographic studies of Hocutt and Wiley (1986) and lepisosteid phylogeography by Sipiorski (2011). Divisions consisted of the Great Lakes (A), Mississippi River drainage (B), Eastern Gulf Coast (C), and Western Gulf Coast (D) regions. Map modified from Becker ('83), Page and Burr (2011), and Sipiorski (2011). (B) Relative haplotype frequency of all loci combined and relative geographic position for each study population of spotted gars. Lowest haplotype diversity was observed in MI-p, with highest haplotype diversity observed in LA-c. TX-c possessed haplotypes unique to the population. Also note continuum of haplotypes and haplotype diversity from LA-c northward to MI-p. Map modified from Becker ('83), Page and Burr (2011), and Sipiorski (2011) [Color figure can be viewed at wileyonlinelibrary.com]

was also observed in all *L. platyrhincus* samples. Due to this intraspecific homogeneity, *16S* data were excluded from gene distance analyses of spotted gar populations, with the exception of a genetic distance comparison with *L. platyrhincus* (uncorrected *p*-distance = 1.09%).

Variation between spotted gar samples was greater in the *COI* and *COII* sequence data. A single *COI* haplotype was observed in all TX-c individuals, in which a single pyrimidine transition was found at base position 291. This transition was also found in one LA-c individual (LA SpG 2736), which additionally showed a single purine transition at base position 634. This haplotype was unique to this individual. For the LE-p sample, only *COI* sequence data were available, which was identical to that obtained from all MI-p, IL-c, and the remaining LA-c specimens.

One *COII* haplotype (Haplotype *COII*-b) was observed in all MI-p individuals, with a single base substitution at position 248; this haplotype was also shared with one LA-c individual and two IL-c individuals. Core populations consisted of two to three haplotypes in each component population, with two haplotypes observed in IL-c, and three in LA-c and TX-c. One TX-c individual showed a unique haplotype, characterized by four base substitutions (at positions 53, 119, 218, and 248).

Concatenated results for all regions revealed seven composite haplotypes for *L. oculatus* (Haplotypes A-G) and also three unique haplotypes for *L. platyrhincus* (Tables 2 and 3 and Figure 2). Of the seven *L. oculatus* haplotypes, three were unique to a single individual. These singletons were represented by one LA-c (Haplotype E) and two TX-c (Haplotypes F and G) fish. Haplotype A was the most common (38% of individuals) and widespread haplotype recovered, occurring in MI-p, IL-c, and LA-c populations, but not in TX-c fish. Haplotype B was the second most common (19%) and was only found in IL-c and LA-c populations. Haplotype C was only found in the remaining LA-c samples, while haplotype D was limited to the remaining TX-c samples.

All MI-p individuals shared the same haplotype (haplotype diversity, $H = 0.00$) for individual regions and concatenated results. LA-c was the most diverse population ($H = 0.80$) with four concatenated haplotypes (A, B, C, E), followed by TX-c ($H = 0.70$) with three haplotypes (D, F, G). Haplotype data were also combined to compare core and peripheral populations (peripheral population was only represented by MI-p except for *COI*, which included LE-p), resulting in zero haplotype diversity for the peripheral population and 0.98 for the core population. Concatenated results for FLG indicated three unique haplotypes from the three different populations sampled, with a haplotype diversity value of 1.00, although these haplotypes differed only in the portion of the alignment representing *COII* sequences (Tables 3 and 4). Average genetic distance (uncorrected *p*-distance) between core and peripheral populations was very low (0.09%), over an order of magnitude less than that seen between *L. oculatus* and *L. platyrhincus* (1.50%).

AMOVA tests indicated that significant variation occurred between core and peripheral populations of spotted gars, as well as within and among component populations ($p < 0.0001$; Table 5). The amount of variation explained by comparison of peripheral versus all core populations (MI-p vs. IL-c, LA-c, TX-c combined) was only 14.42%, with 34.77% of variation coming from comparisons between (core) populations, and 50.81% of variation from within populations. Pairwise comparisons based on F_{ST} values indicated that MI-p was significantly different from LA-c and TX-c populations, but not from IL-c. TX-c was significantly different from MI-p and IL-c, but not LA-c. In comparing peripheral versus core populations (MI-p vs. IL-c, LA-c, TX-c combined), the peripheral population was significantly different from the core population. When comparing each individual population to all population

TABLE 2 Haplotypes for Each Individual Spotted and Florida Gar by Individual and Combined mtDNA Loci

Population	Population Code	Individual Code	*16S* Haplotype	*COI* Haplotype	*COII* Haplotype	Combined Haplotype
Michigan	MI-p	SpG118	a	a	B	A
Michigan	MI-p	SpG120	a	a	B	A
Michigan	MI-p	SpG123	a	a	B	A
Michigan	MI-p	SpG125	a	a	B	A
Michigan	MI-p	SpG130	a	a	B	A
Lake Erie	LE-p	LE SpG	–	a	–	–
Illinois	IL-c	IL SpG1	a	a	A	B
Illinois	IL-c	IL SpG2	a	a	B	A
Illinois	IL-c	IL SpG3	a	a	A	B
Illinois	IL-c	IL SpG4	a	a	B	A
Illinois	IL-c	IL SpG5	a	a	A	B
Louisiana	LA-c	LA SpG2730	a	a	A	B
Louisiana	LA-c	LA SpG2731	a	a	B	A
Louisiana	LA-c	LA SpG2732	a	a	C	C
Louisiana	LA-c	LA SpG2733	a	a	C	C
Louisiana	LA-c	LA SpG2734	a	a	C	C
Louisiana	LA-c	LA SpG2736	a	c	A	E
Texas	TX-c	Tx SpG8164	a	b	A	D
Texas	TX-c	Tx SpG8165	a	b	A	D
Texas	TX-c	Tx SpG8169	a	b	A	D
Texas	TX-c	Tx SpG8455	a	b	D	G
Texas	TX-c	Tx SpG8456	a	b	C	F
Florida	FLG (1)	FLG SRD 18	FLG-a	FLG-a	FLG-a	FLG-A
Florida	FLG (2)	FLG SRD 19	FLG-a	FLG-a	FLG-b	FLG-B
Florida	FLG (3)	FLG SRD 21	FLG-a	FLG-a	FLG-c	FLG-C

Alphabetized haplotype identification indicates level of mutations (base substitutions), with "a" and "A" having no base substitutions, and those following (b, c, B, C, D, etc.) having cumulative base substitutions.

TABLE 3 Haplotype Diversity of Individual and Combined mtDNA Loci for Study Populations of Spotted Gars and Florida Gars

Population	N	*16S*	*COI*	*COII*	Combined	H (*16S*)	H (*COI*)	H (*COII*)	H (Combined)
MI-p	5	1	1	1	1	0.00	0.00	0.00	0.00
LE-p	1	–	1	–	–	–	0.00	–	–
IL-c	5	1	1	2	2	0.00	0.00	0.60	0.60
LA-c	6	1	2	3	4	0.00	0.03	0.73	0.80
TX-c	5	1	1	3	3	0.00	0.00	0.70	0.70
PERI	5 (6)	1	1	1	1	0.00	0.00	0.00	0.00
CORE	16	1	3	4	7	0.00	0.48	0.63	0.98
Total	21 (22)	1	3	4	7	0.00	0.39	0.66	0.81
FLG	3	1	1	3	3	0.00	0.00	1.00	1.00

Number in parenthesis indicates inclusion of LE-p individual sequence data. *N* = number of individuals, followed by number of haplotypes observed for each locus (*16S*, *COI*, *COII*, Combined). *H* = haplotype diversity calculated for individual and combined loci.

data combined, only TX-c was significantly different (Table 6). Mantel tests were significantly positive ($r = 0.8437$, $P = 0.014$) and genetic distance ($F_{ST}/(1 - F_{ST})$) was significantly correlated ($r^2 = 0.70$, $P < 0.05$) with geographic distance (km) between populations, suggesting a pattern of isolation by distance (IBD; Figure 3).

4 | DISCUSSION

Spotted gars from peripheral and core populations exhibited low but significant genetic variation based on analyses of three mitochondrial regions. Among spotted gar populations, seven unique concatenated

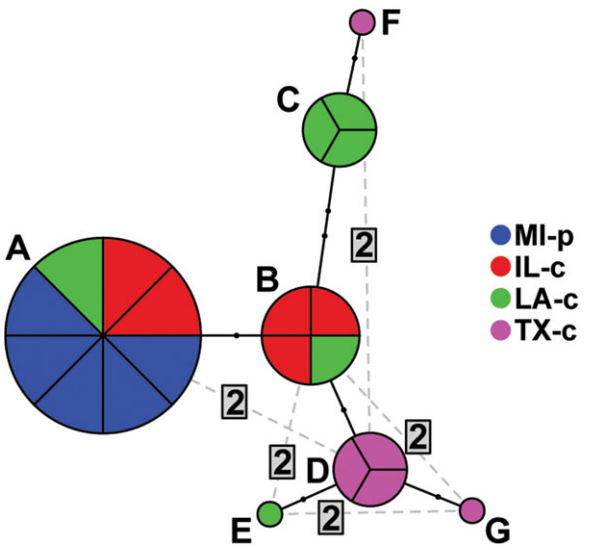

FIGURE 2 Unrooted concatenated haplotype network of the 21 spotted gars examined in this study. Letters correspond to distinct concatenated haplotypes and subdivisions within circles correspond to single individuals. Nucleotide substitutions differentiating haplotypes are indicated by black circles along solid lines. Dashed lines indicate ambiguous connections, with number of nucleotide differences between haplotypes indicated by boxed numbers [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 4 Matrix of Genetic Distances (Uncorrected *p*-Distance Shown as Percent, Below Diagonal) and Geographic Distances (Euclidean distance, km, Above Diagonal) among Study Populations of Spotted Gars and Florida gars

Population	MI-p	IL-c	LA-c	TX-c	FLG-1	FLG-2	FLG-3
MI-p		656	1423	1,930	1,703	1,727	1,797
IL-c	0.03		836	1,273	1,400	1,364	1,482
LA-c	0.11	0.10		815	965	867	1,006
TX-c	0.14	0.10	0.13		1,747	1,627	1,768
FLG-1	1.44	1.40	1.47	1.46		98	41
FLG-2	1.38	1.34	1.41	1.40	0.12		139
FLG-3	1.68	1.64	1.71	1.70	0.24	0.36	

TABLE 5 Results of Analysis of Molecular Variance (AMOVA) Run in Arlequin 3.5 (Excoffier et al. 2010) Comparing Peripheral and Core Populations of Spotted Gars

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation
Among groups	1	3.56	0.15	14.42
Among pop within groups	2	4.96	0.37	34.77
Within populations	17	9.10	0.54	50.81
Total	20	17.62	1.05	
Fixation index, F_{ST}	0.49	$P < 0.0001$		

AMOVA compared peripheral (MI-p) versus core (IL-c, LA-c, TX-c combined) populations. MI-p was significantly different from the core population, however, a large portion of variation remained within groups.

haplotypes were identified, which reflected potential interpopulation-level genetic structuring. The spotted gar and its sister species, the Florida gar, exhibited low levels of variation in genetic comparisons, although interspecific variation was over an order of magnitude larger than intraspecific variation. Interspecific variation (1.50% between *L. oculatus* and *L. platyrhincus*) was similar to that reported among other lepisosteids such as the alligator gar *Atractosteus spatula* and Cuban gar *A. tristoechus*, where genetic distances (uncorrected *p*-distance) between species were low (1.21%) compared with those seen in several other fishes (Barrientos-Villalobos and Monteros, 2008; Borden & Krebs, 2009).

Colonization of the Great Lakes region by extant gars from Mississippian refugia is believed to be relatively recent compared with the age of the family in North America (Bailey & Smith, 1981; Hocutt & Wiley, 1986; Grande, 2010). Mandrak and Crossman (1992) suggested that spotted gars entered the Great Lakes region (and progressed to southwestern Ontario) specifically through the Chicago and Michigan Lower Peninsula glacial outlets (a shorter connection to Lake Erie via the Fort Wayne outlet is believed to have been too cold for the species to use for dispersal). In such a scenario, Great Lakes populations of spotted gars would reasonably be expected to share some genetic similarity with Mississippi River drainage populations (Bailey & Smith, 1981; Bernatchez & Wilson, 1998). We found that, among the seven unique concatenated mitochondrial haplotypes we obtained for spotted gars, Michigan individuals representing the peripheral population all shared the same haplotype (Haplotype A). This haplotype was not unique to Michigan fish, but also shared with core population fish (IL-c and LA-c) from the Mississippi River drainage.

Spotted gars from core populations in the Mississippi River drainage also had other haplotypes not found in any Michigan individuals (Haplotypes B, C, E). The singular but shared (with IL-c and LA-c) haplotype found in MI fish suggests very low genetic diversity in the peripheral population of spotted gars, and given the time period since the most recent glacial recession (~8,000 years), is also consistent with a relatively recent colonization by the species into the Great Lakes region (Bailey & Smith, 1981; Hubbs et al., 2004). Results from our analyses of haplotype diversity and IBD support these theories of dispersal of spotted gars into the Great Lakes region. We found MI-p and LE-p fish to have identical haplotypes (comparing *COI* data), and our isolation by distance regression model showed greater similarity between peripheral and proximal core populations (IL-c, LA-c; Mississippi River drainage), compared with more distal core populations (TX-c; western Gulf Coast drainage).

Low genetic diversity coupled with shared haplotype(s) is consistent with founder effects in the peripheral population colonized by the Mississippi River drainage core population (Hamner, Freshwater, & Whitefield, 2007). Limiting our examination to the *COI* gene, which allowed us to include limited data for the Lake Erie population of spotted gars, also provided evidence for low genetic diversity in peripheral populations as well as recent colonization from Mississippi River refugia, in that sequence data were identical for MI-p and LE-p populations (Welsh, Hill, Quinlan, Robinson, & May, 2008; Borden & Krebs, 2009). This echoes the findings of Glass et al. (2015), which also provided evidence of bottleneck effects in peripheral spotted gar populations

TABLE 6 Matrix of Pairwise Genetic Distances (F_{ST} Values below Diagonal, Significance Values above Diagonal) for Study Populations of Spotted Gars, as well as Comparisons with Core Populations (Combined) and all Populations (all Data Combined)

Population	MI-p	IL-c	LA-c	TX-c	Core	All
MI-p		0.16	**0.01**	**0.01**	**0.00**	0.05
IL-c	0.50		0.16	**0.01**	0.16	0.53
LA-c	**0.46**	0.18		0.07	0.50	0.26
TX-c	**0.77**	**0.55**	0.27		0.11	**0.02**
CORE	**0.33**	0.06	−0.02	0.12		0.68
ALL	0.20	−0.02	0.04	**0.22**	−0.03	

Significant values in bold.

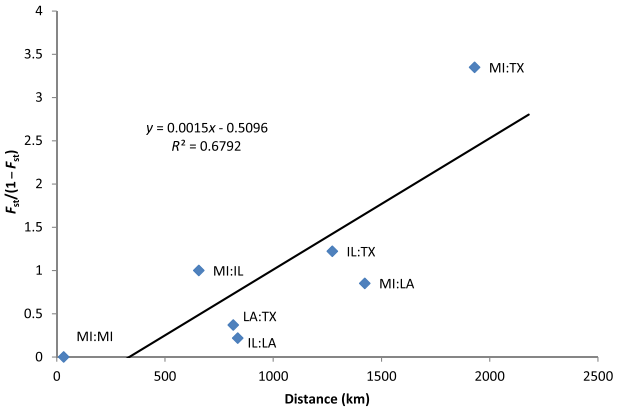

FIGURE 3 Pairwise geographic distance (km) versus genetic distance ($F_{ST}/(1 - F_{ST})$) for spotted gar populations. ANOVA indicated significant positive correlation ($r^2 = 0.68$) between genetic distance and geographic distance, suggesting isolation by distance in spotted gars. "MI:MI" refers to genetic versus geographic distance for the two MI-p subpopulations used in analyses [Color figure can be viewed at wileyonlinelibrary.com]

based on microsatellite markers, as well as admixture between peripheral Great Lakes populations. Glass et al. (2015) also, however, found evidence for population genetic structure within Lake Erie, in contrast to the genetic homogeneity that we observed in our peripheral population mtDNA samples. This is likely due both to the relative rapidity of microsatellite marker evolution versus mtDNA, and our comparatively lower number of individuals sampled from various sub-populations. Nonetheless, our smaller sample size of individuals revealed significant differences in genetic diversity between peripheral and core subpopulations, lending credence to the assertion that genetic diversity in peripheral spotted gar populations is lower than that found in core populations.

The Texas population of spotted gars is the southern-most population in our study, and it is also from a locality (Choke Canyon Reservoir, Nueces River watershed) that lies beyond the southern edge of the widely accepted distribution for this species, which most current distribution maps show as the San Antonio River watershed (Hendrickson & Cohen, 2010; NatureServe, 2011; Page & Burr, 2011). This range discrepancy appears to stem from simple oversight, as a search of the Texas Natural History Collection's Ichthyology holdings revealed at least one collection of spotted gars within the Nueces River watershed in 1947 (TNHC 1529), as well as several additional collections south of the San Antonio River watershed in 2011 and 2012 (TNHC 47748, 47800, 5156). The Texas population also occurs in a separate regional watershed unit from all of the other populations investigated, with TX-c belonging to the western Gulf Coast, and all other populations connected with the Mississippi River regional watershed (either presently or historically). Therefore, geographic isolation between the two major watershed units may have facilitated divergence by genetic drift (Kawamura, Yonekura, Katano, Taniguchi, & Saitoh, 2009).

Sipiorski (2011) found that variation in mtDNA (control region, or "D-loop") of spotted gars was greater between the eastern Gulf Coast watershed and Mississippi River watershed populations than among several populations within the Mississippi River watershed. Bernatchez and Wilson (1998) showed that populations of fishes from western Gulf drainages were more divergent (among populations) than those from eastern Gulf drainages. The Texas population of spotted gars contained three unique haplotypes not found in other study populations, and mutations (based on number of base substitutions in individual loci) were greater in TX-c than other populations, supporting higher levels of divergence in TX-c from other populations (Avise, 2009). According to coalescent theory, rarer haplotypes are likely more recently derived, and older haplotypes (more ancestral) should be more widespread than younger haplotypes (Templeton, 1998; Avise, 2000; Barrientos-Villalobos and Monteros, 2008). TX-c possessed multiple rare haplotypes (three unique to TX-c) compared with other populations and, therefore, may be the most derived of the spotted gar populations in this study. Haplotype A was shared by the most individuals in this study and widespread over three out of four of study populations, therefore it may be the most ancestral haplotype (Avise, 2009). Although population sample sizes were relatively small in our study, the rare haplotypes identified warrant additional investigation of the high diversity and potential divergence of TX-c relative to other populations.

Alternatively to founder effects and recent colonization, low genetic diversity in the Great Lakes Basin population could reflect selection for the most suitable or adaptive genotype for ecologically harsher, high-latitude environments with shorter growing seasons. David et al. (2015) found that spotted gars from the Great Lakes peripheral population (high latitude, shorter growing season) had higher growth rates than those from core populations (lower latitude, longer growing season), suggesting countergradient variation in growth (Conover, Duffy, & Hice, 2009). Scudder (1989) stated that selection in ecologically peripheral environments favors adaptation to a diversity of density-independent factors (as opposed to density-dependent factors in core environments) as well as colonization ability. Other genotypes may have been present when spotted gars initially entered the Great Lakes Basin, but may have been selected against (and therefore eliminated) in the ecologically harsher peripheral environment (Scudder, 1989). Low genetic diversity in ecologically peripheral versus core populations of species has been observed in several other studies supporting the adaptive significance of peripheral populations (see Scudder, 1989 for review). Identification of other peripheral populations of spotted gars, and analysis of

FIGURE 4 Comparison of adult and juvenile spotted gars from core and peripheral populations. Adult spotted gar from Michigan (top photo) compared with adult spotted gar from Louisiana (second photo); young of the year spotted gar from Michigan (third photo) compared with young of the year spotted gar from Louisiana (bottom photo). Note elongate morphology of caudal peduncle in peripheral population specimens compared with shorter and stouter caudal peduncle in core population specimens. Photos by David (2008; 2009) [Color figure can be viewed at wileyonlinelibrary.com]

additional, non-mtDNA sequence data, may further elucidate the relationship between selection and adaptation in ecologically marginal environments.

Comparisons of genetic distances (uncorrected *p*-distance) between spotted gar populations and its sister species, *L. platyrhincus*, indicated interspecific variation was over an order of magnitude greater than intraspecific variation. Previous analyses based on cytochrome *b* (*cyt b*) and *COI* genes by Barrientos-Villalobos and Monteros (2008) showed that *L. oculatus* and *L. platyrhincus* differed by only 0.55% (based on uncorrected *p*-distance). Our analyses based on three regions indicated an overall genetic distance of 1.50% between species. Genetic distance between the sister species may vary depending on the geography of the populations being compared. Sipiorski (2011) found that spotted gars sampled from the Apalachicola River in western Florida (eastern Gulf Coast region), possessed a haplotype (based on mtDNA control region analysis) that grouped more closely with the Florida gar than spotted gars from other regions. The Apalachicola River is considered to be within a potential hybridization zone as the range of both species overlap in the panhandle region of Florida (Becker, 1983; Page & Burr, 2011). Although we did not sample spotted gars from the eastern Gulf Coast region, and our sample size of *L. platyrhincus* was very small ($N = 3$ fish from three localities), it should be noted that three different haplotypes were observed from the three localities sampled, suggesting potential genetic structuring among much more geographically restricted populations. Further investigation of genetic diversity in this introgression zone may reveal higher resolution patterns of variation between these two closely related species.

Other analyses may further elucidate relationships and variation among core and peripheral populations of spotted gars and closely related species. Life history analysis (David, 2012), common garden experiments (David et al., 2015), habitat use modeling (Frenette & Snow, 2016), and morphological analyses may be useful in uncovering patterns of divergence and local adaptation among populations in

different geographic regions. Pope and Wilde (2003) found a significantly high degree of variation in spotted gar mass–length relationships among 49 reservoirs throughout the state of Texas. In our study, Texas spotted gars were the most divergent population in terms of haplotype diversity, and might therefore be considered a "genetically" peripheral population; life history, morphological, genetic, and habitat analyses of additional Texas populations may clarify patterns of variation among spotted gars from the western Gulf Coast and other regions.

Bernatchez and Wilson (1998) found that populations of species from previously glaciated regions may have different morphologies (morphotypes) than those from unglaciated regions. Lesica and Allendorf (1995) also noted that morphological characters are expected to diverge more rapidly in peripherally isolated populations. Spotted gars from peripheral and core populations may also differ morphologically, as individuals from peripheral populations appear to have more elongate caudal peduncles than those from core populations (personal observation; Figure 4). Morphologically, only a single diagnostic character, the presence or absence of bony plates on the isthmus, separates spotted gars from Florida gars, therefore a combination of genetic and morphological analyses may provide further insight into divergence or similarities within and between species (Trautman, 1981; Grande, 2010; Page & Burr, 2011).

From a conservation perspective, phylogeographic studies can be important in identifying evolutionarily significant units such as distinct population segments (Ryder, 1986; Bernatchez & Wilson, 1998). Peripheral populations of species often experience very low gene flow and high degrees of genetic drift, leading to divergence from core populations (Jones, Gliddon, & Good, 2001; Lammi et al., 2001). Additionally, populations of species with very low genetic diversity have been shown to be much more vulnerable to perturbations such as habitat loss, invasive species, and overfishing (Garcia de Leaniz et al., 2007). Peripheral populations of spotted gars in this study were found to share a single haplotype (i.e., MI-p), therefore exhibiting extremely low genetic diversity. Furthermore, the peripheral population is completely disjunct from the core population, therefore gene flow is likely nonexistent.

Spotted gars are currently listed as threatened and are therefore protected throughout their range in Canada (COSEWIC, 2005; Glass, Corkum, & Mandrak, 2011), but were only listed as a "species of greatest conservation need" in Michigan (revised; Michigan Department of Natural Resources, 2014), where a large portion of the peripheral population resides in inland lakes (Carman, 2002; Hubbs et al., 2004; Page & Burr, 2011). Spotted gars are dependent on aquatic vegetation for multiple life stages, and loss of habitat is believed to be the largest threat to peripheral populations of the species (Trautman, 1981; Carman, 2002; COSEWIC, 2005). Loss of essential habitat coupled with very low genetic diversity make peripheral populations of spotted gars highly susceptible to local extinction, which has already been recorded in localities within Ohio and Michigan (Trautman, 1981; Carman, 2002). Additional investigations into habitat use, abundance, and effective population size are recommended to protect potentially vulnerable peripheral populations of spotted gars, and therefore contribute to the conservation of local biodiversity.

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CONFLICT OF INTEREST

None.

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LITERATURE CITED

Amores, A., Catchen, J., Ferrara, A., Fontenot, Q., & Postlethwait, J. H. (2011). Genome evolution and meiotic maps by massively parallel DNA sequencing: Spotted gar, an outgroup for the teleost genome duplication. *Genetics*, *188*, 1–10.

Avise, J. C. (2000). *Phylogeography: The history and formation of species*. Cambridge, Massachusetts: Harvard University Press.

Avise, J. C. (2009). Phylogeography: Retrospect and prospect. *Journal of Biogeography*, *36*, 3–15.

Avise, J. C., Arnold, J., Ball, Jr. R. M., Bermingham, E., Lamb, T., Neigel, N. E., … Saunders, N. C. (1987). Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, *18*, 489–522.

Bailey, R. M., & Smith, G. R. (1981). Origin and geography of the fish fauna of the Laurentian Great Lakes basin. *Canadian Journal of Fisheries and Aquatic Sciences*, *38*, 1539–1561.

Barrientos-Villalobos, J., & De Los Monteros, E. (2008). Genetic variation and recent population history of the tropical gar *Atractosteus tropicus* Gill (Pisces: Lepisosteidae). *Journal of Fish Biology*, *73*, 1919–1936.

Becker, G. C. (1983). *Fishes of Wisconsin. Madison*, Wisconsin: University of Wisconsin Press.

Berendzen, P. B., Simons, A. M., & Wood, R. M. (2003). Phylogeography of the northern hogsucker, *Hypentelium nigricans* (Teleostei: Cypriniformes): Genetic evidence for the existence of the ancient Teays River. *Journal of Biogeography*, *30*, 1139–1152.

Bermingham, E., & Avise, J. C. (1986). Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics*, *113*, 939–965.

Bernatchez, L., & Wilson, C. C. (1998). Comparative phylogeography of Nearctic and Palearctic fishes. *Molecular Ecology*, *7*, 431–452.

Borden, W. C., & Krebs, R. A. (2009). Phylogeography and postglacial dispersal of smallmouth bass (*Micropterus dolomieu*) into the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*, 2142–2156.

Braasch, I., Gehrke, A. R., Smith, J. J., Kawasaki, K., Manousaki, T., Pasquier, J., Amores, A. Desvignes, T., Batzel, P., Catchen, J., et al. (2016). The spotted gar genome illuminates vertebrate evolution and facilitates human-teleost comparisons. *Nature Genetics*, *48*, 427–437.

Carman, S. M. (2002). *Special animal abstract for Lepisosteus oculatus (spotted gar)* (pp. 1–3). Lansing, Michigan: Michigan Natural Features Inventory. 1–3.

Conover, D. O., Duffy, T. A., & Hice, L. A. (2009). The covariance between genetic and environmental influences across ecological gradients. *Annals of New York Academy of Sciences*, *1168*, 100–129.

COSEWIC. (2005). *COSEWIC assessment and update status report on the spotted gar* Lepisosteus oculatus *in Canada* (pp. 1–17). Ottawa: Committee on the Status of Endangered Wildlife in Canada.

David, S. R. (2012). *Life history, growth, and genetic diversity of the Spotted Gar Lepisosteus oculatus from peripheral and core populations* (Doctoral dissertation). Ann Arbor, Michigan: University of Michigan.

David, S. R., Kik IV, R. S., Diana, J. S., Rutherford, E. S., & Wiley, M. J. (2015). Evidence of countergradient variation in growth of Spotted Gar *Lepisosteus oculatus* from core and peripheral populations. *Transactions of the American Fisheries Society*, *144*, 837–850.

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, *131*, 479–491.

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite version 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*, 564–567.

Frenette, B. D., & Snow, R. (2016). Natural habitat conditions in a captive environment lead to spawning of Spotted Gar. *Transactions of the American Fisheries Society*, *145*, 835–838.

Garcia de Leaniz, C., Fleming, I. A., Einum, S., Verspoor, E., Jordan, W. C., Consuegra, S., … Quinn, T. P. (2007). A critical review of adaptive genetic variation in Atlantic salmon: Implications for conservation. *Biological Reviews*, *82*, 173–211.

Glass, W. R., Corkum, L. D., & Mandrak, N. E. (2011). Pectoral fin ray aging: An evaluation of a non-lethal method for aging gars and its application to a population of the threatened spotted gar. *Environmental Biology of Fishes*, *90*, 235–242.

Glass, W. R., Walter, R. P., Heath, D. D., Mandrak, N. E., & Corkum, L. D. (2015). Genetic structure and diversity of spotted gar (*Lepisosteus oculatus*) at its northern range edge: Implications for conservation. *Conservation Genetics*, *16*, 889–899.

Earth, Google (2011). Retrieved from https://www.earth.google.com.

Grande, L. (2010). *An empirical synthetic pattern study of gars (Lepisosteiformes) and closely related species, based mostly on skeletal anatomy. The resurrection of holostei* (Special Publication 6:i-x, 1–871; supplementary issue of Copeia 10:2A). Lawrence, KS: American Society of Ichthyologists and Herpetologists.

Hamner, R. M., Freshwater, D. W., & Whitefield, P. E. (2007). Mitochondrial cytochrome *b* analysis reveals two invasive lionfish species with strong founder effects in the western Atlantic. *Journal of Fish Biology*, *71*, 214–222

Hendrickson, D. A., & Cohen, A. E. (2010). *Fishes of Texas Project and Online Database*. Retrieved from https://www.fishesoftexas.org, Austin, TX: Published by Texas Natural History Collection, a division of Texas Natural Science Center, University of Texas at Austin.

Hocutt, C. H., & Wiley, E. O. (1986). *The zoogeography of North American Freshwater Fishes*. New York: John Wiley and Sons.

Hubbs, C. L., Lagler, K. F., & Smith, G. R. (2004). *Fishes of the Great Lakes Region, revised edition*. Ann Arbor, Michigan: The University of Michigan Press.

Inoue, J. G., Miya, M., Tsukamoto, K., & Nishida, M. (2003). Basal actinopterygian relationships: A mitogenomic perspective on the phylogeny of the "ancient fish". *Molecular Phylogenetics and Evolution*, *26*, 110–120.

Jenkins, D. G., Carey, M., Czerniewska, J., Fletcher, J., Hether, T., Jones, A., … Tursi, R. (2010). A meta-analysis of isolation by distance: Relic or reference standard for landscape genetics? *Ecography*, *33*, 315–320.

Johannesson, K., & Andre, C. (2006). Life on the margin: Genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. *Molecular Ecology*, *15*, 2013–2029.

Jones, B., Gliddon, C., & Good, J. E. G. (2001). The conservation of variation in geographically peripheral populations: *Lloydia serotina* (Liliaceae) in Britain. *Biological Conservation*, *101*, 147–156.

Kawamura, K., Yonekura, R., Katano, O., Taniguchi, Y., & Saitoh, K. (2009). Phylogeography of the Bluegill Sunfish, *Lepomis macrochirus*, in the Mississippi River Basin. *Zoological Science*, *26*, 24–34.

Lammi, A., Siikamaki, P., & Mustajarvi, K. (2001). Genetic diversity, population size, and fitness in central and peripheral populations of a rare plant *Lychnis viscaria*. *Conservation Biology*, *13*, 1069–1078.

Lesica, P., & Allendorf, F. W. (1995). When are peripheral populations valuable for conservation? *Conservation Biology*, *9*, 753–760.

Mandrak, N. E., & Crossman, E. J. (1992). Postglacial dispersal of freshwater fishes into Ontario. *Canadian Journal of Zoology*, *70*, 2247–2259.

Mayden, R. L. (1988). Vicariance biogeography, parsimony, and evolution in North American freshwater fishes. *Systematic Zoology*, *37*, 329–355.

Michigan Department of Natural Resources. (2014). *Species of greatest conservation need. Michigan's wildlife action plan 2015–2025*. Lansing, MI: Michigan Department of Natural Resources. Retrieved from https://www.michigan.gov/documents/dnr/18_appendix2_sgcn_rationales_500079_7.pdf

NatureServe. (2011). *NatureServe Explorer: An online encyclopedia of life [web application]*. Version 7.1. Virginia: NatureServe, Arlington. Retrieved from https://www.natureserve.org/explorer

Nei, M., & Tajima, F. (1981). DNA polymorphism detectable by restriction endonucleases. *Genetics*, *97*(1)145–163.

Normark, B. B., McCune, A. R., & Harrison, R. G. (1991). Phylogenetic relationships of neopterygian fishes, inferred from mitochondrial DNA sequences. *Molecular Biology and Evolution*, *8*, 819–834.

Palumbi, S. R. (1996). Nucleic acids II: The polymerase chain reaction. In D. M., Hillis, C., Moritz, & B. K., Mable (Eds.), *Molecular systematics* (pp. 205–247). Sunderland, Massachusetts: Sinauer and Associates, Inc..

Page, L. M., & Burr, B. M. (1991). *A Field Guide to Freshwater Fishes. Boston*, Massachusetts: Houghton Mifflin Company.

Page, L. M., & Burr, B. M. (2011). *A Field Guide to Freshwater Fishes, second edition. Boston*, Massachusetts: Houghton Mifflin Company.

Parichy, D. M. (2016). The gar is a fish…is a bird…is a mammal? *Nature Genetics*, *48*(4), 344–345.

Pennsylvania Code. (2011). *58 Pa. Code § 75.1 Endangered species*. Harrisburg, PA: Commonwealth of Pennsylvania. Retrieved from https://pacode.com/index.html

Pope, K. L., & Wilde, G. R. (2003). Variation in spotted gar (*Lepisosteus oculatus*) mass-length relationships in Texas reservoirs. *Texas Journal of Science*, *55*, 43–48.

Rambaut, A. (1996). *Se-Al:* Sequence Alignment Editor [computer program]. Oxford: University of Oxford. Retrieved from https://evolve.zoo.ox.ac.uk/

Rousset, F. (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, *145*(4), 1219–1228.

Ryder, O. A. (1986). Species conservation and systematics: The dilemma of subspecies. *Trends in Ecology and Evolution*, *1*, 9–10.

Scudder, G. G. E. (1989). The adaptive significance of marginal populations: A general perspective. In C. D. Levings, L. G. Holtby, & M. A. Henderson (Eds.), *Proceedings of the national workshop on effects of habitat alteration on salmonid stocks* (Vol. *105*, pp. 180–185). Ottawa, ON: Canadian Special Publication of Fisheries and Aquatic Sciences.

Sipiorski, J. T. (2011). *The world according to gars: The molecular systematics and comparative phylogeography of living gars (Actinopterygii: Lepisosteidae)*. Carbondale, IL: Doctoral dissertation, Southern Illinois University Carbondale.

Suttkus, R. D.Order Lepisostei. In H. B. Bigelow (Ed.), et al. (1963). *Fishes of the Western Atlantic; Part three, Soft-rayed fishes* (pp. 61–88). New Haven, Connecticut: Yale University Memoir Sears Foundation for Marine Research.

Swofford, D. L. (2003). *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sunderland, Massachusetts: Sinauer Associates.

Trautman, M. B. (1981). *The Fishes of Ohio (revised edition).Columbus*: Ohio State University Press.

Templeton, A. R. (1998). Nested clade analyses of phylogeographic data: Testing hypotheses about gene flow and population history. *Molecular Ecology*, *7*, 381–397. Oxford, United Kingdom.

U.S. Environmental Protection Agency. (2007). *Biological indicators of watershed health*. Washington D.C. Retrieved from https://www.epa.gov/national-aquatic-resource-surveys/indicators-fish-assemblage

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society of London. *Series B: Biological Sciences*, *360*, 1847–1857.

Welsh, A., Hill, T., Quinlan, H., Robinson, C., & May, B. (2008). Genetic assessment of lake sturgeon population structure in the Laurentian Great Lakes. *North American Journal of Fishries Management*, *28*, 572–591.

Wiley, E. O. (1976). *The phylogeny and biogeography of fossil and recent gars (Actinopterygii: Lepisosteidae)* (Vol. *64*, pp. 1–111). Lawrence, KS: University of Kansas Museum of Natural History Miscellaneous Publication.

Wright, J. J., David, S. R., & Near, T. J. (2012). Gene trees, species trees, and morphology converge on a similar phylogeny of living gars (Actinopterygii: Holostei: Lepisosteidae), an ancient clade of ray-finned fishes. *Molecular Phylogenetics and Evolution*, *63*, 848–856.

Wright, S. (1942). Isolation by distance. *Genetics*, *28*, 114–138

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