2018. Proceedings of the Indiana Academy of Science 127(1):89–95

GENETIC IDENTITY OF THE LEAST BROOK LAMPREY (*LAMPETRA AEPYPTERA*) IN INDIANA

Rex Meade Strange[1] and **Alanna Noland**: Department of Biology, University of Southern Indiana, Evansville, IN 47712 USA

ABSTRACT. The Least Brook Lamprey (*Lampetra aepyptera*) is a common inhabitant of small streams throughout the southeast United States and reaches its northern-most extent near the boundary of the glacial till plains of southern Indiana, Ohio, and western Pennsylvania. Previous genetic studies found that populations from eastern Kentucky and Ohio were distinct from other populations of *L. aepyptera*, suggesting that these populations from the upper Ohio River basin were isolated in their current locations well before the Pleistocene. However, samples from Indiana (or elsewhere in the lower Ohio River basin) were not included in these studies. As the modern Ohio River system was established in the late Pleistocene (or after), samples from Indiana will be critical to our understanding of the historical factor(s) giving rise to the distribution of *L. aepyptera* in the Ohio River basin. Sequence variation of the mitochondrial NADH dehydrogenase subunit 3 gene from specimens of *L. aepyptera* collected from across its distribution were examined to better understand the phylogeographic position of the Indiana populations. Specimens collected from southern Indiana, Illinois, and the Green River of Kentucky (the lower Ohio River basin) formed a well-supported monophyletic group with specimens collected from the upper Ohio River basin. Deeper relationships within the species remain unresolved. The Ohio River clade shows evidence of reduced genetic heterogeneity relative to more southerly populations, consistent with an assemblage of populations that has recently expanded. Our results suggest that the contemporary distribution of *L. aepyptera* in the Ohio River basin was established after the integration of the modern Ohio River system in the late Pleistocene.

Keywords: Ohio River drainage, *Lampetra aepyptera*, biogeography, mtDNA, Pleistocene

INTRODUCTION

The Least Brook Lamprey, *Lampetra aepyptera* (Abbott 1860), is a non-parasitic species that occurs in headwater streams of the southeastern United States and reaches its northern-most extent in the Ohio River basin (Rhode & Jenkins 1980). The species was originally described as '*Ammocoetes aepyptera*' from a single specimen from 'the Ohio River' near Meigs, Ohio (Abbott 1860; see species account in Trautman 1981), and was first reported in Indiana by Jordan (1918) from Griffith's Creek (Monroe County) and by Creaser (1939) from Lick Creek (Orange County). Although subsequent surveys have established the presence of *L. aepyptera* in southern Indiana (viz. Simon 2011), no other information regarding its natural history is available for the populations that occur in the State.

The life history of the *L. aepyptera* was documented in Maryland (Seversmith 1953) and Kentucky (Walsh & Burr 1981), and is known to include a filter-feeding larval (ammocoete) stage and a short lived (non-feeding) adult stage that dies after spawning in the spring. Other information regarding the natural history of *L. aepyptera* includes descriptions of its karyotype (Alabama: Howell & Denton 1969), demographic structure and sex ratios (Alabama, Delaware, Kentucky, Maryland, and Tennessee: Docker & Beamish 1994), and its phylogenetic relationships with other lamprey species (Docker et al. 1999; Lang et al. 2009). Finally, Martin & White (2008) inferred the phylogeographic structure of *L. aepyptera* from mitochondrial DNA (mtDNA) variation, but did not include samples from Indiana (see also White & Martin 2009).

MtDNA-based studies such as Martin & White (2008) have revealed evidence of Pleistocene vicariance and post-Pleistocene expansion of many North American fishes (e.g., Strange & Burr 1997; Near et al. 2001), and thereby provide important insights into the processes that gave rise to contemporary patterns of biodiversity. A major Pleistocene event that may have influenced the distribution of *L. aepyptera* was the formation of the modern Ohio River basin by the integration of components of the ancient Teays River system to the east (including the modern tributary streams in eastern Kentucky and southern Ohio)

[1] *Corresponding author:* Rex Meade Strange, 812-465-1008 (phone), rmstrange@usi.edu.

with the Old Ohio River system to the west (including the modern Wabash River of Indiana, the Green River of Kentucky, and the smaller Ohio River tributaries in southern Indiana and Illinois (Melhorn & Kempton 1991; Hoagstrom et al. 2014)). Martin & White (2008) found that lamprey populations from the upper Ohio River basin (eastern Kentucky and southern Ohio) were genetically divergent from other populations and conjectured that this 'Ohio River Clade' was a relict of a pre-Pleistocene distribution in the ancient Teays River system. However, the distinctiveness of their 'Ohio River Clade' may be an artifact of incomplete sampling, as samples from Indiana, Illinois, and central Kentucky (the lower Ohio River basin = Old Ohio) were not available for Martin & White's (2008) analysis (Fig. 1). Thus, samples from Indiana will provide a better understanding of the biogeographic history of *L. aepyptera* in the Ohio River basin.

Herein, we supplement the mitochondrial data set collected by Martin & White (2008; available on GenBank) with new samples from southern Indiana, Illinois, and central Kentucky to assess the historical biogeography of *L. aepyptera*. The primary objective of this study was to determine the genetic identity of the populations that occur in Indiana and assess the roles of pre-Pleistocene vicariance and/or post-Pleistocene dispersal in shaping its present distribution within the Ohio River basin. In particular, we test whether populations of *L. aepyptera* from Indiana form a monophyletic group with those from the upper Ohio River (consistent with a post-Pleistocene dispersal), or if the Indiana populations are more closely related to more southerly populations (consistent with pre-Pleistocene distributions in both the Old Ohio and Teays systems).

METHODS

Adult lampreys were collected by seine and ammocoetes by electrofishing from various tributaries of the lower Ohio River basin, including streams in southern Illinois, Indiana, and Kentucky (Table 1). Specimens of *Lethenteron appendix* (formerly *Lampetra appendix*) were collected for outgroup comparison in the phylogenetic analyses (below). Tissues (fin and muscle) were fixed in the field with ethanol and brought to the laboratory for processing. Voucher specimens were formalin-fixed and deposited in the Natural History Museum of the University of Southern Indiana. Whole genomic DNA was extracted from the ethanol-fixed tissues by a standard phenol-chloroform extraction procedure.

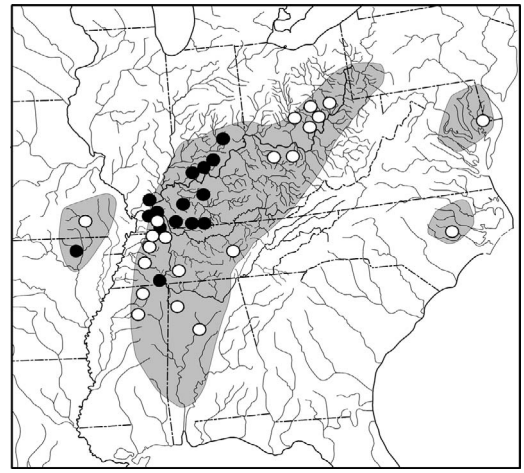

Figure 1.—Distribution of *Lampetra aepyptera* (shaded areas) following Rhode & Jenkins (1980). White spots represent sample localities from Martin & White (2008) and black spots represent sample localities collected for this study.

The polymerase chain reaction (PCR) was used to amplify the mitochondrial NADH dehydrogenase subunit 3 (ND3) gene with the primers ND3-F and ND3-R originally developed by Docker et al. (1999). PCR reactions consisted of a 25µl volume with concentrations of 1.5 mM $MgCl_2$, 200 µM of each dNTP, 1.0 µM of each primer, and 1.0 unit of *Taq* polymerase. An initial denaturation at 94° C for 2 min was followed by 35 cycles of denaturation (94° C, 1 min), annealing (52° C, 1 min), and polymerase extension (72° C, 1 min). A final extension at 72° C for 7 min was included to reduce the number of partial strands. Amplification products were purified using spin-columns (Qiagen), and resuspended in ddH_2O prior to automated sequencing on an ABI 3700 genetic analyzer.

Trace files for all sequences were edited using BioEdit (Hall 1999) and initial alignments were made with CLUSTALX (Thompson et al. 1997). Final alignments included ND3 sequences from Martin & White (2008) and Docker et al. (1999; Table 1). Phylogenetic analyses were performed on the DNA sequence data with a combination of parsimony and likelihood approaches. Maximum Parsimony (MP) analyses were performed using PAUP* (Swofford 2002). All characters were treated as unweighted, and searches were heuristic with starting trees obtained by stepwise addition,

Table 1.—Collection localities and GenBank accesson numbers for NADH dehydrogenase subunit 3 (ND3) sequences from *Lampetra aepyptera*. Samples new for this study are indicated by asterisks (*).

Locality	System/Drainage	GenBank
Big Creek, Hardin Co., IL*	Lower Ohio	MH177976
Anderson River, Perry Co., IN*	Lower Ohio	MH177977
Stinking Fork, Crawford Co., IN*	Little Blue/Lower Ohio	MH177978
Patoka River, Orange Co., IN*	Wabash/Lower Ohio	MH177979
Vernon Fork, Jackson Co., IN*	White/Wabash/Lower Ohio	MH177980
West Fork, Ohio Co., KY*	Green/Lower Ohio	MH177981
W. Fork Pond River, Christian Co., KY*	Green/Lower Ohio	MH177982
Donaldson Cr., Trigg Co., KY*	Cumberland/Lower Ohio	MH177983
Big Sinking Creek, Carter Co., KY	Little Sandy/Upper Ohio	DQ532792
Big Caney Creek, Elliott Co., KY	Little Sandy/Upper Ohio	DQ532798
Strouds Run, Athens Co., OH	Hocking/Upper Ohio	DQ532801
Camp Creek, Pike Co., OH	Scioto/Upper Ohio	DQ532788
M. Branch Shade River, Athens Co., OH	Shade/Upper Ohio	DQ532800
Spring Cr., Todd Co., KY*	Red/Cumberland	MH177984
L. Whippoorwill Cr., Logan Co., KY*	Red/Cumberland	MH177985
Cane Creek, Putnam Co., TN	Caney Fork/Cumberland	AF177965
Trace Creek, Graves Co., KY*	Clarks/Tennessee	MH177986
Panther Creek, Graves Co., KY*	Clarks/Tennessee	MH177987
Wildcat Creek, Calloway Co., KY*	Blood/Tennessee	MH177988
McCollough Fork, Calloway Co., KY	Blood/Tennessee	DQ532795
Bear Creek, Henry Co., TN	Big Sandy/Tennessee	DQ532793
Weatherford Creek, Wayne Co., TN	Indian/Tennessee	DQ532790
Robinson Creek (#1), Hardin Co., TN*	Tennessee	MH177989
Robinson Creek (#2), Hardin Co., TN*	Tennessee	MH177990
Little Bear Creek, Franklin Co., AL	Bear/Tennessee	DQ532789
Little Black River, Ripley Co., MO	Black/White	DQ532799
Mill Creek, Sharpe Co., AR*	Strawberry/White	MH177991
Terrapin Creek, Graves Co., KY	Obion/Mississippi	DQ532803
Middle Fork Obion Creek, Henry Co., TN	Obion/Mississippi	DQ532794
Tar Creek, McNairy Co., TN	Forked Deer/Mississippi	DQ532802
Gaylor Creek, Hardeman Co., TN	Hatchie/Mississippi	DQ532785
Yellow Leaf Creek, Lafayette Co., MS	Yazoo/Mississippi	DQ532786
Kettle Creek, Lafayette Co., MS	Yazoo/Mississippi	DQ532787
Schultz Creek, Bibb Co., AL	Cahaba/Mobile	DQ532796
Davis Mill Creek, Dorchester Co., MD	Chesapeake/Atlantic	DQ532797
Neuse River, Johnston Co., NC	Atlantic	DQ532791
Lethenteron appendix (outgroup)		
Driftwood River, Bartholomew Co., IN*	White/Wabash/Lower Ohio	MH177992

1000 random addition sequence replicates, and TBR branch swamping. Support for nodes was assessed by bootstrap resampling (Felsenstein 1985) with 1000 pseudoreplicates using the same parameters as for the parsimony analysis. For the Maximum Likelihood (ML) analysis, the best-fitting model of nucleotide substitution was chosen with jModelTest (Darriba et al. 2012) following Akaike's (1974) information criterion. PHYML 3.0 (Guindon & Gascuel 2003) was then used with the specified optimal model to infer the most likely set of phylogenetic relationships. Branch support for the ML analysis was estimated by bootstrap resampling with 100 pseudoreplicates.

Finally, the patterns of nucleotide diversity and mismatch distributions were examined to evaluate evidence of recent population expansion with tests implemented in DNASP (Librado & Rozas 2009). Nucleotide diversity (π) is the average number of nucleotide differences between sequences within a sample and is analogous to heterozygosity at the nucleotide level (Nei 1987); recently founded populations are expected to have lower levels of nucleotide diversity than older populations (Avise 2000). The frequency

distribution of pairwise differences (mismatches) between haplotypes is another estimate of population history, wherein historically stable populations are expected to exhibit multimodal mismatch distributions, while those that have undergone a recent expansion should show unimodal distributions (Slatkin & Hudson 1991; Rogers & Harpending 1992). Results of the mismatch analysis were assessed with Tajima's *D*, wherein positive values represent a decrease in population size, negative values represent a recent population expansion, and a value of '0' is consistent with a population in mutation-drift equilibrium (Tajima 1989).

RESULTS

The aligned data set consisted of 351 bp of the mitochondrial ND3 gene, with 72 polymorphic sites, 49 of which were parsimony-informative; the remaining sites were invariant. Among the polymorphic sites, 17 (23.61%) were at the first, 9 (12.50%) at the second, and 46 (63.89%) were at the third codon position. Base composition was similar to that previously reported for lamprey mitochondrial sequences (e.g., Caputo et al. 2009; Lang et al. 2009; Strange et al. 2016), with a low guanine content (13.11%) relative to the proportions of adenine (25.94%), cytosine (28.03%), and thymine (32.92%) residues. All sequences passed the X^2 test for homogeneity of nucleotide composition ($X^2 = 6.97$, df = 108, $p > 0.99$) and showed no evidence of transition or transversion saturation. Average sequence divergence between all *L. aepyptera* ND3 haplotypes was 3.74% (range 0.3–6.3%).

Both the MP and ML analyses of the ND3 sequence data yielded similar phylogenetic topologies within *L. aepyptera*, although both analytical methods failed to resolve deeper relationships within the species (Fig. 2). Parsimony analysis resulted in 766 equally parsimonious trees with 140 steps each (CI = 0.614; RI = 0.784). Likelihood analysis (using the GTR model identified by jModelTest) identified a single phylogenetic tree with a negative log likelihood score of -1222.925 and 144 parsimony steps. The Ohio River Clade (previously identified by Martin & White 2008) was well supported by both analyses (> 92% bootstrap support) and included samples from eastern Kentucky and southern Ohio as well as our samples from Indiana, central Kentucky (Green River system), southern Illinois, and one sample from the lower Cumberland River system (Donaldson Creek). Samples from the

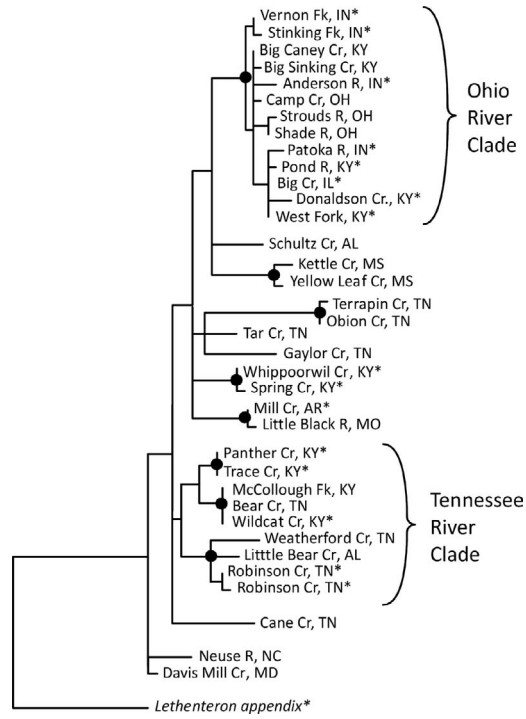

Figure 2.—Phylogenetic relationships of NADH dehydrogenase subunit 3 (ND3) sequences from *Lampetra aepyptera* as inferred from parsimony and likelihood analyses. Filled circles at nodes represent bootstrap support greater than 90% in both analyses; branch lengths are proportionate to the likelihood estimates of the number of substitutions per site. Samples new for this study are indicated by asterisks (*).

Tennessee River drainage formed a monophyletic group in all of the equally parsimonious trees and likelihood analysis, but received < 50% bootstrap support in both MP and ML analyses. Samples within the Tennessee River Clade fell into two subclades, one corresponding to samples from the lower Tennessee River system and another from tributaries of the middle Tennessee River.

Demographic data from the Ohio River Clade (which is distributed along the glacial boundary) and the Tennessee River Clade (which occurs in unglaciated portions of western Kentucky and Tennessee) revealed very different patterns. Haplotypes of the Ohio River Clade exhibit lower nucleotide diversity ($\pi = 0.012$) than do sequences of the Tennessee River Clade ($\pi = 0.020$). Within the Ohio River Clade, nucleotide diversity among the samples from the lower Ohio River basin was higher ($\pi = 0.012$) than that of the samples from

the upper Ohio River basin ($\pi = 0.006$). Mismatch distribution plots for the entire sample (species-wide) showed a multimodal distribution (Fig. 3A), consistent with a model of non-expanding populations at mutation-drift equilibrium (Tajima's D = -0.899; $p > 0.10$). Similarly, the Tennessee River Clade also exhibited a multimodal distribution with no significant difference from expectations of a stable set of populations (Fig. 3B; Tajima's $D = 0.123$; $p > 0.10$). In comparison, mismatch distribution for the Ohio River Clade was unimodal, as expected for a recent population expansion (Fig. 3C). Although Tajima's D was negative (-0.879) for the Ohio River Clade, the value did not differ significantly from zero ($p > 0.10$).

DISCUSSION

Our samples from southern Indiana, Illinois, and central Kentucky demonstrate that the 'Ohio River Clade' is not restricted to southern Ohio and eastern Kentucky (= Teays River System), but is broadly distributed throughout the Ohio River basin above the Cumberland and Tennessee rivers. The close relationship between lampreys from the lower and upper Ohio River basin is further reflected in low levels of nucleotide diversity and a unimodal mismatch distribution analysis (Fig. 3B), consistent with a recent (post-Pleistocene) range expansion of the Ohio River Clade following the integration of the modern Ohio River system. Tajima's D for the Ohio River Clade did not differ significantly from zero ($p > 0.10$), but this may have been the result of the small sample size (n = 13 haplotypes). In contrast, samples collected from the Tennessee River system and elsewhere show deeper divergences with unresolved relationships among drainages, higher levels nucleotide diversity, and a multimodal mismatch distribution, as expected for resident populations of drainage systems that presumably predate the glacial activities of the Pleistocene. In short, mtDNA variation in *L. aepyptera* is consistent with a recent dispersal within the Ohio River system after the establishment of the modern drainage pattern.

Criteria for recognizing glacial refugia and the paths of post-Pleistocene dispersal of fishes typically include patterns of monophyly among mtDNA haplotypes and relative levels of genetic diversity in putative source and dispersant populations (Avise 2000). We postulate that the Pleistocene refugium from which the northern clade emerged was located in the Old Ohio River

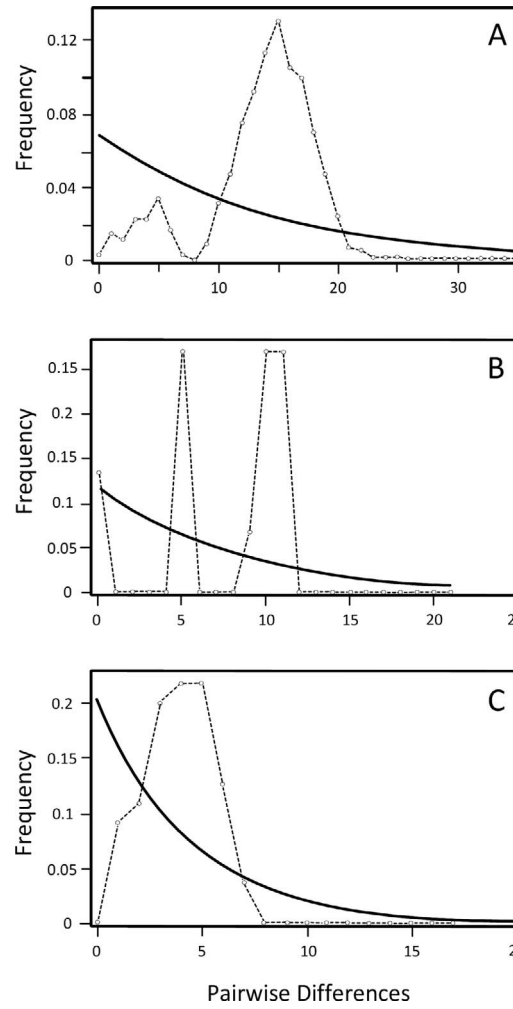

Figure 3.—Mismatch-distribution of pairwise differences of NADH dehydrogenase subunit 3 (ND3) haplotypes of *Lampetra aepyptera*. Shown are observed (dashed lines) frequencies for (A) the entire sample, (B) the Tennessee River Clade, and (C) the Ohio River Clade. Expected frequency distributions under a model of population expansion are shown by solid lines.

system rather than the Teays River system for two reasons. First, although there is little phylogenetic structure within the Ohio River Clade, samples from the lower Ohio River basin show evidence of greater nucleotide diversity ($\pi = 0.012$) than samples collected from the upper Ohio River basin ($\pi = 0.006$). Second, tributaries of the lower Ohio River basin are adjacent to the remainder of the species' distribution and it seems likely the Old Ohio River was part of a historically contiguous

distribution. Although the data presented here do not falsify the hypothesis that *L. aepyptera* was part of an ancient Teays fauna, dispersal from the lower Ohio River basin (Old Ohio system) into the upper Ohio River basin appears to be the most parsimonious explanation for the origin for the Ohio River Clade.

Other stream fishes native to southern Indiana show similar phylogeographic patterns as reported here for *L. aepyptera*. For example, Strange & Burr (1997) examined mtDNA variation in the Streamline Chub, *Erimystax dissimilis* (Cyprinidae), and found evidence for a Pleistocene refugium in the Green River and Tennessee River drainages, followed by a post-Pleistocene dispersal into the newly integrated Ohio River (see also Simons 2004). Likewise, Berendzen et al. (2003) hypothesized that the Northern Hog Sucker, *Hypentelium nigricans* (Catostomidae), dispersed from the Old Ohio into the upper Ohio River system following the retreat of the glaciers. Thus, our conclusions regarding the history of *L. aepyptera* in the Ohio River system are consistent with other fishes, yet differ from that of Martin & White (2008). Given the large hiatus between their collection localities and the unresolved relationships among major drainage populations, it is understandable that Martin & White (2008) interpreted the upper Ohio River Clade as a relict of the pre-Pleistocene Teays River fauna.

In conclusion, it is clear that populations of *L. aepyptera* that occur in Indiana are closely related to other populations in the Ohio River basin. Although the use of a single (and relatively short) genetic marker makes any assessment of the deeper relationships among drainage populations premature, our analysis (and that of Martin & White 2008) suggests that *Lampetra aepyptera* represents a species complex with more taxonomic diversity to be described outside of the Ohio River basin (Boschung & Mayden 2004). Future investigations into the deeper divergences within *L. aepyptera* should include longer (> 1000 nucleotides) regions of the mitochondrial genome and/or nuclear genes (viz. Espanhol et al. 2007; Caputo et al. 2009; Docker et al. 2012).

ACKNOWLEDGMENTS

We would like to thank the many friends and students who helped collect or provided specimens used in this project, including J. Bandoli, C. Beachum, B. Burr, K. Lawrence, H. Mattingly, and the USI Ichthyology class. B. Fisher provided distributional maps for *L. aepyptera* in Indiana and D. Eisenhour provided collection data for Kentucky. J. Bandoli provided comments and editorial changes to the manuscript. A Faculty Research and Creative Work Award from the University of Southern Indiana supported this work.

LITERATURE CITED

Abbott, C.C. 1860. Descriptions of new species of American fresh-water fishes. Proceedings of the Academy of Natural Sciences of Philadelphia 12:327–328.

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19:716–723.

Avise, J.C. 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, Massachusetts. 464 pp.

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

Boschung, H.T. & R.L. Mayden. 2004. Fishes of Alabama. Smithsonian Books, Washington D.C. 736 pp.

Caputo, V., M. Giovannotti, P.N. Cerioni, A. Splendiani, M. Marconi & J. Tagliavini. 2009. Mitochondrial DNA variation of an isolated population of the Adriatic brook lamprey *Lampetra zanandreai* (Agnatha: Petromyzontidae): phylogeographic and phylogenetic inferences. Journal of Fish Biology 75:2344–2351.

Creaser, C.W. 1939. A lamprey of the genus *Lampetra* from Indiana. Copeia 3:169.

Darriba, D., G.L. Taboada, R. Doallo & D. Pasada. 2012. jModelTest 2: more models, new heuristics, and parallel computing. Nature Methods 9:772.

Docker, M.F. & F.W.H. Beamish. 1994. Age, growth, and sex ratio among populations of least brook lamprey, *Lampetra aepyptera*, larvae: an argument for environmental sex determination. Environmental Biology of Fishes 41:191–205.

Docker, M.F., N.E. Mandrak & D.D. Heath. 2012. Contemporary gene flow between "paired" silver (*Ichthyomyzon unicuspis*) and northern brook (*I. fossor*) lampreys: implications for conservation. Conservation Genetics 13:823–835.

Docker, M.F., J.H. Youson, R.J. Beamish & R.H. Devlin. 1999. Phylogeny of the lamprey genus *Lampetra* inferred from mitochondrial cytochrome b and ND3 sequences. Canadian Journal of Fisheries and Aquatic Sciences 56:2340–2349.

Espanhol, R., P.R. Almeida & M.J. Alves. 2007. Evolutionary history of lamprey paired species *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch) as inferred from mitochondrial DNA variation. Molecular Ecology 16:1909–1924.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Guindon, S. & O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52:696–704.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

Hoagstrom, C.W., V. Ung & K. Taylor. 2014. Miocene rivers and taxon cycles clarify the comparative biogeography of North American highland fishes. Journal of Biogeography 41:644–658.

Howell, W.M. & T.E. Denton. 1969. Chromosomes of ammocoetes of the Ohio brook lamprey, *Lampetra aepyptera*. Copeia 1969:393–395.

Jordan, D.S. 1918. The freshwater lampreys of the eastern United States. Copeia 1918:93–96.

Lang, N.J., K.J. Roe, C.B. Renaud, H.S. Gill, I.C. Potter, J. Freyhof, A.M. Naseka, P. Cochran, H.E. Perez, E.M. Habit, B.R. Kuhajda, D.A. Neely, Y.S. Reshetnikov, V.B. Salnikov, M.T. Stoumboudi & R.L. Mayden. 2009. Novel relationships among lampreys (Petromyzontiformes) revealed by a taxonomically comprehensive molecular data set. American Fisheries Society Symposium 72:41–55.

Librado, P. & J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

Martin, H. & M.M. White. 2008. Intraspecific phylogeography of the least brook lamprey (*Lampetra aepyptera*). Copeia 2008:579–585.

Melhorn, W.N. & J.P. Kempton. 1991. The Teays system: a summary. Geological Society of America Special Paper 258:125–128.

Near, T.J., L.M. Page & R.L. Mayden. 2001. Intraspecific phylogeography of *Percina evides* (Percidae: Etheostomatinae): an additional test of the Central Highlands pre-Pleistocene vicariance hypothesis. Molecular Ecology 10:2235–2240.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, New York. 512 pp.

Rhode, F.C. & R.E. Jenkins. 1980. *Lampetra aepyptera* (Abbott), least brook lamprey. P. 21. *In* Atlas of North American Freshwater Fishes. (D.S. Lee, C.R. Gilbert, C.H. Hocutt, R.E. Jenkins, D.E. McAllister & J.R. Stauffer, Eds.). North Carolina State Museum of Natural History, Raleigh, North Carolina. 854 pp.

Rogers, A.R. & H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552–569.

Seversmith, H.F. 1953. Distribution, morphology, and life history of *Lampetra aepyptera*, a brook lamprey, in Maryland. Copeia 1953:225–232.

Simon, T.P. 2011. Fishes of Indiana: A Field Guide. University of Indiana Press, Bloomington, Indiana. 345 pp.

Simons, A.M. 2004. Phylogenetic relationships in the genus *Erimystax* (Actinopterygii: Cyprinidae) based on the cytochrome b gene. Copeia 2004:351–356.

Slatkin, M. & R.R. Hudson. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129:555–562.

Strange, R.M. & B.M. Burr. 1997. Intraspecific phylogeography of North American highland fishes: a test of the Pleistocene vicariance hypothesis. Evolution 51:885–897.

Strange, R.M., V.T. Truong & K.J. Delaney. 2016. The mitogenome of the southern brook lamprey, *Ichthyomyzon gagei* (Cyclostomata: Petromyzontidae). Mitochondrial DNA Part B: Resources 1:903–904.

Swofford, D.L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin & D.G. Higgins. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25:4876–4882.

Trautman, M.B. 1981. The fishes of Ohio, revised edition. Ohio University Press, Columbus, Ohio. 782 pp.

Walsh, S.J. & B.M. Burr. 1981. Distribution, morphology, and life history of the least brook lamprey, *Lampetra aepyptera* (Pisces: Petromyzontidae), in Kentucky. Brimleyana 6:83–100.

White, M.M. & H.R. Martin. 2009. Structure and conservation of tandem repeats in the mitochondrial DNA control region of the least brook lamprey (*Lampetra aepyptera*). Journal of Molecular Evolution 68:715–723.

Manuscript received 27 April 2018, revised 20 June 2018.