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Original Article

Assessing Genomic Diversity, Connectivity, and Riverscape Genetics Hypotheses in the Endangered Rough Hornsnail, *Pleurocera foremani*, Following Habitat Disruption

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Abstract

The southeastern United States is home to some of the richest biodiversity in the world. Over the last 200 years, however, rapid industrialization and urbanization have threatened many natural areas, including freshwater habitats. River impoundments have also rapidly altered freshwater habitats, often resulting in species extirpation or extinction. The Coosa River in Alabama experienced one of the largest faunal declines in modern history after impoundment, making it an ideal system for studying how invertebrate species are affected by reservoir creation. One such species, the Rough Hornsnail, *Pleurocera foremani*, is an endangered freshwater snail in the family Pleuroceridae. We sampled all known localities of *P. foremani* and used 2bRAD-seq to measure genetic diversity. We assessed riverscape genomic patterns across the current range of *P. foremani* and measured gene flow within and between impoundments. We also investigated the degree to which *P. foremani* displays an isolation by distance pattern and conforms to broad hypotheses that have been put forth for population genetics of riverine species like the Mighty Headwater Hypothesis that predicts greater genetic diversity in headwater reaches compared with mainstem populations. Like most other freshwater species, a pattern of isolation by distance was observed in *P. foremani*. We also found that Coosa River dams are a barrier to gene flow, and genetic fragmentation of *P. foremani* is likely to increase. However, gene flow appeared common within reservoirs and tributaries. Additionally, we found that spatial genetic structure of *P. foremani* deviates from what is expected under the Mighty Headwaters Hypothesis, adding to a growing body of research suggesting that the majority of genetic diversity in low-dispersing gastropods is found in mainstem populations.

Keywords: population genomics, pleurocerid, conservation, Coosa River

The southeastern United States is a global hotspot of freshwater invertebrate biodiversity (Neves et al. 1997). Understanding genetic patterns associated with such biodiversity is a major goal of population and landscape genetics (Davis et al. 2018). Furthermore, determining genetic responses to anthropogenic activities is central to conservation genetics (Khan et al. 2016; Allendorf 2017). Despite well-documented declines of freshwater fauna in the southeastern United States (Williams et al. 1993; Lydeard and Mayden 1995; Neves et al. 1997; Johnson et al. 2013), little or no data exist about riverscape genetics of most species (but see Fluker et al. 2014; Whelan et al. 2019; Barnett et al. 2020; Wright et al. 2020). This hinders broad understanding of how genetic patterns in a global biodiversity hotspot are influenced by human activity. Furthermore, population genetic studies are needed to inform conservation efforts and understand whether broad hypotheses about riverscape genetic patterns apply to dispersal limited freshwater invertebrates. One such hypothesis is the Mighty Headwaters Hypothesis (Finn et al. 2011), which postulates that headwaters will harbor greater genetic diversity than mainstem rivers. Many freshwater species also display an isolation by distance (IBD) pattern (Hänfling and Weetman 2006; Dehais et al. 2010; Gouskov et al. 2015; Rougemont et al. 2020). However, most past studies have focused on vagile fish species, and freshwater invertebrates with limited dispersal capabilities may not display the same patterns of genetic variation, especially in modified habitats.

Rapid changes in land use and river impoundment for navigation and hydropower in the southeastern Unites States over the last 200 years have caused considerable declines in freshwater ecosystem biodiversity (Lydeard and Mayden 1995; Strong et al. 2008; Tolley-Jordan et al. 2015). For example, impoundment of the Coosa River in Alabama caused at least 29 freshwater gastropod extinctions, including an entire genus of freshwater snails, *Gyrotoma* (Lydeard and Mayden 1995). Remaining taxa often persist in highly modified river systems that may influence contemporary genetic patterns. For example, Fluker et al. (2014) showed that a single hydropower impoundment on the Tallapoosa River resulted in decreased gene flow among populations of the fishes *Etheostoma tallapoosae* and *Cyprinella gibbsi*. Similarly, Barnett et al. (2020) recently established that within timeframes as short as 36 years after impoundment, crayfish experienced significant genetic fragmentation in the Bear Creek and Cahaba River drainages in Alabama and Mississippi. Outside of the southeastern United States, fragmentation has been shown to hinder gene flow and decrease genetic diversity of fish (Blanchet et al. 2010; Dehais et al. 2010; Faulks et al. 2011; Gouskov et al. 2015; Sotola et al. 2017; Yamamoto et al. 2019) and mussels (Liu et al. 2019). More research is needed, however, to assess patterns in understudied groups like freshwater gastropods.

The freshwater gastropod family Pleuroceridae is one group that has suffered immensely from habitat modification in the eastern United States. This family consists of at least 162 species and is found in rivers and streams of North America, east of the Rocky Mountains (Strong and Köhler 2009; Johnson et al. 2013). As a result of anthropogenic activity, at least 79% of pleurocerid species are imperiled, including 7 species listed as threated or endangered under the U.S. Endangered Species Act and 33 extinct species (Johnson et al. 2013). Despite the high imperilment rate of pleurocerids, only 2 pleurocerid species have been the focus of population genomic studies (Whelan et al. 2019; Wright et al. 2020), and both studies focused on species in the Cahaba River, one of the few major rivers in the southeastern United States that has not been impounded (Ward et al. 2005). Whelan et al. (2019) showed that riverscape genomic patterns in the pleurocerid *Leptoxis ampla* did not follow patterns predicted by the Mighty Headwaters Hypothesis, but more studies are needed to determine whether patterns seen in *L. ampla* are universal to pleurocerids. Furthermore, Wright et al. (2020) focused on a range-restricted pleurocerid, *L. compacta*, only found at mainstem sites in the Cahaba River. Additionally, we have no information on how pleurocerid genomics are influenced by impoundments. Thus, extrapolating patterns seen in 2 species from the same river system to other pleurocerids may not be appropriate.

One pleurocerid that persists in the highly modified Coosa River system is the rough hornsnail, *Pleurocera foremani* (Figure 1). This species is endemic to the lower and middle Coosa River drainage, but loss of habitat from impoundments resulted in drastic range reduction and the species being federally listed as endangered in 2010 (United States Fish and Wildlife Service 2010). The historical range of *P. foremani* spanned the Coosa River at Wetumpka, Alabama, upstream to Etowah County, Alabama, including several major tributaries such as Hatchet Creek and Yellowleaf Creek. Unlike many pleurocerid species from the Coosa River that went extinct as a

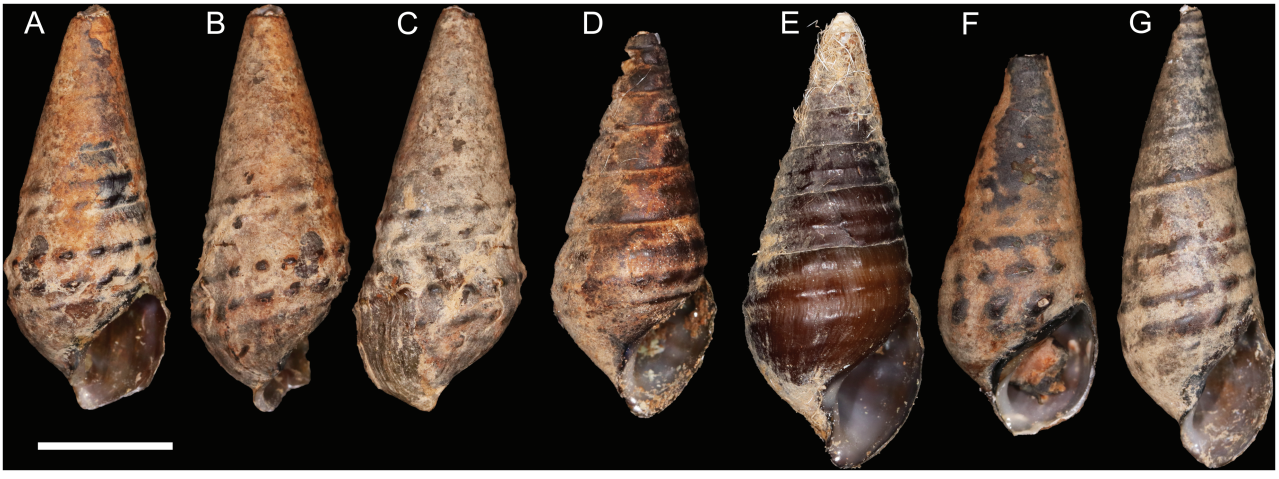

Figure 1. Representative individuals of sequenced *P. foremani*. (A–C) Same individual with different views of the shell. (D–G) Different individuals representing the range of observed morphological variation. Scale bar = 1 cm.

result of river impoundments, *P. foremani* has persisted in a highly modified habitat. The species is currently known from Mitchell Lake, Yellowleaf Creek, and the Coosa River at Wetumpka in the Jordan Dam tailwaters (Figure 2).

Pleurocera foremani is an opportune species for testing how genetic diversity and riverscape genetic patterns of invertebrates with low dispersal ability can be influenced by impoundments. Furthermore, population genomic information is needed for *P. foremani* to obtain quantitative measures of gene flow and to assess how habitat fragmentation and environmental degradation affect the species. Genetic data are also necessary for identifying *P. foremani* populations with adequate diversity to help species recovery efforts, particularly captive propagation and reintroduction (Bert et al. 2007; Moyer and Williams 2012; Whelan et al. 2019). We used a 2bRAD-seq approach (Wang et al. 2012) to assess patterns of genetic diversity across the current range of *P. foremani*. Specifically, we tested for patterns of IBD and those predicted by the Mighty Headwaters Hypothesis, such as increased biodiversity within headwaters compared with mainstem populations. We also measured genetic diversity and fragmentation in the presence of drastic habitat modification over the last 100 years. Broadly, our analyses will inform management strategies and provide insight into how impoundments influence riverscape genetic patterns of low-dispersing freshwater invertebrates.

Methods

Sample Collection

Pleurocera foremani was collected from 15 sites within the Coosa River drainage (Table 1 and Figure 2). These sites included 11 locations within Lake Mitchell both on the previous linear path of the river and in the surrounding tributary areas, 3 locations in Yellowleaf Creek, and 1 location in the Coosa River at Wetumpka. Sampling was done in October 2018 during reservoir drawdowns that Alabama Power Company does for maintenance purposes every 5 years. Sampling at a single site in the Coosa River at Wetumpka was done in June 2019 and required SCUBA diving. We collected 10 individuals from each site. Where found, *P. foremani* was abundant, but we did not perform quantitative measurements of density. During the Coosa River reservoir drawdowns, considerable sampling effort was expended to locate *P. foremani* in Lay Lake (approximately 271 person hours), but no individuals were found. The collections made here represent the known contemporary range of *P. foremani* (Figure 2). Animals were preserved following Fukuda et al. (2008), placed in 100% EtOH, and kept at −20 °C until DNA was extracted. All sequenced specimens have been individually deposited as vouchers at the Auburn University Museum of Natural History (AUMNH; Table 1).

Data Generation

Foot tissue was removed from samples, and DNA was extracted using the Qiagen DNeasy Plant Kit, with a minor modification to the manufacturer's protocol to facilitate a proteinase K tissue digestion. A plant kit was used because it handles mucus polysaccharides better than standard extraction methods for animals (Whelan et al. 2019). We prepped samples for 2bRAD sequencing following the protocol of Wang et al. (2012). Briefly, DNA was digested using the AlfI enzyme, a IIb restriction enzyme. Following Whelan et al. (2019), we used a 1/16 reduction scheme during sample preparation by ligating adaptor oligos with "NC" overhangs (see Wang et al. 2012). Libraries were sent to the University of Oregon Genomic and Cell Characterization Core Facility for sequencing on Illumina HiSeq 4000 using single-end 75 bp chemistry.

Raw reads that had more than 5 base pairs with Phred quality scores less than 20 and those that did not include the AlfI cut fragment were discarded (scripts can be found at https://github.com/NathanWhelan/2bRAD-processing). Filtered reads were assembled using Stacks 1.48 (Catchen et al. 2011, 2013). We used the denovo_map.pl script with default distances between stacks (M = 2) and specified distance between catalog loci (n = 2) and minimum stack depth (m = 5). These parameters were selected after preliminary runs based on protocols for optimization described by Paris et al. (2017). After assembly, loci were passed through the *populations* program within Stacks. Sequences were filtered to allow for a single SNP per locus using the "-write-single-snp" option, a minimum minor allele frequency of 0.025, and a maximum observed heterozygosity of 0.5. Loci had to be present in 10 of the 15 sample sites and 75% of individuals per site to be retained.

Population Genetic Analyses

Nucleotide diversity, observed heterozygosity, expected heterozygosity, and number of private alleles per collection site were calculated by Stacks. We calculated allelic richness per collection site in R (R Core Team 2020) with the package *diveRsity* (Keenen et al. 2013). Pairwise F_{ST} among collection sites was calculated using the Weir and Cockerman's (1984) method in R with the package *hierfstat* (Goudet 2005). Distances between collection sites were measured by tracing river paths or straight distances across lakes using Google Earth (Table 3). Three Mantel tests with F_{ST} values and geographic distances (using the "mantel.randtest" method with 1000 permutations in package ade4; Dray and Dufour 2007) were conducted to assess IBD. One test included all sites, the second test included only Lake Mitchell and the Coosa River at Wetumpka sites, and the third test included only Lake Mitchell sites. Although Mantel tests are a common method for testing IBD, they have been criticized (Legendre et al. 2015, Meirmans 2015). Therefore, we also tested for IBD with multiple regressions using the package *ecodist* (Goslee and Urban 2007) in R. This was done using the same distance matrices and F_{ST} calculations used for Mantel tests and with the MRM function and 1000 permutations.

Genomic admixture was investigated with the program ADMIXTUTRE 1.3 (Alexander et al. 2009) using the AdmixPipe pipeline (Mussmann et al. 2020). The best-fit number of genetic clusters (K) was selected with 20% cross-validation, testing K from 1 to 15. We also analyzed genetic clustering using discriminant analysis of principal components (DAPC) and the R package *adegenet* (Jombart and Ahmed 2011). We used the function "find.clusters," retaining all principle components, and Bayesian information criteria (BIC) to test for the best-fit K within the principal component analysis, up to 25 clusters. The K with the lowest BIC was chosen as the best-fit K. The *adegenet* command "dapc" was used with the best-fit K, retaining 2 principle components and all discriminant functions. DAPC results were plotted in R.

We further investigated if collection sites were in complete admixture to delineate populations and assess migration among populations using migrate-n (Beerli and Palczewski 2010). We randomly selected 100 loci out of the full dataset to decrease computational time required for migrate-n analyses. Because migrate-n has been more thoroughly tested and validated on sequences than SNPs (Beerli and Palczewski 2010), we utilized the entire 36 bp sequences associated with the randomly selected loci. Geographic distances between

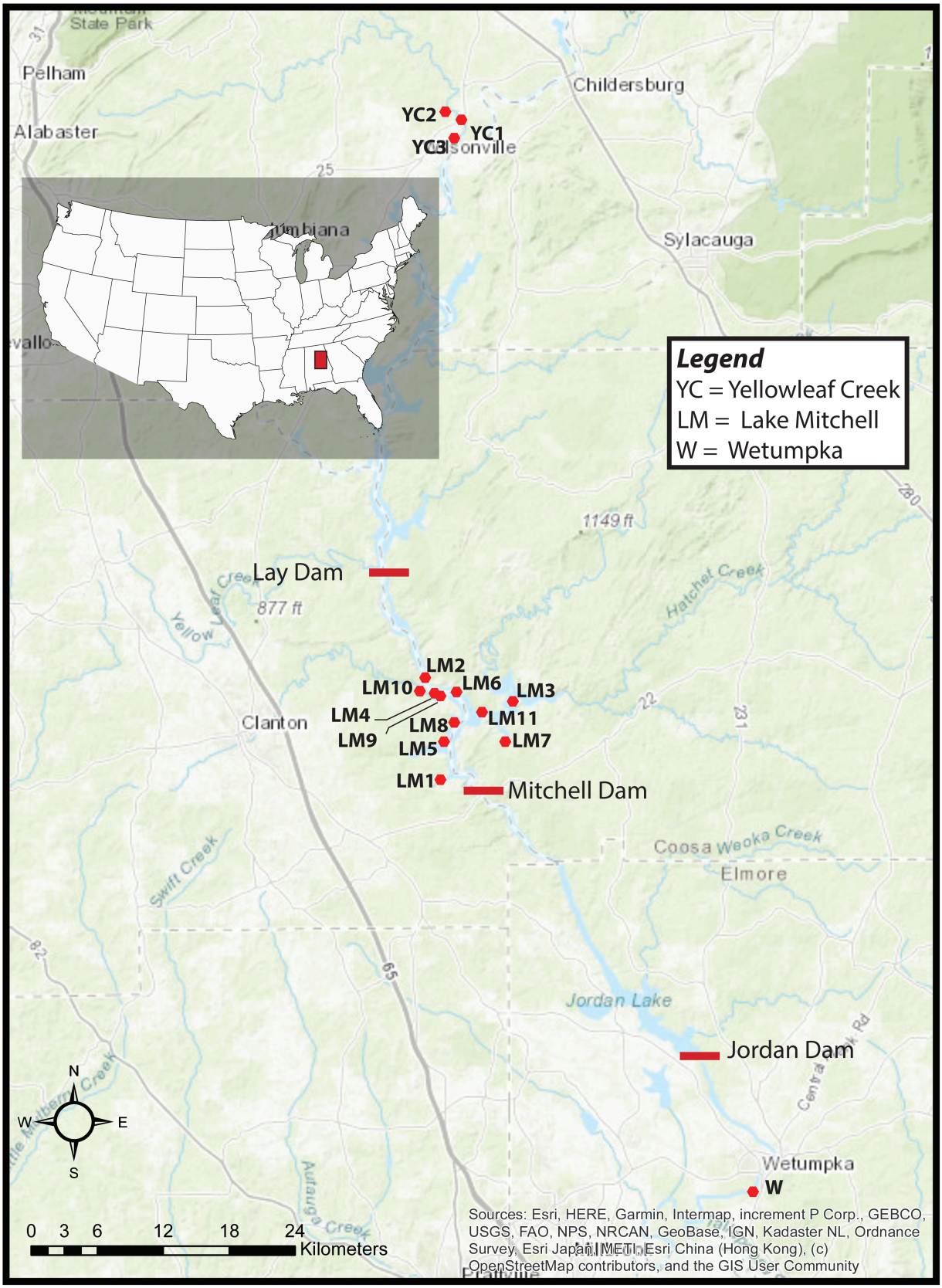

Figure 2. Map of collection localities and impoundments. Red box on inset map represents collection area.

sites were used in the same way as measured for IBD analyses. We ran migrate-n using the Bayesian inference strategy with uniform prior distributions for both theta (min = 0, mean = 0.1, max = 0.2) and migration rate (min = 0, mean = 25 000, max = 50 000). Prior distribution values were determined after testing parameters on initial runs to make sure priors were not too restrictive. Each model

Table 1. Collection and locality information for all sites and shell voucher catalog numbers

Population	Latitude	Longitude	AUMNH catalog numbers
Yellowleaf Creek 1 (YL1)	33.2602	−86.4512	45732–45741
Yellowleaf Creek 2 (YL2)	33.2657	−86.4645	45772–45781
Yellowleaf Creek 3 (YL3)	33.2478	−86.4572	45802–45811
Lake Mitchell 1 (LM1)	32.8087	−86.4683	45742–45751
Lake Mitchell 2 (LM2)	32.8787	−86.4811	45752–45761
Lake Mitchell 3 (LM3)	32.8624	−86.4094	45762–45771
Lake Mitchell 4 (LM4)	32.8681	−86.4733	45782–45791
Lake Mitchell 5 (LM5)	32.8348	−86.4655	45792–45801
Lake Mitchell 6 (LM6)	32.8689	−86.4554	45812–45821
Lake Mitchell 7 (LM7)	32.8348	−86.4155	45822–45831
Lake Mitchell 8 (LM8)	32.8481	−86.4571	45831–45841
Lake Mitchell 9 (LM9)	32.8662	−86.4686	45841–45851
Lake Mitchell 10 (LM10)	32.8696	−86.4854	45852–45861
Lake Mitchell 11 (LM11)	32.8553	−86.4344	45872–45881
Coosa River at Wetumpka (W)	32.5257	−86.2132	45862–45871

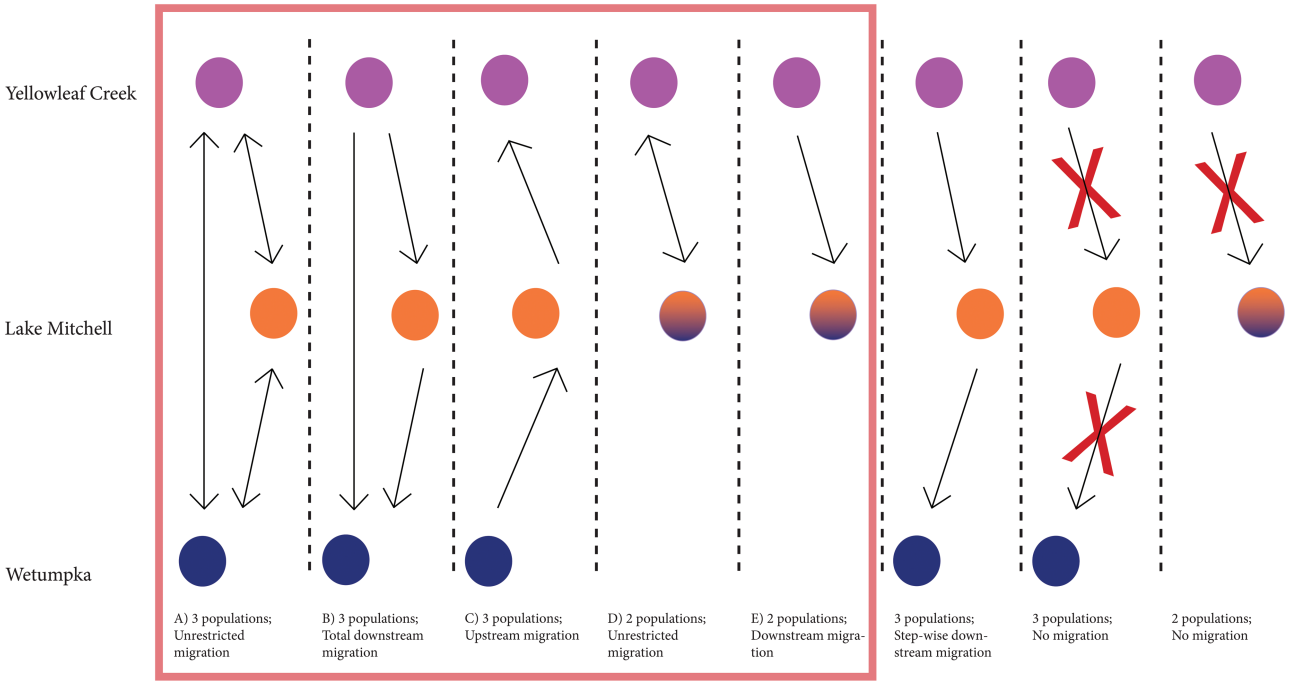

Figure 3. Models tested in migrate-n. Models included in the final analysis are within the red box. Circles represent populations, with arrows showing upstream (toward top of figure) and downstream (toward bottom of figure) migration. Orange circles correspond to Yellowleaf Creek sites, purple corresponds to Lake Mitchell sites, and blue is associated with Coosa River at Wetumpka. Circles with both purple and blue represent models where Lake Mitchell and Coosa River at Wetumpka sites are treated as one population. The number and color of circles correspond to the number of populations given to the migrate-n model, and colored based on sites included in those populations. Models are assigned letters corresponding to Table 4.

was run with 4 metropolis-coupled Markov chain Monte Carlo runs and a static heating scheme. Each chain was heated using default parameters and ran for 110 000 steps, recorded every 100 steps, and the first 10 000 steps were discarded as burn-in. Default parameters were used for all other model specifications. Initial analyses were done using models that represented several possible movement patterns between collection sites, including upstream and downstream movement, and movement within- and between-single sites (Figure 3). However, lack of convergence for some models after 100 000 generations suggested not enough information was available for precise parameter estimation. Model runs that did not converge were not considered further. Ultimately, 5 migration models were investigated covering realistic movement patterns between either 2 populations or 3 populations (Figure 3). For the 2 population models, one population was comprised of all Yellowleaf Creek sites and the other population included all Lake Mitchell and the Coosa River at Wetumpka sites. For the 3-population model, individuals were grouped as one population from Yellowleaf Creek, a second that included all Lake Mitchell sites, and a third consisting of the Coosa River at Wetumpka site. We selected the best-fit model using Bezier approximation scores as output by migrate-n, and log Bayes factors (Beerli and Palczewski 2010) (Table 4).

Results

Molecular Analysis

Data from all 150 individuals collected were included in the molecular data set. We recovered an average of 3 583 250 raw reads

from each individual, ranging from 307 416 to 6 947 036 reads. After filtering, we recovered an average of 3 287 029 reads per individual (range 169 286–6 703 259). After using STACKS denovo_map.pl and filtering with *populations*, we retained 2866 polymorphic loci.

Population Genetic Analyses

We recovered a range of private alleles from different localities (0 at Lake Mitchell sites LM3–8 and LM11 to 24 at the Coosa River at Wetumpka; Figure 2 and Table 2). Each Yellowleaf Creek site contained private allele counts ranging from 11 to 21 (Figure 2 and Table 2). Observed heterozygosity ranged from 0.0974 at site YC2 within Yellowleaf Creek up to 0.2299 at site LM11 within Lake Mitchell (Figure 2 and Table 2). Expected heterozygosity ranged from 0.083 at YC2 to 0.1813 at site LM11 (Figure 2 and Table 2). Nucleotide diversity ranged from 0.0877 in Yellowleaf Creek site YC2 to 0.1919 in Lake Mitchell site LM11 (Figure 2 and Table 2). Values for expected heterozygosity, observed heterozygosity, and nucleotide diversity were lower at Yellowleaf Creek sites than at Lake Mitchell and the Coosa River at Wetumpka (Table 2). Allelic richness was lowest at Lake Mitchell site LM11 (0.856) and highest at site LM8 (1.48; Figure 2 and Table 2). Notably, the site with the lowest allelic richness value, LM11, also had the largest observed heterozygosity, expected heterozygosity, and nucleotide diversity.

Pairwise F_{ST} values ranged from 0.013 between 2 of the Lake Mitchell sites (LM6 and LM8) to 0.719 between Yellowleaf Creek site YM2 and Lake Mitchell site LM10 (Table 3). Mantel tests indicated a significant signal of IBD when all sites were included ($P = 0.001$), as well as when just the Lake Mitchell and the Coosa River at Wetumpka sites were included ($P = 0.002$). The Mantel Test of only Lake Mitchell sites indicated a lack of IBD in Lake Mitchell ($P = 0.06$). Similarly, multiple regression of distance between all sites and pairwise F_{ST} showed a significant signal of IBD ($R^2 = 0.752$, $P = 0.001$). Multiple regression of Lake Mitchell and the Coosa River at Wetumpka sites also had a significant signal of IBD ($R^2 = 0.338$, $P = 0.002$), while multiple regression of just Lake Mitchel sites was not significant for a signal of IBD ($R^2 = 0.128$, $P = 0.095$).

Cross-validation model testing with ADMIXTURE indicated that the best-fit number of genetic clusters in our data was 2. This model grouped the Yellowleaf Creek individuals at all 3 sites together in one genetic cluster and all Lake Mitchell and the Coosa River at Wetumpka individuals together (Figure 4). There was little to no signal of admixture in any individuals, except for very low amounts in the Coosa River at Wetumpka individuals and 4 members of the most upstream Lake Mitchell site, ML2 (Figures 2 and 4). Discriminant analyses of principle components indicated 2 clusters of genetic groups, explained by a single discriminant function (Figure 5).

Contrary to results from DAPC and ADMIXTURE, the best-fit model from our migrate-n analysis indicated the presence of 3 populations, rather than 2 populations (Table 4). The best-fit model also only allowed for downstream migration and was favored with a model probability of 0.999 compared with the next highest model probability of 1.17e-06. Mutation-scaled effective population size (Θ) in the Yellowleaf Creek population (mean: 0.00135; 95% confidence interval [CI]: 0.000–0.00453) and the Coosa River at Wetumpka population (mean: 0.00009; 95% CI: 0.000–0.00280) were both less than within the geographically larger Lake Mitchell population (mean: 0.00923; 95% CI: 0.00573–0.01253).

Discussion

Across the current range of *P. foremani*, a complex interplay of historical processes and contemporary habitat modification drive genetic patterns. IBD influences genetic diversity across the riverscape, which is likely a phenomenon that predates dam construction as IBD is common in freshwater taxa from non-modified habitats (Blanchet et al. 2010; Meffe and Vrijenhoek 1988). Higher levels of genetic diversity in Lake Mitchell and the Coosa River at Wetumpka compared with sites in the Coosa River tributary Yellowleaf Creek are also likely a result of historical genetic diversity, and fragmentation does not appear to be causing decreases in genetic diversity of remaining *P. foremani* populations compared to other freshwater species (Dehais et al. 2010; Faulks et al. 2011; Fluker et al. 2014; Sotola et al. 2017; Yamamoto et al. 2019; Barnett et al. 2020; Rougemont et al. 2020). For example, Barnett et al. (2020) observed a decrease in genetic diversity of crayfish across impoundments in as little as 36 years, about 36 generations, while *P. foremani* has been impounded for almost 100 years, around 100 generations. Similarly, Faulks et al (2011) demonstrated that in as little as 10 years, or 3 generations, the Macquarie perch (*Macquaria australasica*) experienced significant bottlenecks and population diversity declines. With a median impoundment age of 82 years, fish populations within the upper Rhine catchment underwent a decrease in connectivity even when provided with fish passage systems to mediate the effects of impoundments (Gouskov et al. 2015). Thus, the relatively widespread range of *P. foremani* in Lake Mitchell, which likely allows for a larger overall population in Lake Mitchell, may insulate remaining *P. foremani* populations from immediate effects of fragmentations. A similar phenomenon has been seen in some fish (Hudman and Gibo 2012; Sotola et al. 2017). Of course, genetic

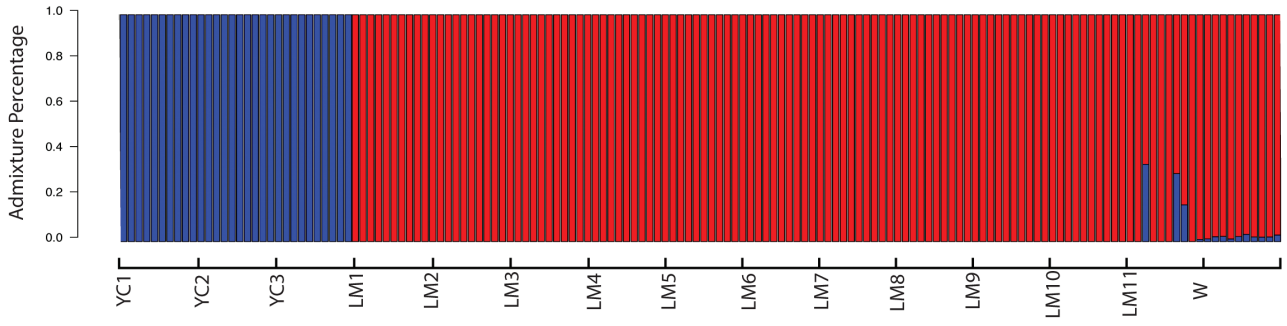

Figure 4. Admixture plot ($K = 2$) of individuals from each site. Each column represents an individual. LM, Lake Mitchell; W, Coosa River at Wetumpka; YC, Yellowleaf Creek.

Table 2. Estimates of genetic diversity for all sites

Population	Private alleles	Allelic richness	Observed heterozygosity	Expected heterozygosity	Nucleotide diversity
Yellowleaf Creek 1	17	1.13 (0.545)	0.0999 (0.0041)	0.0890 (0.0032)	0.094 (0.0034)
Yellowleaf Creek 2	21	1.13 (0.542)	0.0974 (0.0042)	0.0830 (0.0031)	0.0877 (0.0033)
Yellowleaf Creek 3	11	1.10 (0.574)	0.1071 (0.0043)	0.0927 (0.0033)	0.0978 (0.0039)
Lake Mitchell 1	2	1.29 (0.566)	0.1526 (0.0044)	0.1395 (0.0037)	0.1471 (0.0039)
Lake Mitchell 2	2	1.41 (0.533)	0.1555 (0.0039)	0.1571 (0.0035)	0.1656 (0.0037)
Lake Mitchell 3	0	1.39 (0.546)	0.1608 (0.0040)	0.1569 (0.0036)	0.1655 (0.0038)
Lake Mitchell 4	0	1.40 (0.515)	0.1545 (0.0039)	0.1495 (0.0035)	0.1577 (0.0037)
Lake Mitchell 5	0	1.38 (0.557)	0.1602 (0.0040)	0.1562 (0.0035)	0.1647 (0.0037)
Lake Mitchell 6	0	1.45 (0.520)	0.1645 (0.0039)	0.1632 (0.0034)	0.1721 (0.0036)
Lake Mitchell 7	0	1.39 (0.537)	0.1572 (0.0039)	0.1512 (0.0035)	0.1595 (0.0036)
Lake Mitchell 8	0	1.48 (0.502)	0.1719 (0.0039)	0.1676 (0.0034)	0.1767 (0.0036)
Lake Mitchell 9	1	1.39 (0.533)	0.1561(0.004)	0.1503 (0.0035)	0.1584 (0.0037)
Lake Mitchell 10	1	1.28 (0.532)	0.1309 (0.0041)	0.1219 (0.0034)	0.1286 (0.0036)
Lake Mitchell 11	0	0.856 (0.843)	0.2299 (0.0066)	0.1813 (0.0045)	0.1919 (0.0048)
Coosa River at Wetumpka	24	1.38 (0.548)	0.1542 (0.004)	0.1499 (0.0035)	0.1581 (0.0037)

Allelic richness, observed heterozygosity, expected heterozygosity, and nucleotide diversity are collection site means. Standard deviations are in parentheses.

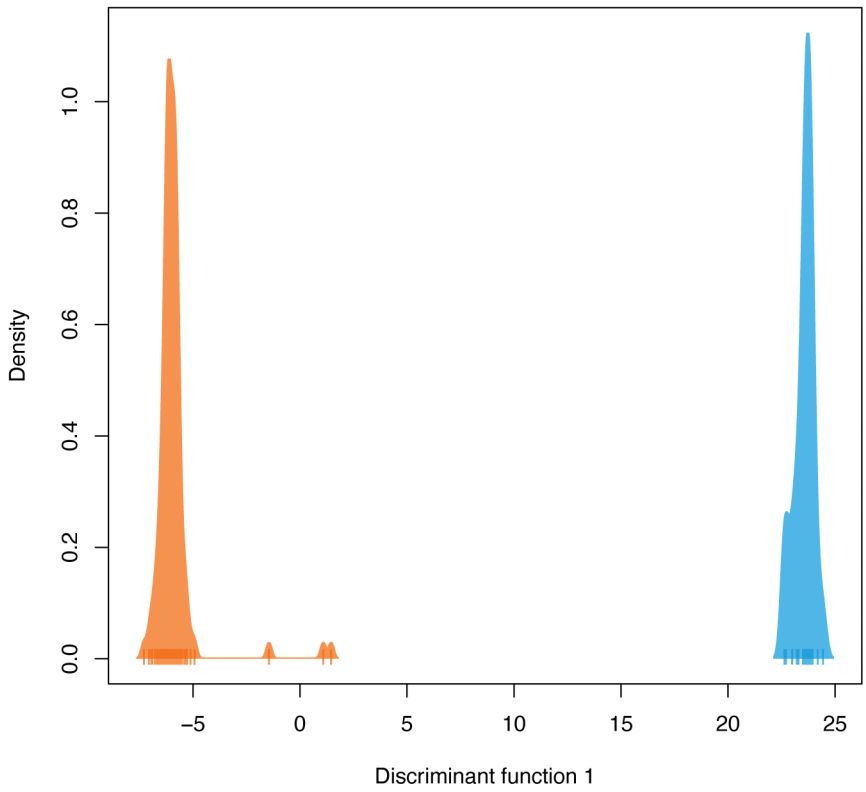

Figure 5. DAPC plot showing distribution of discriminant function 1. Different shading represent unique genetic clusters, and tick marks are individuals within these clusters.

diversity has decreased to zero in many places of the Coosa River system where *P. foremani* is extirpated. The contrast between relatively high genetic diversity at mainstem sites where *P. foremani* persists versus severe range contraction is striking. Coupled with well-documented extinctions of other pleurocerids from the Coosa River (Lydeard and Mayden 1995), our data indicate that habitat degradation causes an all-or-nothing response in pleurocerids where surviving populations retain genetic diversity, but declining populations are rapidly extirpated. Therefore, conservation planning for pleurocerids should consider the potential for rapid population loss. Nevertheless, our data offer promise for reintroduction efforts as remaining pleurocerid populations can likely serve as genetically diverse broodstock sources.

The inferred signal of connectivity between Lake Mitchell and the Coosa River at Wetumpka sites indicated by ADMXITURE and DAPC (Figures 4 and 5) suggests that full genetic fragmentation has yet to occur. However, *P. foremani* has both limited dispersal ability and a 1-year generation time, indicating that the inferred signal of connectivity between Mitchell Lake and the Coosa River at Wetumpka in ADMIXTURE and DAPC analyses are a relic of

Table 3. Pairwise geographic distance and F_{ST} values

	YC 1	YC 2	YC 3	ML 1	ML 2	ML 3	ML 4	ML 5	ML 6	ML 7	ML 8	ML 9	ML 10	ML 11	W
YC 1	—	2.09	2.08	66.5	55.0	64.7	56.3	62.8	59.5	65.0	60.8	57.3	59.4	61.5	112
YC 2	0.055	—	4.17	68.6	57.1	66.8	58.4	64.9	61.6	67.1	62.9	59.4	61.5	63.6	114
YC 3	0.048	0.097	—	64.2	52.9	62.4	54.2	60.7	57.6	62.9	58.7	55.4	57.3	59.2	110
ML 1	0.689	0.7	0.671	—	11.4	11.1	9.83	3.65	8.09	11.4	5.52	9.30	11.2	7.85	46.0
ML 2	0.666	0.677	0.643	0.141	—	9.64	1.34	7.74	2.98	9.98	5.86	2.33	4.31	6.42	57.5
ML 3	0.663	0.675	0.641	0.155	0.102	—	7.81	7.42	6.87	4.60	5.52	7.48	9.37	3.22	57.1
ML 4	0.675	0.686	0.651	0.175	0.042	0.129	—	6.26	3.12	8.38	4.47	0.853	2.78	4.59	52.3
ML 5	0.663	0.676	0.639	0.147	0.067	0.097	0.084	—	4.79	7.82	1.88	5.78	7.71	4.20	49.7
ML 6	0.658	0.671	0.637	0.128	0.06	0.075	0.09	0.064	—	7.21	3.17	1.27	3.20	3.65	51.0
ML 7	0.67	0.684	0.653	0.148	0.075	0.077	0.101	0.077	0.054	—	5.86	7.82	9.70	3.56	53.2
ML 8	0.653	0.666	0.631	0.126	0.055	0.065	0.082	0.06	0.013	0.043	—	3.99	5.92	2.30	48.4
ML 9	0.672	0.686	0.653	0.162	0.101	0.103	0.126	0.098	0.082	0.094	0.068	—	1.93	4.26	52.5
ML 10	0.707	0.719	0.692	0.244	0.177	0.187	0.197	0.179	0.172	0.175	0.156	0.097	—	6.15	54.4
ML 11	0.533	0.549	0.531	0.086	0.035	0.04	0.05	0.037	0.029	0.028	0.022	0.043	0.096	—	53.9
W	0.664	0.676	0.641	0.252	0.195	0.199	0.211	0.178	0.171	0.173	0.168	0.195	0.268	0.072	—

Geographic distances (km) are above the diagonal and F_{ST} values are below. YC, Yellowleaf Creek; ML, Mitchell Lake; W, Coosa River at Wetumpka.

Table 4. Model output and probability for different migration models inferred through migrate-n

Model	Bezier	Harmonic	Log Bayes factor	Rank	Model probability
3pop_downall (B)	−13 379	−6588.7	0	1	0.99999
3pop_upstream (C)	−13 392	−6147.7	−13.660	2	<0.001
3pop_unrestrict (A)	−13 444	−6496.0	−64.740	3	<0.001
2pop_unrestrict (D)	−13 764	−6704.8	−385.17	4	<0.001
2pop_down (E)	−13 776	−6790.5	−397.26	5	<0.001

Probability was calculated as outlined in Beerli and Palczewski (2010). Shaded row is model used to calculate the log bayes factor as described in Beerli and Palczewski (2010) as well. Letters correspond to model letters in Figure 4.

historical connectivity that stopped with the construction of Mitchell Dam in 1923. Of course, this assumes that contemporary gene flow is not occurring between the 2 locations. We argue that such an assumption is reasonable given the inability of pleurocerids to migrate over land, their lack of a highly vagile life history stage like a veliger larva, and the absence of *P. foremani* in Jordan Lake, which sits between Lake Mitchell and the Coosa River at Wetumpka. Moreover, migrate-n analyses indicated 3 distinct populations with some downstream mediated gene flow (Figure 3 and Table 4). Mantel tests and multiple regression indicated a signal of IBD between most sites. When all 3 populations were analyzed together and when only Lake Mitchell and the Coosa River at Wetumpka sites were analyzed, a significant IBD effect was detected. This further indicates that despite clustering together in ADMIXTURE and DAPC analyses (Figures 4 and 5), these 2 sites are at least partly isolated. Additionally, the higher number of private alleles in the Coosa River at Wetumpka site compared with any other (Table 2) supports the presence of unique populations in Lake Mitchell and the Coosa River at Wetumpka. The lack of IBD and low numbers of private alleles at any given site within Lake Mitchell (Table 2) indicated a single *P. foremani* population with genetic admixture between the different collection sites, regardless of geographic location within the lake. Although there may be gradients of increased relatedness between geographically proximate sites, we found no evidence for a significant barrier to reproduction within Lake Mitchell. An alternative explanation for the lack of IBD within Lake Mitchell is that there was a severe bottleneck with the introduction of the Lake Mitchell impoundment. This appears unlikely given high heterozygosity and nucleotide diversity at most sites within the Lake Mitchell (Table 2). However, low allelic richness at Lake Mitchell site LM11 may represent a recent colonization to that specific area within Lake Mitchell, but high heterozygosity and nucleotide diversity give evidence for a population expansion, possibly rapidly occurring after colonization.

Agreement among all methods that Yellowleaf Creek represents a distinct population is likely a result of little historical connectivity between tributary and mainstem sites. The relative isolation of the Yellowleaf Creek individuals corroborates the hypothesis that tributary populations of pleurocerids are relatively isolated from mainstem population and less diverse (Whelan et al. 2019). Migrate-n analyses also suggest that *P. foremani* has downstream-biased migration patterns, similar to what has been documented in *L. ampla*. Given the unlikely probability of *P. foremani* individuals traversing 2 dams and a reservoir, our results indicate the observed signal of downstream migration predates dam construction. This historical migration pattern likely explains the inference of 2 genetic clusters with ADMIXTURE and DAPC. Therefore, the inference of 3 populations by migrate-n analyses indicates contemporary fragmentation that has the potential to get worse with time following impoundment. For the time being, the relatively high amount of genetic diversity of *P. foremani* from the Coosa River at Wetumpka and Lake Mitchell will likely insulate populations from the more drastic effects of genetic fragmentation, such as inbreeding. However, increasing population divergence will likely continue in the presence of current impoundments.

Observed spatial genetic diversity in *P. foremani* was similar to riverscape genetic patterns seen in previously studied pleurocerids

(Whelan et al. 2019; Wright et al. 2020). This was surprising because the 2 previous population genomic studies on pleurocerids focused on species from one of the least modified major rivers in the southeastern United States. For example, *L. ampla* populations in tributaries of the Cahaba River in central Alabama displayed lower levels of genetic diversity, on average, than mainstem populations (Whelan et al. 2019). Furthermore, Wright et al. (2020) found that *Leptoxis compacta*, a species restricted to the mainstem Cahaba River, had relatively high levels of genetic diversity when compared with tributary populations of *L. ampla*, and our results show similarity with spatial genetic diversity in *P. foremani*. Thus, low genetic diversity of *P. foremani* in Yellowleaf Creek compared with Lake Mitchell and the Coosa River at Wetumpka is likely the result of natural processes. For example, our migrate-n analyses indicate that gene flow in *P. foremani* is biased toward downstream movement from the Yellowleaf Creek tributary to mainstem sites, like Lake Mitchell and the Coosa River at Wetumpka. Migration from mainstem sites to tributaries has also been shown to be rare in *L. ampla* (Whelan et al. 2019). Thus, the inferred lack of gene flow toward Yellowleaf Creek from other populations likely predates dam construction.

Broadly, our study adds to a growing body of evidence that pleurocerids do not conform to the Mighty Headwaters Hypothesis (Finn et al. 2011). All 3 population genomics studies done to date on pleurocerids suggest the opposite: both summary genetic diversity measurements like heterozygosity and allelic richness, and overall genomic diversity were higher in large rivers compared with headwaters (Table 2; Whelan et al. 2019; Wright et al. 2020). A similar pattern has been documented for the unionid mussel *Popenaias popeii* from the Rio Grande drainage in Texas (Inoue et al. 2015). The Mighty Headwaters Hypothesis could still explain diversity patterns in other macroinvertebrates, particular insects where migration patterns are expected to differ given that part of their life cycle is out of the water. Downstream-biased migration seen in pleurocerids likely explains the greater genetic diversity seen in downstream populations as downstream sites will have more migrants contributing to the gene pool. Therefore, we conclude that pleurocerids follow an upstream to downstream "source-sink" pattern of genetic diversity, similar to what has been documented in other freshwater taxa with downstream-biased migration (Hanfling and Weetman 2006; Barson et al. 2009; Blanchet et al. 2010; Yamamoto et al. 2019). Notably, our conclusions conflict with past inferences that pleurocerid movement was upstream biased (Houp 1970; Krieger and Burbank 1976; Huryn and Denny 1997; Stewart 2007), emphasizing the utility of population genomics data for revealing basic biology of understudied invertebrates. Future studies should assess whether any noninsect macroinvertebrates have greater diversity in headwaters compared to mainstem reaches. Such information is needed to inform management choices about which habitats deserve conservation priority given limited resources.

Pleurocera foremani has seen a massive range reduction in the last 150 years and observed riverscape genetic patterns offer no explanation for why *P. foremani* persists in Lake Mitchell but not the next upstream or downstream reservoirs (i.e., Lay Lake and Jordan Lake). Conservation efforts for *P. foremani* should emphasize maintaining genetic diversity and creating resiliency to extinction. Whereas *P. foremani* is widespread and genetically diverse in Lake Mitchell, all individuals from Lake Mitchell represent a single population. Furthermore, the species is geographically and genetically isolated in the Coosa River at Wetumpka and especially within Yellowleaf Creek (Figure 2 and Table 2). Given the relatively small geographic area of Yellowleaf Creek and the Coosa River at Wetumpka that is inhabited by *P. foremani* and the absence of current migration between populations, a single catastrophic event could cause considerable decline or extirpation. Such an event would result in a considerable loss of species-wide evolutionary potential. Unfortunately, management options for mitigating long-term effects of fragmentation that have been successful for more mobile species, like fish passage systems (Gouskov et al. 2015; Rougemont et al. 2021), will likely not be useful for *P. foremani*. Moreover, the Yellowleaf Creek population must be prioritized for protection to maintain evolutionary potential of *P. foremani*. A captive breeding and reintroduction program to reintroduce populations within the historic range of *P. foremani* from which the species has been extirpated, like at sites in Jordan Lake and in the middle the Coosa River, could mitigate risks of extinction. Before such efforts, habitat suitability at potential reintroduction sites would need to be assessed, and future studies on why *P. foremani* persists in Lake Mitchell but not in other Coosa River reservoirs would aid in such an effort.

This study provides the first genetic diversity estimates of *P. foremani*, which will be crucial for future conservation monitoring. With estimates of current diversity and relatedness of populations, managers can begin to make informed decisions about broodstock choice for reintroduction efforts and prioritize remaining populations. Protection of key areas like Yellowleaf Creek and the Coosa River at Wetumpka will be critical to the survival of *P. foremani*, and the Lake Mitchell population is likely a good broodstock choice given high genetic diversity and ease of access. Our analyses reject the Mighty Headwaters Hypothesis for *P. foremani* and support a broader pattern suggesting that it does not apply to pleurocerids. Furthermore, detrimental effects of fragmentation may accumulate more slowly in invertebrates with low dispersal abilities but large population sizes. Broadly, our study demonstrates the importance of including low-dispersing invertebrates, especially those in highly modified environments, when postulating about general expectations of riverscape genetics.

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Data Availability

All raw, demultiplexed sequencing data are available on NCBI under BioProject PRJNA771623. All assembled data used for data analyses in various file formats for each analysis are available on FigShare (DOI: 10.6084/m9.figshare.13705009).

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