Received: 16 October 2021 | Revised: 23 March 2022 | Accepted: 18 May 2022

DOI: 10.1002/aqc.3849

RESEARCH ARTICLE

WILEY

Population genomics reveal low differentiation and complex demographic histories in a highly fragmented and endangered freshwater mussel

Nicholas S. Gladstone[1] | Nicole L. Garrison[2] | Tim Lane[3] | Paul D. Johnson[4] | Jeffrey Garner[5] | Nathan V. Whelan[1,6]

[1]School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, Auburn, AL, USA

[2]Department of Biomedical Sciences, West Liberty University, West Liberty, WV, USA

[3]Aquatic Wildlife Conservation Center, Virginia Department of Wildlife Resources, Marion, VA, USA

[4]Alabama Aquatic Biodiversity Center, Alabama Division of Conservation and Natural Resources, Marion, AL, USA

[5]Alabama Department of Conservation and Natural Resources, Florence, AL, USA

[6]Southeast Conservation Genetics Laboratory, United States Fish and Wildlife Service, Auburn, AL, USA

Correspondence
Nicholas Gladstone, School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, Auburn, AL 36849, USA.
Email: nscottgladstone@gmail.com

Abstract

1. Freshwater mussels are an important element of freshwater biodiversity and provide essential ecosystem services. However, mussels are among the most imperilled groups of organisms in the world. Although research has increased in recent years, information about range-wide genetic diversity and historical demography of most species is lacking. One such species is Cumberlandian combshell *Epioblasma brevidens*, which is listed as endangered under the US Endangered Species Act.
2. Genetic diversity of *E. brevidens* was analysed using a high-resolution RADseq approach and included the previously overlooked Bear Creek population. Hypotheses were tested about population decline, comparative genetic diversity and population structure with model-based approaches enabled by a genome-scale dataset.
3. Estimates of genetic differentiation among populations of *E. brevidens* were lower than past analyses, suggesting higher historical population connectivity than previously known. Demographic analyses indicate relatively recent splits among *E. brevidens* populations in the late Pleistocene to early Holocene, with clear founder effects in two populations. The Clinch River population has the highest genetic diversity and effective population size, despite demographic analyses revealing decline of this population since the Pleistocene. Analyses of both population structure and migration show evidence of past gene flow, but all populations are currently isolated by artificial barriers.
4. Analyses indicate that populations began to decline before industrialization, but fragmentation and population extirpation has been exacerbated by modern habitat destruction. Relatively high genetic diversity in the Bear Creek population indicates that water quality improvements in the last 20 years have had a positive impact on population viability, offering promise for targeted management actions. In contrast, the Big South Fork population that has been presumed stable showed low genetic diversity and effective population size. Furthermore, genetic structure among sampled populations indicates that reintroduction efforts should use broodstock from as close to the reintroduction site as possible.

KEYWORDS
conservation, *Epioblasma*, genetic diversity, population connectivity, Unionidae

1 | INTRODUCTION

Freshwater rivers and lakes are considered among the most imperilled ecosystems globally, and many species have sustained steep declines in response to human demands for water resources (Strayer & Dudgeon, 2010). Given that freshwater biota often play important roles in maintaining the function and integrity of lotic and lentic systems, continued loss of freshwater biodiversity is of concern to societal wellbeing (Dudgeon et al., 2006; Reid et al., 2019). Freshwater mussels (order Unionida) are a considerably biodiverse group of freshwater organisms with more than 1,000 described species, and global hotspots of mussel diversity occur in the south-eastern USA (Graf & Cummings, 2007; Haag & Williams, 2014). Freshwater mussels have a unique life cycle that includes a parasitic larval stage (i.e. glochidia). As such, their biology, dispersal capabilities and demography are tied to their co-distributed freshwater vertebrate hosts, which are primarily fish. Furthermore, mussels provide an array of functional roles and ecosystem services such as nutrient recycling, habitat structuring and biofiltration (Vaughn, 2018). Unfortunately, North American mussel biodiversity is critically imperilled (Williams et al., 1993). Over 60% of the more than 300 freshwater mussel species in the USA and Canada are listed as threatened under NatureServe criteria (NatureServe, 2021), and over one-third of all species in the USA are listed as threatened or endangered under the US Endangered Species Act. Among the factors that have led to the decline of freshwater mussels are range fragmentations, extirpations or extinctions, in response to dam construction, channelization and pollution (Haag & Williams, 2014).

Active research and management of imperilled freshwater mussels increased in the USA after the development of a national strategy for mussel conservation in the late 1990s (National Native Mussel Conservation Committee, 1998; Freshwater Mollusk Conservation Society, 2016). However, population genetic studies of freshwater mussels have been limited, despite the importance of such research for informing conservation efforts (but see, for example, Berg, Christian & Guttman, 2007; Elderkin et al., 2008; Inoue et al., 2014). Nearly all population genetic studies of freshwater mussels have used allozyme markers or microsatellites, which can display higher error rates during genotyping, have limited capacity to detect fine-scale geographical patterns of genetic differentiation and possess increased susceptibility to homoplasy compared with next-generation sequencing approaches (Pasqualotto, Denning & Anderson, 2007; Jeffries et al., 2016; Garrison, Johnson & Whelan, 2021). Furthermore, larger sample sizes are generally needed to accurately assess allele frequency and genetic diversity of individual populations when using microsatellites (e.g., Hale, Burg & Steeves, 2012), which may be difficult to obtain for endangered species that are difficult to sample. Therefore, high-resolution population genetic studies that use genomic approaches are needed to better quantify geographical patterns of genetic variation, estimate genetic diversity across populations and examine demographic history, all of which are important to understanding freshwater mussel biology and conservation (Hohenlohe, Funk & Rajora, 2021).

Cumberlandian combshell, *Epioblasma brevidens*, is a freshwater mussel that is native to the Cumberland River and Tennessee River drainages (USFWS, 2004). This species was listed as endangered under the US Endangered Species Act in 1997 and Critically Endangered on the International Union for Conservation of Nature (IUCN) Red List in 2000 (USFWS, 2004; IUCN, 2021). Although *E. brevidens* was once distributed throughout the Tennessee and Cumberland River systems, extensive dam construction and subsequent habitat loss in the last century has led to a range reduction of more than 90% (USFWS, 2004). Similar range reductions have occurred for other *Epioblasma* species, and the genus has sustained more extinctions than any other freshwater mussel genus in North America (Williams et al., 1993). Only five disjunct populations of *E. brevidens* remain in: (i) Bear Creek in north-western Alabama and north-eastern Mississippi; (ii) Buck Creek and (iii) Big South Fork Cumberland River in north-central Tennessee and south-central Kentucky; and (iv) the Clinch River and (v) Powell River in north-eastern Tennessee and south-western Virginia (Figure 1; USFWS, 2004).

Past studies have indicated that the Clinch River maintains the highest census size of *E. brevidens* with estimates typically ranging from several thousand to >10,000 individuals (Lane et al., 2021). The other four populations are thought to be relatively small, possibly not exceeding a few thousand individuals (McGregor & Garner, 2003; Jones & Neves, 2011; Ahlstedt et al., 2016). Despite the impacts of adjacent coal mining operations, the Big South Fork Cumberland River population is considered stable owing to recent surveys finding evidence of reproduction and recruitment (Ahlstedt et al., 2005; USFWS, 2019). In contrast, the Bear Creek population is considered of questionable long-term viability because of complete isolation via dam construction and human disturbance from impacts such as gold and coal mining, agro-chemical runoff, and sedimentation (McGregor & Garner, 2003). Monitoring, propagation, translocation and reintroduction efforts for *E. brevidens* through the Kentucky Department of Fish and Wildlife Resources, the Virginia Department of Wildlife Resources, and the Tennessee Wildlife Resource Agency have been continuing since listing (USFWS, 2006; USFWS, 2019; Hubbs, 2020). However, such efforts have often not been informed by genetic data, which could limit their utility (Strayer et al., 2019).

Unlike most mussel species in the Tennessee and Cumberland River drainages, *E. brevidens* has been the focus of several demographic and population genetic studies (Jones & Neves, 2011; Jones, Neves & Hallerman, 2012; Jones, Neves & Hallerman, 2015;

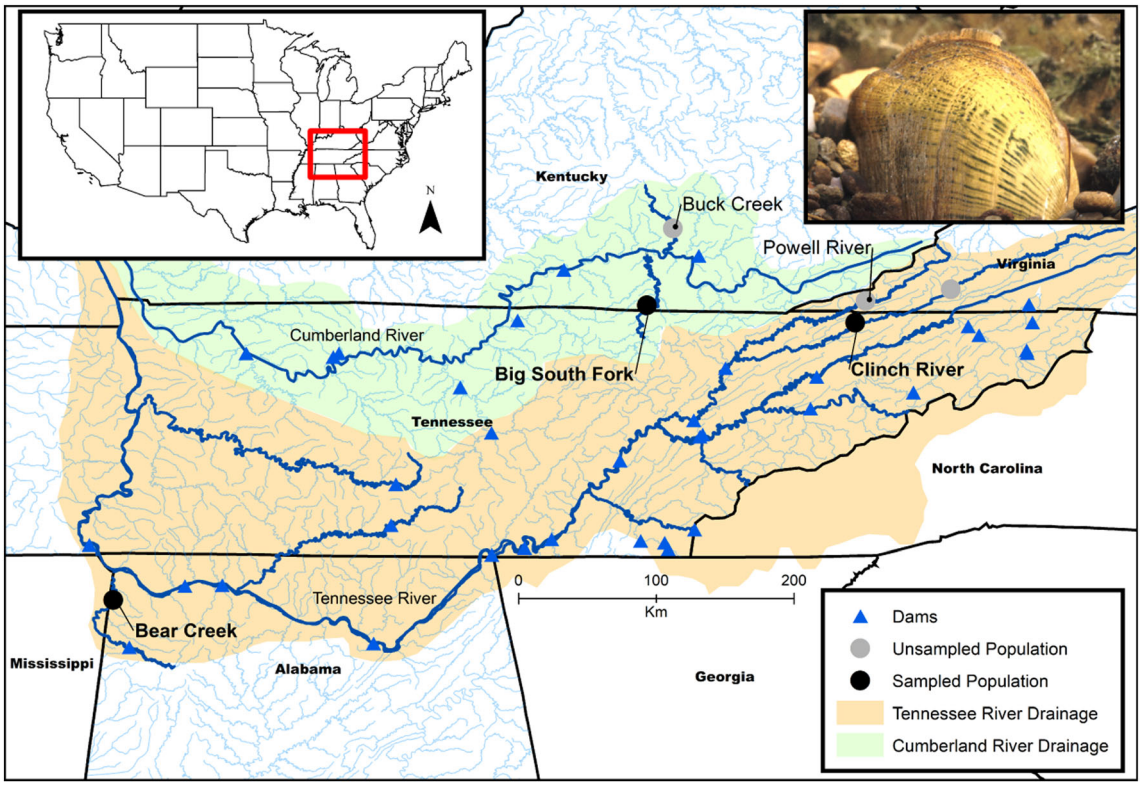

FIGURE 1 Geographical range of remaining *Epioblasma brevidens* populations and photograph of *E. brevidens* in the wild. Sampled populations, unsampled populations, dams and river drainages are delineated by colour

Jones et al., 2018; Lane et al., 2021). These studies generally indicate that *E. brevidens* has been declining steadily in the Clinch River since the late Pleistocene epoch in response to early human exploitation (Peacock, Haag & Warren, 2005; Jones, Neves & Hallerman, 2015). Census size (N_c) of *E. brevidens* in the Clinch River is probably lower than for other sympatric species, (Jones et al., 2014; Lane et al., 2021). For effective population size (N_e), past estimates in the Clinch River had a 95% confidence interval that spanned 941–2,823 individuals (Jones, Neves & Hallerman, 2015). Jones et al. (2021) also generated N_e estimates for *E. brevidens*, but the confidence intervals spanned infinity and as such the estimates are uninterpretable (Marandel et al., 2019). Compared with the Clinch River, Jones et al. (2021) indicated that *E. brevidens* in the Big South Fork Cumberland River and Powell River had smaller, less genetically diverse populations. Despite concerns regarding the long-term genetic viability of *E. brevidens* from Bear Creek in Alabama and Mississippi (McGregor & Garner, 2003), no previous genetic analysis has included individuals from this tributary. Thus, research is needed to assess genetic diversity of the Bear Creek population. Furthermore, high-resolution genomic markers offer promise for finer-scale and more accurate understanding of the molecular ecology of *E. brevidens* that will enable better understanding of population demography and better inform management efforts.

The goals of this study were to use a restriction-site associated DNA sequencing (RADseq) approach to assess population structure, genetic diversity and demographic history of *E. brevidens* in the Clinch River, Big South Fork Cumberland River and Bear Creek. Given past population genetic studies on *E. brevidens*, three hypotheses were investigated: (i) *E. brevidens* populations exhibit high genetic structure owing to geographical barriers, and that migration is limited within and across river drainages; (ii) the Clinch River population harbours greater genetic diversity and has higher N_e compared with other populations; and (iii) each population has undergone steady population decline. Population genetic patterns revealed here, especially regarding the previously unstudied Bear Creek population, will enhance understanding of population genetic patterns across the distribution of *E. brevidens*. Moreover, data from these analyses can be used to guide management and recovery efforts.

2 | METHODS

2.1 | Study design

2.1.1 | Sample collection and DNA sequencing

Individuals were collected from three locations: Bear Creek (BEA; n = 29); Clinch River (CLI; n = 14); and Big South Fork Cumberland River (BSF; n = 14) (Table 1). The sampling locations nearly span the full extent of the remaining range of *E. brevidens* (Figure 1). For practical purposes, collection sites are referred to as distinct populations. Sites were selected owing to high relative abundance of

TABLE 1 Population summary statistics of the *Epioblasma brevidens* populations, including sample size after filtering (N), private alleles (PA), allelic richness (AR), observed heterozygosity (H_o), expected heterozygosity (H_e), nucleotide diversity (Π), coefficient of inbreeding (F_{IS}) and effective population size (N_e)

Population	N	PA	AR (SD)	H_o (SD)	H_e (SD)	Π (SD)	F_{IS} (SD)	N_e (CI)
Big South Fork	14	1,454	1.516 (0.458)	0.150 (0.178)	0.171 (0.183)	0.178 (0.190)	0.082 (0.248)	59 (58.6–59.3)
Bear Creek	27	2,580	1.637 (0.400)	0.164 (0.154)	0.195 (0.169)	0.198 (0.172)	0.120 (0.241)	274.3 (262.9–286.6)
Clinch River	11	1,331	1.612 (0.438)	0.174 (0.179)	0.201 (0.179)	0.211 (0.188)	0.104 (0.284)	692.0 (653.2–735.5)

Abbreviations: CI, 95% confidence interval, SD, standard deviation.

E. brevidens, and prioritization of the previously unsampled BEA population. Mussels were collected from natural populations by hand while snorkelling. All individuals appeared to be adults, but some size variation was observed, indicating multiple age classes. Genetic material was obtained through a non-lethal foot swab with an Isohelix DNA buccal swab. Swabs were immediately placed into Isohelix BuccalFix Stabilization and Lysis Buffer. Individuals were returned to the point of collection after swabbing. All individuals were collected under required state and federal permits.

2.1.2 | Laboratory methods

DNA was extracted with the Isohelix Xtreme DNA isolation kit, which was previously shown to result in high-quality DNA from mussel foot swabs appropriate for RADseq (Garrison, Johnson & Whelan, 2021). After DNA extraction, samples were quantified using a Qubit Fluorometer and normalized to 20 ng μL^{-1}. Normalized DNA was treated with RNase A (ThermoFisher) by adding 100 $\mu g\ mL^{-1}$ to the sample and incubating the sample at 37°C for 15 min. DNA was sent to Floragenex for single enzyme RADseq. Reduced representation genomic libraries were prepared using the *Pst*I restriction enzyme following Baird et al. (2008). Size selection during library prep ranged from 300 to 500 bp. Samples were tagged with unique barcode identifiers and sequenced on three Illumina HiSeq 4,000 lanes using 150-bp paired-end chemistry.

2.2 | Data analysis

2.2.1 | Genomic data assembly

Raw sequence data were processed and assembled using STACKS v2.3 (Rochette, Rivera-Colón & Catchen, 2019), following the approach of Garrison, Johnson & Whelan (2021) and by trimming poorly sequenced individuals following Cerca et al. (2021). See Supporting Information for more details. Two datasets were then created using the STACKS *populations* program: one with multiple single nucleotide polymorphisms (SNPs) per locus (denoted 'EpioM') and one that only allowed for single SNPs per locus (denoted 'EpioS'). The EpioS dataset was used for downstream analyses that assumed unlinked loci, whereas the EpioM dataset was used for analyses that do not assume SNPs are unlinked. File formats for downstream analyses were produced directly by the *populations* program or converted using *PGDSpider* (Lischer & Excoffier, 2012).

2.2.2 | Genetic diversity and effective population size

The number of private alleles, observed heterozygosity, expected heterozygosity, nucleotide diversity, inbreeding coefficients (F_{IS}) and pairwise F_{ST} were calculated for the EpioM dataset in *populations*. The R package *diveRsity* (Keenan et al., 2013) was used to quantify allelic richness of each population. To estimate N_e, the EpioS dataset was used, separated by population with additional filtering steps using *GBS_SNP_Filter* v1.17 (available from http://github.com/laninsky/GBS_SNP_filter) and *BayeScan* v2.1 (Foll & Gaggiotti, 2008) to meet assumptions of the linkage disequilibrium method and to lower missing data. First, using *GBS_SNP_Filter*, only SNPs present within each population were used and loci that were not in Hardy–Weinberg equilibrium were removed using a *P*-value of 0.05 and an R^2 cutoff of 0.2. Then, to identify loci that may be under selection, *BayeScan* v2.1 (Foll & Gaggiotti, 2008) was used. *BayeScan* analyses were run with prior odds of neutrality at 1,000 and 10,000 owing to the large genetic structure expected among populations. All other *BayeScan* parameters were set to defaults. No loci were removed following the use of *BayeScan* as no loci were shown to be under selection. N_e estimates were calculated with the filtered dataset using *NeEstimator* v2 (Do et al., 2014) for each population independently with a minimum minor allele frequency critical value of 0.05.

2.2.3 | Population structure

Population structure was assessed with several methods. Using the EpioM dataset, analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992) was performed using the *poppr.amova* function in the R package *adegenet* (Jombart, 2008; Kamvar, Tabima & Grünwald, 2014). Individuals were stratified by population and whether they were in the Tennessee River drainage or Cumberland River drainage. Significance was tested with a 999-permutation randomization test. Using the EpioS dataset, potential genetic admixture was assessed using the sparse non-negative matrix factorization (sNMF) algorithm in the R package *LEA* (Frichot & François, 2015). This program is robust to violations of demographic

assumptions of Hardy–Weinberg and linkage equilibrium (Frichot et al., 2014). The number of best-fit clusters (K) was assessed by running 10 replicates of each K value from 1 to 5 and assessing fit with the cross-entropy criterion. The lowest cross-entropy score, averaged among runs for each K, was selected for downstream analysis. Genome ancestry coefficients with the best-fit K were visualized with *LEA* using the run with the lowest cross-entropy.

Discriminant analyses of principal components (DAPC) was performed using the EpioM dataset in *adegenet*, which does not make assumptions of underlying population genetic processes (e.g. linkage disequilibrium). The number of clusters was determined using K-means clustering and Bayesian information criteria (Supporting Information). To determine the optimum number of principal components to retain, the *optim.a.score* function in *adegenet* was used (Supporting Information). Genomic co-ancestry among individuals was also assessed using the EpioM dataset and *fineRADstructure* (Malinsky et al., 2018). This method can use linked SNPs, potentially allowing enhanced assessment of fine-scale patterns of relatedness among individuals (Malinsky et al., 2018). A co-ancestry matrix was inferred using the *RADpainter* script, and clustering was performed using the Markov chain Monte Carlo method with default parameters.

To test for a signature of isolation-by-distance (IBD) a Mantel test of correlation between the F_{ST} values generated by STACKS and the geographical distances between each population was done in the R package *ade4* (Dray & Dufour, 2007). To quantify the geographical distance between sites, river paths were measured directly in ArcMap v10.7.1 by ESRI. Significance of the Mantel test was evaluated with 1,000 permutations. A multiple regression on the F_{ST} and geographical matrices was also performed using the MRM function of the *ecodist* R package (Goslee & Urban, 2007). Significance of the multiple regression was evaluated with 10,000 permutations. Although MANTEL and MRM analyses may have limited statistical power with only three collection sites, a strong signal of IBD would still be possible to observe.

2.2.4 | Migration

The coalescence-based program *MIGRATE* v.3.7.2 (Beerli & Palczewski, 2010) was used with the EpioM dataset to explicitly test different models of migration and population admixture (see Table 2). Given large computational requirements of *MIGRATE*, 99 polymorphic loci present in 100% of individuals were randomly selected from the EpioM dataset for use. The best-fit migration model was determined by calculating Bayes factors using *BF.py* in *MIGRATE* with the marginal likelihood of each model, which was measured with thermodynamic integration (Beerli & Palczewski, 2010). Additional details on *MIGRATE* analyses are in Supporting Information.

2.2.5 | Demographic history

To estimate the demographic history of the three *E. brevidens* populations, the ordinary differential equations method of modelling

TABLE 2 Models assessed by *MIGRATE*, sorted by model fit. Log Bayes factors were used to rank models

Description	Log Marginal Likelihood	Log Bayes Factor	Rank
Downstream migration from Clinch River to Bear Creek	−81372.79	0	1
Bidirectional migration from Bear Creek and Clinch River	−81607.48	−234.69	2
Bear Creek and Clinch River panmictic; migration from the Tennessee River drainage to the Cumberland River drainage	−82013.84	−641.05	3
Bear Creek and Clinch River panmictic; migration from the Cumberland River drainage to the Tennessee River drainage	−82035.62	−662.83	4
Bidirectional migration from Bear Creek and Clinch River; migration from Big South Fork to Clinch River	−82059.7	−686.91	5
Downstream migration from Clinch River to Bear Creek; migration from Big South Fork to Clinch River	−82078.01	−705.22	6
Downstream migration from Clinch River to Bear Creek; migration from Clinch River to Big South Fork	−82090.79	−718	7
Bidirectional migration from Bear Creek and Clinch River; migration from Clinch River to Big South Fork	−82112.96	−740.17	8
Upstream migration from Bear Creek to Clinch River	−82120.23	−747.44	9
Bidirectional migration from Bear Creek and Clinch River; bidirectional migration from Clinch River and Big South Fork	−82146.37	−773.58	10
Downstream migration from Clinch River to Bear Creek; bidirectional migration from Clinch River and Big South Fork	−82157.31	−784.52	11
Full migration	−82201.93	−829.14	12

the evolution of allele frequencies was applied in the *moments* software (Jouganous et al., 2017) implemented in the program *GADMA* (Noskova et al., 2020). *GADMA* applies a heuristic genetic algorithm for global optimization of parameter values to infer automatically the best demographic model given the joint allele frequency spectrum, an initial demographic model structure, and a final demographic model structure (Noskova et al., 2020). For *GADMA* analyses, a reduced EpioS dataset was created in *populations* with a 100% missingness threshold and a minimum minor allele frequency of 0.01 because site frequency spectrum methods perform best with information on rare alleles (details in Supporting Information). Three demographic models were inferred with generation times of 4.0, 6.0 and 8.0 years for *E. brevidens*. Three generation times were run because of uncertainty in the generation times of *E. brevidens*. The generation time of *E. penita* has been estimated to be 6 years (Jones & Neves, 2011), but *E. penita* can reach sexual maturity in 3 years, at least in captivity (P. Johnson, unpublished data). While using *E. penita* as a proxy, running analyses with a range of generation times allows uncertainty in generation times to be captured for *E. brevidens*. Each demographic model was run with 50 repeats. Although the demographic modelling produces a time estimate of events, the inherent bias of using variable sites, in addition to having no accurate estimate of theta available (i.e. the expected number of mutations that occur in one chromosome in one generation in the infinite-sites model), may cause potential error in absolute timing of demographic events (Noskova et al., 2020). Thus, although the exact time intervals produced by *GADMA* are reported, we only make conclusions in regard to broad geological epochs.

3 | RESULTS

3.1 | Genomic data assembly

The 57 sequenced individuals had an average of 25,825,582 reads (range 2,275,874 – 47,770,067) retained of the total ~1.5 billion raw reads. Sequence reads filtered out during demultiplexing comprised ~1.6% of the raw reads. Upon obtaining the initial assembly, five individuals (two from BEA, three from CLI) were removed after not passing the <50% missingness threshold, which left 52 individuals in the final datasets. Filtering implemented in *populations* resulted in 16,556 loci with one SNP in the EpioS dataset. When allowing multiple SNPs per locus in the EpioM dataset, 28,954 SNPs were assembled with an average of 1.42 SNPs per locus.

3.2 | Genetic diversity and effective population size

The number of private alleles present in each population ranged from 1,331 in CLI to 2,580 in BEA (Table 1). Observed heterozygosity was lower than expected heterozygosity across all populations (H_o = 0.15–0.17; H_e = 0.17–0.20). Average nucleotide diversity was highest in CLI (0.21) and lowest in BSF (0.18), whereas BEA had the highest allelic richness (1.64) and BSF had the lowest (1.52). The separation of each population and additional filtering procedures for estimating N_e resulted in datasets comprising 21,083, 4,249 and 29,073 loci for the BSF, BEA and CLI populations, respectively. The lowest estimate of N_e was the BSF population at 59 (95% confidence interval 58.6–59.3), whereas the N_e estimate of the CLI population was 692 (95% confidence interval 653.2–735.5; Table 1).

3.3 | Population structure

F_{ST} values (Table 3) were similar (range 0.07–0.11) to freshwater mussels with much smaller geographical ranges than *E. brevidens* (e.g. *Margaritifera hembeli*; Garrison, Johnson & Whelan, 2021). F_{IS} estimates were low (range 0.08–0.12), being highest in BEA (Table 1). AMOVA was significant at all hierarchical levels ($P < 0.001$); 8.41% of genetic variation was explained by river drainage, 13.72% of the variation was explained by population, and 64.24% of variation was explained by within-individual variability (Table 4). Both the Mantel

TABLE 3 Pairwise F_{ST} (above diagonal) and geographical distance (river km) between populations in km (below diagonal)

	Big South Fork, KY	Bear Creek, AL	Clinch River, TN
Big South Fork, KY	-	0.09762	0.1125
Bear Creek, AL	1,064.30	-	0.07144
Clinch River, TN	1,779.70	747.8	-

TABLE 4 Analysis of molecular variance (AMOVA) showing genetic variation among and within populations nested by major river drainage (i.e. Tennessee River or Cumberland River)

Source of variation	df	Sum sq	% total variation	Φ-statistic	*P*-value
Between major river drainage	1	36645.91	8.41%	0.0841	0.001
Between population within major river drainage	1	21638.22	13.72%	0.1498	0.001
Between samples within population	49	186370.51	13.63%	0.1751	0.001
Within samples	52	138852.15	64.24%	0.3576	0.001

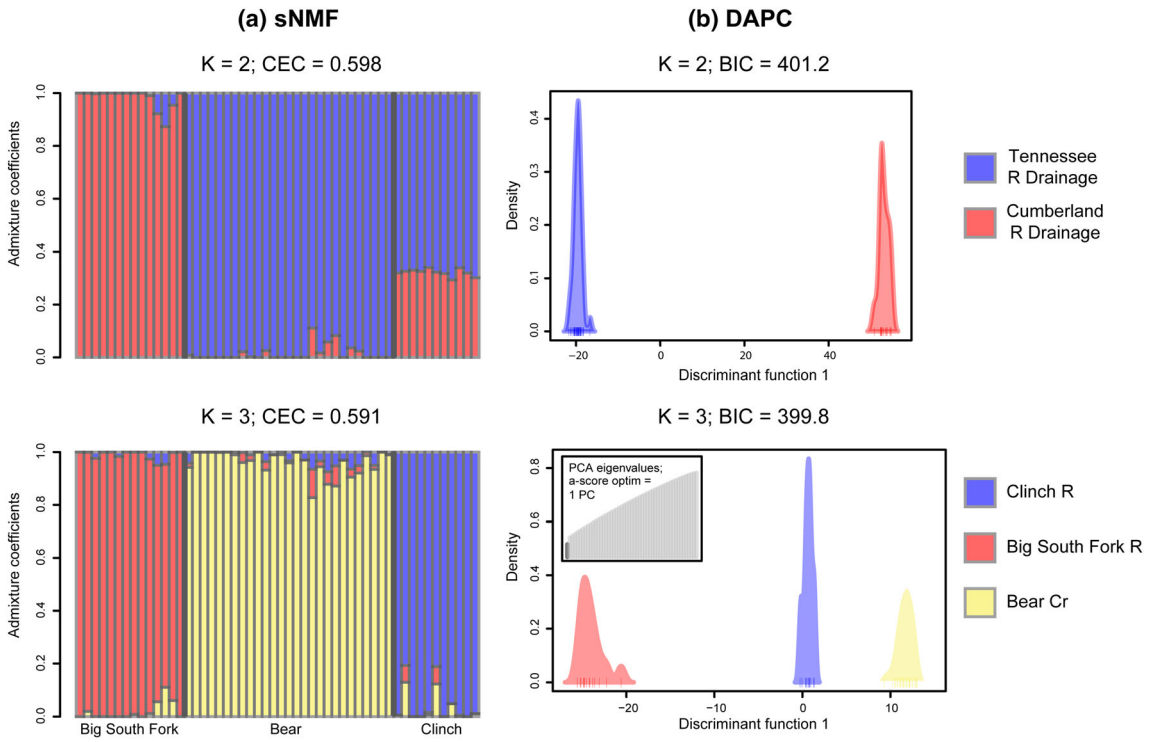

FIGURE 2 (a) Discriminant analysis of principal components (DAPC) and (b) genetic admixture inferred by the sparse non-negative matrix factorization (sNMF) algorithm implemented LEA. Populations are coloured by population. Top: sNMF and DAPC using the second-best-K (K = 2). Bottom: sNMF and DAPC using the best-fit K (K = 3)

test and multiple regression for IBD were not significant ($P > 0.05$), indicating a lack of IBD, but the pattern could be obscured by the low number of sites.

Both sNMF and DAPC analyses indicated that the best-fit number of genetic clusters was three (K = 3; Figure 2). However, these methods revealed differing depictions of genetic admixture, and K = 3 fit the data only narrowly better than K = 2 for both analyses (Figure 2, Supporting Information). DAPC shows three distinct clusters with no evidence of admixture at K = 3, whereas all samples from the Tennessee River drainage are clustered together and distinct from the Cumberland River drainage at K = 2. In contrast, the sNMF analysis shows admixture of approximately 65% between the two Tennessee River drainage populations at K = 2, as well as admixture of up to approximately 35% between BSF and CLI (Figure 2). At K = 3, sNMF shows population structure among each site with little to no admixture among populations (Figure 2). The *fineRADstructure* analyses also inferred three distinct genetic clusters representing each population with greatest genetic similarity between the two Tennessee River drainage populations (Figure 3).

3.4 | Migration and demographic history

Among the 12 *MIGRATE* models assessed, the model of no migration among BSF and the other sites coupled with downstream migration from the CLI to BEA was the best fit. The second best-fit model had no migration among BSF and other sites and bidirectional migration in the Tennessee River drainage (Table 2).

The best demographic models inferred by *GADMA*, for each generation time used, have an initially low N_e beginning in the late Pleistocene followed by a population split between the Tennessee and Cumberland River drainages. The demographic model with a generation time of 6.0 years is shown in Figure 4, and each additional model is available within the Supporting Information. Following the initial split between the two major river drainages, the ancestral population in the Tennessee palaeodrainage expanded substantially. Upon the splitting of the two Tennessee River drainage populations, the CLI population maintained a higher N_e followed by constant, linear decline to the present, whereas the BEA population began as a much smaller population followed by gradual increase in N_e to the present. These results were consistent for each generation time model. In contrast, for the 3.0-year generation time model, upon splitting away from the Tennessee River populations, the BSF population showed maintenance of a similarly small N_e to the BEA population, followed by population growth into the early Holocene, followed then by population decline into the present. For the 6.0-year generation time model BSF underwent a decline following the split from the Tennessee River drainage ancestral population that was later followed by gradual population growth beginning in the late Pleistocene. In both models, all three populations were inferred to have gene flow events among each other after splitting (Figure 4; Supporting Information).

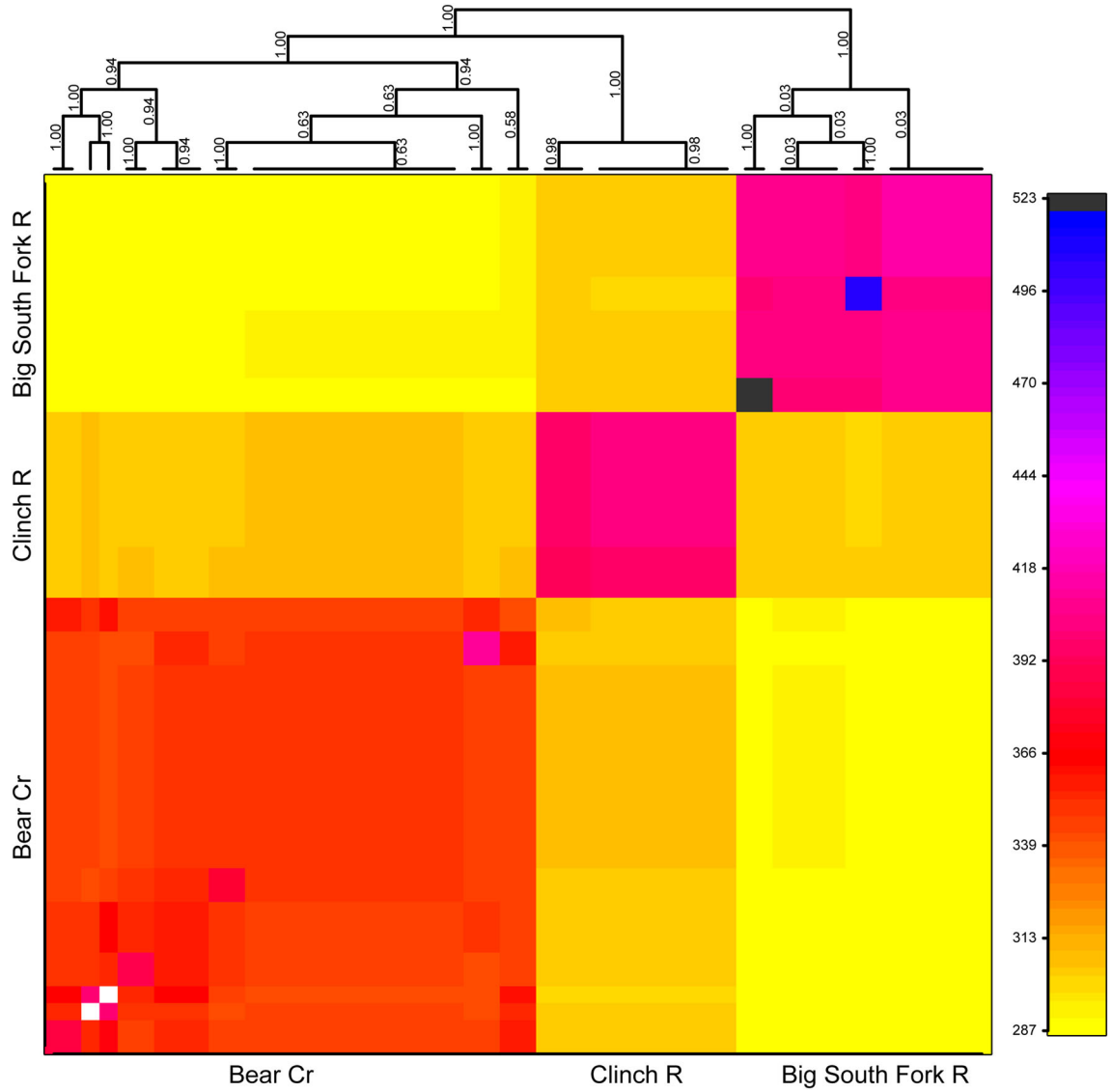

FIGURE 3 Hierarchical heatmap and inferred simple tree generated by fineRADstructure of all individuals from the three sampled populations. Colours represent relative co-ancestry values. Numerical values next to each branch of the tree are posterior population assignment probabilities

4 | DISCUSSION

By using high resolution genomic data, novel insights were generated into *E. brevidens* genetic diversity, population differentiation, migration patterns and demography. Despite high population fragmentation and human disturbance, all *E. brevidens* populations sampled exhibited similar or higher levels of genetic diversity compared with other freshwater mussel species for which RADseq data have been generated (Garrison, Johnson & Whelan, 2021; Kim & Roe, 2021; Meyer, 2021). However, the CLI population has higher genetic diversity and N_e relative to other sampled *E. brevidens* populations (Table 1), corroborating past studies. Compared with previous population genetic analyses of *E. brevidens* with mitochondrial and microsatellite data, estimates of genetic differentiation among populations were lower, suggesting higher historical population connectivity than previously known. Although analyses of both population structure and migration show evidence of past gene flow, contemporary river fragmentation and impoundments have isolated each population. Past studies of the CLI population inferred continual population decline since the Pleistocene epoch (Peacock, Haag & Warren, 2005; Jones, Neves & Hallerman, 2015), and demographic inferences corroborate these findings (Figure 4). In addition, analysis of the BEA population shows population growth through time, whereas the BSF population was inferred to have either historical population growth followed by more recent decline or the inverse. Analyses done here also indicate a relatively recent split among *E. brevidens* populations in the late Pleistocene to early Holocene, which may contribute to the low genetic differentiation despite high geographical separation.

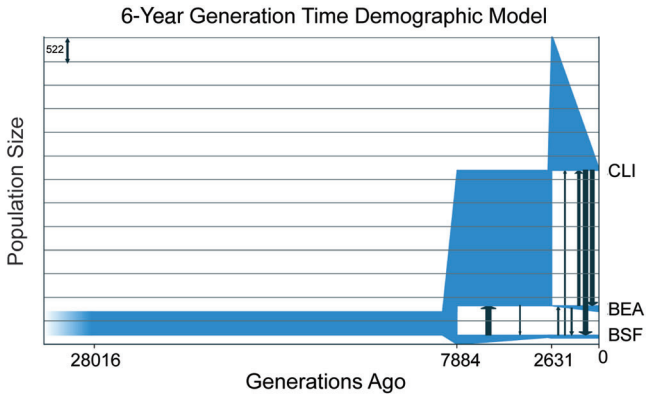

FIGURE 4 Demographic history for the three *Epioblasma brevidens* populations inferred by GADMA. Demographic model of the Clinch River (CLI), Bear Creek (BEA), and Big South Fork (BSF) populations. Values on y-axis correspond to inferred effective population size; values on x-axis correspond to generation time before present. Thickness of black arrows between populations indicates migration rates

4.1 | Genetic diversity and effective population size

Previous studies using mitochondrial and microsatellite data reported that the CLI population of *E. brevidens* had less genetic diversity than other sympatric species (i.e. *Epioblasma capsaeformis*, *Lampsilis fasciola*; Jones, Neves & Hallerman, 2015; Jones et al., 2021). However, estimates of genetic diversity for all three *E. brevidens* populations were similar, or higher, than other freshwater mussel species (Garrison, Johnson & Whelan, 2021; Kim & Roe, 2021; Meyer, 2021). For example, *E. brevidens* exhibits higher allelic richness and heterozygosity than *Cyprogenia stegaria*, *Cyprogenia aberti* or *Dromus dromas*, which have all also experienced declines due to river fragmentation and human disturbance (Kim & Roe, 2021). Furthermore, all three *E. brevidens* populations had higher genetic diversity than any population of *M. hembeli* analysed by Garrison, Johnson & Whelan (2021). Although the above comparisons do not include other CLI species given difficulties comparing absolute genetic diversity estimates made with microsatellites versus SNPs, genetic diversity of the three populations sampled here suggest that *E. brevidens* may not be as imperilled as other, similarly restricted freshwater mussels. Nevertheless, all three populations had lower observed heterozygosity than expected heterozygosity (Table 1), suggesting that inbreeding or genetic drift could be having an impact. However, estimates of F_{IS} were low for all populations compared with other freshwater mussels (Garrison, Johnson & Whelan, 2021; Kim & Roe, 2021), so inbreeding is likely to be less of a factor for *E. brevidens* than at least some other imperilled mussels.

The CLI population has been the focus of extensive traditional surveys, and N_c estimates range from a few thousand to over 10,000 (Jones & Neves, 2011; Lane et al., 2021), but the most recent estimates of N_e with genetic data were potentially inaccurate as they had confidence intervals that were either large or spanned infinity (Jones, Neves & Hallerman, 2015; Jones et al., 2021). In contrast, precise estimates (95% confidence intervals spanning less than 101) with non-infinite confidence intervals for each sampled population were generated here (Table 1). That said, the method used to obtain N_e estimates may be downwardly biased because *E. brevidens* has overlapping generations (Waples, Larson & Waples, 2016), and as such the N_e estimates are best interpreted in a comparative, rather than absolute, context. The N_e estimates corroborate past hypotheses that the CLI population is larger than other remaining *E. brevidens* populations (Jones et al., 2021). Furthermore, these estimates provide reliable baseline data that can be used for long-term monitoring in the face of human stressors or population improvements as a result of management actions.

4.2 | Population structure and historical demography

Despite considerable geographical separation among the three *E. brevidens* populations, estimates of population differentiation are similar to freshwater mussels with much smaller ranges. For example, the freshwater mussel *M. hembeli* had pairwise F_{ST} estimates of 0.043–0.082 among populations separated by a stream distance of less than 105 km (Garrison, Johnson & Whelan, 2021), whereas *E. brevidens* shows pairwise F_{ST} of 0.071 between the two Tennessee River drainage populations that are separated by a stream distance of ~750 km. This may be attributed to higher historical connectivity among *E. brevidens* populations compared with *M. hembeli* or differences in life history. Even between The Tennessee and Cumberland River drainages, *E. brevidens* shows relatively low pairwise F_{ST} (<0.112 Table 3).

Analyses of population structure generally depict three genetic populations; however, the two clusters model had only a slightly worse fit to the data. When $K = 2$ there was much higher admixture detected between the BSF and CLI populations compared with BSF and BEA. The $K = 2$ analyses may therefore suggest historical connectivity between the Upper Tennessee and Cumberland, which is also supported by the historical fluxes of migration from the CLI to BSF inferred by GADMA. Previous studies have suggested that the Powell River (which also maintains a contemporary *E. brevidens* population) was once connected to the Cumberland River before being captured by a CLI tributary and diverting to the Tennessee River drainage (Ross, 1972; Starnes & Etnier, 1986), which would potentially explain the observed pattern. However, geological and phylogeographical evidence generally refutes the hypothesis that the Upper Tennessee and Cumberland were connected, despite faunal similarities (Ross, 1971; Starnes & Etnier, 1986; Kozak, Blaine & Larson, 2006). Thus, it is unclear how dispersal events occurred for *E. brevidens* between river drainages following the evolution of the contemporary Tennessee River drainage beyond fish migration through the geographically proximate mouths of both the Tennessee and Cumberland rivers at the confluence of the Ohio River. Historical connectivity among populations may also be obscured by the inability

to sample extirpated populations, and routes of connectivity between other parts of the Tennessee and Cumberland River drainages (e.g. between Duck River and Harpeth or Collins rivers) may have existed. Owing to the limited study of freshwater invertebrate phylogeography in the Cumberland and Tennessee River drainages, we advocate for greater taxon sampling and the use of similarly high-resolution data to address these questions.

Dispersal capability of *E. brevidens* is directly related to that of its host fish, so fish movement patterns may be driving the downstream-biased migration inferred by *MIGRATE*. However, movement studies of putative *E. brevidens* host fish in the genera *Percina* and *Etheostoma* do not indicate significant differences in upstream and downstream dispersal (Schwalb, Poos & Ackerman, 2011; Roberts, Angermeier & Hallerman, 2016). Other studies have shown limited upstream migration bias in *Cottus* sculpins (McCleave, 1964; Breen et al., 2009; Lamphere & Blum, 2012), which *E. brevidens* can use as a host (Yeager & Saylor, 1995). *Percina* are probably the primary host used by *E. brevidens* in the wild, as the violent capture event that *E. brevidens* uses to parasitize fish will often kill *Etheostoma* spp., and *Cottus* heads may be too wide to be easily captured (Barnhart, Haag & Roston, 2008). As such, it is likely that past migration was tied to *Percina* movement patterns.

The stepping-stone model of dispersal (Kimura & Weiss, 1964) has been shown to provide the best explanation of population structure in other freshwater mussels, including in *E. triquetra* (Berg, Christian & Guttman, 2007; Beaver, Woolnough & Zanatta, 2019). Movement by host fish among suitable habitat patches for *E. brevidens* in the Tennessee River prior to dam construction may have similarly facilitated stepping-stone migration of *E. brevidens*. However, such a pattern would be obscured as mainstem Tennessee River and Cumberland River populations of *E. brevidens* have been extirpated. Thus, extirpated populations (i.e. 'ghost' populations) could be biasing migration inference (Beerli, 2004; Delser et al., 2019) and also obscuring a historical pattern of IBD. Nevertheless, both analyses of genetic structure and migration analyses indicate population fragmentation, and, if migration between sites is currently occurring, it must be rare.

Demographic modelling suggests a geologically recent division between *E. brevidens* in the Tennessee and Cumberland River drainage that occurred in the late Pleistocene (Figure 4). This finding corroborates previous molecular clock estimates for *E. brevidens* and mirrors patterns seen in some fish (Berendzen, Gamble & Simons, 2008; Hollingsworth & Near, 2009; Jones, Neves & Hallerman, 2015). Notably, both BEA and BSF populations had a founder effect followed by gradual population growth after splitting. BEA was inferred to continue expansion until present day in both *GADMA* models, which may not accurately reflect recent decline in the last century (McGregor & Garner, 2003). Recent and severe decline may, in fact, also be why BEA is inferred to have such a small population size since the split of CLI and BEA. The concurrent decline or limited population growth of the CLI and BSF populations beginning in the late Pleistocene to early Holocene is possibly related to the advent of using freshwater mussels as a food resource by indigenous peoples (Peacock, Haag & Warren, 2005; Jones, Neves & Hallerman, 2015). Inference from relict shell deposits show that freshwater mussels, particularly *Epioblasma*, have sustained deleterious impacts from early civilizations manipulating river-adjacent landscapes for agricultural development and from use as food resources and shell harvesting (Peacock, Haag & Warren, 2005). Continued human pressure until now has only worsened the conditions for remaining *E. brevidens* (McGregor & Garner, 2003; USFWS, 2006; Ahlstedt et al., 2016), which is reflected by the inferred sustained decline of N_e to the present day in CLI and BSF (Figure 4).

4.3 | Conservation implications

Epioblasma brevidens is at high risk of extinction given recent extirpations and the persistence of only a few fragmented populations. However, data generated here indicate that genetic diversity is maintained within the remaining populations. These analyses also provide important information about relative population sizes, particularly for the previously understudied BEA population. Such information is essential for long-term monitoring, conservation prioritization and planning practical management actions. The generation of genomic data for *E. brevidens* will provide reliable comparative material for other freshwater mussel species to identify species of conservation concern and broadly enhance the understanding of population genetic patterns in freshwater mussels. Moreover, although the following information is relevant for guiding management for *E. brevidens*, the methods used in this study and their implications can be applied to other freshwater fauna of conservation concern.

Captive propagation is an increasingly common conservation tool for freshwater mussels, but care must be taken that reintroductions accomplish recovery goals without unintended consequences (Strayer et al., 2019). Many freshwater species display an IBD pattern, leading to recommendations that source populations for reintroduction through captive propagation or translocation come from as close to the reintroduction site as possible (IUCN/SSC, 2013; Strayer et al., 2019; Garrison, Johnson & Whelan, 2021). Although IBD was not detected in *E. brevidens*, we also recommend that source populations come from as close to reintroduction sites as possible because *E. brevidens* has strong genetic structure, and a pattern of IBD may be obscured by the inability to sample extirpated populations.

A recent status review of *E. brevidens* reported that the BSF population is stable and potentially growing (USFWS, 2019), but conservation genomic analyses indicate that BSF has the least genetic diversity and smallest N_e of sampled populations. The immediate cause for this lower genetic diversity is unknown. Demographic analysis showed that the BSF population had very large reductions in N_e starting around the beginning of the Holocene, but this is also true for the CLI population. The F_{IS} estimate for BSF was the lowest among all sampled populations, suggesting that inbreeding is not likely

to be causing a reduction of genetic diversity. Nevertheless, the data indicate that the BSF population may be of greatest concern for decline. In addition to monitoring efforts within the BSF drainage by the Kentucky Department of Fish and Wildlife Resources, more than 2,000 individuals have been propagated and released back into the BSF since 2015, and we advocate continued propagation with broodstock from the Cumberland River drainage. Data generated here should also serve as a baseline goal for genetic diversity at reintroduction sites. Inferred genetic diversity and N_e suggest that the BEA population is in better shape than previously indicated (McGregor & Garner, 2003), which could be a result of recent improvements to water quality in Bear Creek (ADEM, 2014). Given these findings, we suggest that propagation efforts could begin using the BEA population as a genetically diverse broodstock to re-establish mussel beds in other portions of Bear Creek drainage in Alabama and Mississippi and possibly other geographically proximate sites within the historical range of *E. brevidens*.

The Clinch River has been referred to as "arguably the most important river for freshwater mussel conservation in the United States" (Zipper et al., 2014), and the high genetic diversity of the CLI population indicates that the population is essential for long-term survival and maintaining evolutionary potential of *E. brevidens*. The Virginia Department of Wildlife Resources and the Tennessee Wildlife Resource Agency have worked to monitor existing populations as well as propagate and release thousands of *E. brevidens* CLI broodstock back into several sites within the Clinch River (USFWS, 2019; Hubbs, 2020). CLI-sourced individuals have also been used in reintroduction efforts at several other historical sites, including in the Duck River, Elk River and Nolichucky River (USFWS, 2006; Hubbs, 2020). The use of the most genetically diverse possible broodstock, which would come from the Clinch River, has potential advantages for long-term survival of *E. brevidens*. However, the Duck and Elk rivers are more geographically proximate to the BEA population, and the use of BEA broodstock probably should have been prioritized under widely accepted best practices for introductions (IUCN/SSC, 2013; McMurray & Roe, 2017). Future efforts should consider whether using CLI or BEA broodstock is ideal for the target reintroduction site based upon geographical proximity between the reintroduction sites and potential broodstock source.

ACKNOWLEDGEMENTS

Funding was provided by a USFWS 1311 System-wide Funding Competitive Grant to N.V.W. We thank Jess Jones, Monte McGregor and Jessie Holifield for assistance with sample collection. David Zanatta, Philip Boon and an anonymous reviewer provided comments that improved the manuscript. The findings and conclusions in this article are those of the authors and do not necessarily represent the U.S. Fish and Wildlife Service.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare associated with this research.

DATA AVAILABILITY STATEMENT

Raw, demultiplexed sequence data are available from NCBI SRA under BioProject PRJNA819001. Other data used for each analysis are available from FigShare (https://figshare.com/s/d9281418c302b8731dee). R code available at https://github.com/ngladstone/Epioblasma-brevidens-population-genomics.

ORCID

Nicholas S. Gladstone https://orcid.org/0000-0001-5799-9992

REFERENCES

Ahlstedt, S.A., Bakaletz, S., Fagg, M.T., Hubbs, D., Treece, M.W. & Butler, R.S. (2005). Current status of freshwater mussels (Bivalvia: Unionidae) in the Big South Fork National River and Recreation Area of the Cumberland River, Tennessee and Kentucky (1999-2002) (evidence of faunal recovery). *Walkerana*, 14(31), 33–77.

Ahlstedt, S.A., Fagg, M.T., Butler, R.S., Connell, J.F. & Jones, J.W. (2016). Quantitative monitoring of freshwater mussel populations from 1979–2004 in the Clinch and Powell Rivers of Tennessee and Virginia, with miscellaneous notes on the fauna. *Freshwater Mollusk Biology and Conservation*, 19(2), 1–18. https://doi.org/10.31931/fmbc.v19i2.2016.1-18

Alabama Department of Environmental Management (ADEM). (2014). Delisting decision for Bear Creek assessment unit ID # AL06030006–0103-103 metals (Aluminum). Montgomery, AL: ADEM Water Quality Branch.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A. et al. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE*, 3(10), e3376. https://doi.org/10.1371/journal.pone.0003376

Barnhart, M.C., Haag, W.R. & Roston, W.N. (2008). Adaptations to host infection and larval parasitism in Unionoida. *Journal of the North American Benthological Society*, 27(2), 370–394. https://doi.org/10.1899/07-093.1

Beaver, C.E., Woolnough, D.A. & Zanatta, D.T. (2019). Assessment of genetic diversity and structure among populations of *Epioblasma triquetra* in the Laurentian Great Lakes drainage. *Freshwater Science*, 38(3), 527–542. https://doi.org/10.1086/704886

Beerli, P. (2004). Effect of unsampled populations on the estimation of population sizes and migration rates between sampled populations. *Molecular Ecology*, 13(4), 827–836. https://doi.org/10.1111/j.1365-294X.2004.02101.x

Beerli, P. & Palczewski, M. (2010). Unified framework to evaluate panmixia and migration direction among multiple sampling locations. *Genetics*, 185(1), 313–326. https://doi.org/10.1534/genetics.109.112532

Berendzen, P.B., Gamble, T. & Simons, A.M. (2008). Phylogeography of the bigeye chub *Hybopsis amblops* (Teleostei: Cypriniformes): early Pleistocene diversification and post-glacial range expansion. *Journal of Fish Biology*, 73(8), 2021–2039. https://doi.org/10.1111/j.1095-8649.2008.02046.x

Berg, D.J., Christian, A.D. & Guttman, S.I. (2007). Population genetic structure of three freshwater mussel (Unionidae) species within a small stream system: Significant variation at local spatial scales. *Freshwater Biology*, 52(8), 1427–1439. https://doi.org/10.1111/j.1365-2427.2007.01756.x

Breen, M.J., Ruetz, C.R., Thompson, K.J. & Kohler, S.L. (2009). Movements of mottled sculpins (*Cottus bairdii*) in a Michigan stream: How restricted are they? *Canadian Journal of Fisheries and Aquatic Sciences*, 66(1), 31–41. https://doi.org/10.1139/F08-189

Cerca, J., Maurstad, M.F., Rochette, N.C., Rivera-Colón, A.G., Rayamajhi, N., Catchen, J.M. et al. (2021). Removing the bad apples: A

simple bioinformatic method to improve loci-recovery in de novo RADseq data for non-model organisms. *Methods in Ecology and Evolution*, 12(5), 805–817. https://doi.org/10.1111/2041-210X.13562

Delser, P.M., Corrigan, S., Duckett, D., Suwalski, A., Veuille, M., Planes, S. et al. (2019). Demographic inferences after a range expansion can be biased: The test case of the blacktip reef shark (*Carcharhinus melanopterus*). *Heredity*, 122(6), 759–769. https://doi.org/10.1038/s41437-018-0164-0

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J. & Ovenden, J.R. (2014). NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources*, 14(1), 209–214. https://doi.org/10.1111/1755-0998.12157

Dray, S. & Dufour, A.-B. (2007). The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22(4), 1–20. https://doi.org/10.18637/jss.v022.i04

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C. et al. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews*, 81(2), 163–182. https://doi.org/10.1017/S1464793105006950

Elderkin, C.L., Christian, A.D., Metcalfe-Smith, J.L. & Berg, D.J. (2008). Population genetics and phylogeography of freshwater mussels in North America, *Elliptio dilatata* and *Actinonaias ligamentina* (Bivalvia: Unionidae). *Molecular Ecology*, 17(9), 2149–2163. https://doi.org/10.1111/j.1365-294X.2008.03745.x

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(2), 479–491. https://doi.org/10.1093/genetics/131.2.479

Foll, M. & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics*, 180(2), 977–993. https://doi.org/10.1534/genetics.108.092221

Freshwater Mollusk Conservation Society. (2016). A national strategy for the conservation of native freshwater mollusks. *Freshwater Mollusk Biology and Conservation*, 19(1), 1–21. https://doi.org/10.31931/fmbc.v19i1.2016.1-21

Frichot, E. & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution*, 6(8), 925–929. https://doi.org/10.1111/2041-210X.12382

Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G. & François, O. (2014). Fast and efficient estimation of individual ancestry coefficients. *Genetics*, 196(4), 973–983. https://doi.org/10.1534/genetics.113.160572

Garrison, N.L., Johnson, P.D. & Whelan, N.V. (2021). Conservation genomics reveals low genetic diversity and multiple parentage in the threatened freshwater mussel, *Margaritifera hembeli*. *Conservation Genetics*, 22(2), 217–231. https://doi.org/10.1007/s10592-020-01329-8

Goslee, S.C. & Urban, D.L. (2007). The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, 22(1), 1–19. https://doi.org/10.18637/jss.v022.i07

Graf, D.L. & Cummings, K.S. (2007). Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). *Journal of Molluscan Studies*, 73(4), 291–314. https://doi.org/10.1093/mollus/eym029

Haag, W.R. & Williams, J.D. (2014). Biodiversity on the brink: An assessment of conservation strategies for North American freshwater mussels. *Hydrobiologia*, 735(1), 45–60. https://doi.org/10.1007/s10750-013-1524-7

Hale, M.L., Burg, T.M. & Steeves, T.E. (2012). Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. *PLoS ONE*, 7(9), e45170. https://doi.org/10.1371/journal.pone.0045170

Hohenlohe, P.A., Funk, W.C. & Rajora, O.P. (2021). Population genomics for wildlife conservation and management. *Molecular Ecology*, 30(1), 62–82. https://doi.org/10.1111/mec.15720

Hollingsworth, P.R. & Near, T.J. (2009). Temporal patterns of diversification and microendemism in Eastern Highland endemic barcheek darters (Percidae: Etheostomatinae). *Evolution*, 63(1), 228–243. https://doi.org/10.1111/j.1558-5646.2008.00531.x

Hubbs, D. (2020). *Tennessee Wildlife Resources Agency annual mussel recovery activity report for project 7775*. Nashville, TN: Tennessee Wildlife Resource Agency.

Inoue, K., Monroe, E.M., Elderkin, C.L. & Berg, D.J. (2014). Phylogeographic and population genetic analyses reveal Pleistocene isolation followed by high gene flow in a wide ranging, but endangered, freshwater mussel. *Heredity*, 112(3), 282–290. https://doi.org/10.1038/hdy.2013.104

International Union for the Conservation of Nature (IUCN). (2021). The IUCN Red List of Threatened Species. Version 2021–3. Available at: https://www.iucnredlist.org [Accessed 27 December 2021]

International Union for the Conservation of Nature (IUCN) Species Survival Commission (SSC). (2013). Guidelines for reintroductions and other conservation translocations. Version 1.0. Available at: https://portals.iucn.org/library/efiles/documents/2013-009.pdf [Accessed 27 December 2021]

Jeffries, D.L., Copp, G.H., Handley, L.L., Olsén, K.H., Sayer, C.D. & Hänfling, B. (2016). Comparing RADseq and microsatellites to infer complex phylogeographic patterns, an empirical perspective in the Crucian carp, *Carassius carassius*, L. *Molecular Ecology*, 25(13), 2997–3018. https://doi.org/10.1111/mec.13613

Jombart, T. (2008). Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24(11), 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jones, J., Ahlstedt, S., Ostby, B., Beaty, B., Pinder, M., Eckert, N. et al. (2014). Clinch River freshwater mussels upstream of Norris Reservoir, Tennessee and Virginia: A quantitative assessment from 2004 to 2009. *JAWRA Journal of the American Water Resources Association*, 50(4), 820–836. https://doi.org/10.1111/jawr.12222

Jones, J., Lane, T., Ostby, B., Beaty, B., Ahlstedt, S., Butler, R. et al. (2018). Collapse of the Pendleton Island mussel fauna in the Clinch River, Virginia: Setting baseline conditions to guide recovery and restoration. *Freshwater Mollusk Biology and Conservation*, 21(2), 36–56. https://doi.org/10.31931/fmbc.v21i2.2018.36-56

Jones, J.W., Lane, T.W., Johnson, N. & Hallerman, E.M. (2021). Guiding species recovery through assessment of spatial and temporal population genetic structure of two critically endangered freshwater mussel species (Bivalvia: Unionidae) [Preprint]. In Review. https://doi.org/10.21203/rs.3.rs-282423/v1

Jones, J.W. & Neves, R.J. (2011). Influence of life-history variation on demographic responses of three freshwater mussel species (Bivalvia: Unionidae) in the Clinch River, USA. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 21(1), 57–73. https://doi.org/10.1002/aqc.1161

Jones, J.W., Neves, R.J. & Hallerman, E.M. (2012). Population performance criteria to evaluate reintroduction and recovery of two endangered mussel species, *Epioblasma brevidens* and *Epioblasma capsaeformis* (Bivalvia: Unionidae). *Freshwater Mollusk Biology and Conservation*, 15(1), 27–44. https://doi.org/10.31931/fmbc.v15i1.2012.27-44

Jones, J.W., Neves, R.J. & Hallerman, E.M. (2015). Historical demography of freshwater mussels (Bivalvia: Unionidae): Genetic evidence for population expansion and contraction during the late Pleistocene and Holocene. *Biological Journal of the Linnean Society*, 114(2), 376–397. https://doi.org/10.1111/bij.12437

Jouganous, J., Long, W., Ragsdale, A.P. & Gravel, S. (2017). Inferring the joint demographic history of multiple populations: Beyond the diffusion approximation. *Genetics*, 206(3), 1549–1567. https://doi.org/10.1534/genetics.117.200493

Kamvar, Z.N., Tabima, J.F. & Grünwald, N.J. (2014). Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, 2, e281. https://doi.org/10.7717/peerj.281

Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W. & Prodöhl, P.A. (2013). diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, 4(8), 782–788. https://doi.org/10.1111/2041-210X.12067

Kim, K.S. & Roe, K.J. (2021). Genome-wide SNPs redefines species boundaries and conservation units in the freshwater mussel genus *Cyprogenia* of North America. *Scientific Reports*, 11(1), 10752. https://doi.org/10.1038/s41598-021-90325-0

Kimura, M. & Weiss, G.H. (1964). The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, 49(4), 561–576. https://doi.org/10.1093/genetics/49.4.561

Kozak, K.H., Blaine, R.A. & Larson, A. (2006). Gene lineages and eastern North American palaeodrainage basins: Phylogeography and speciation in salamanders of the *Eurycea bislineata* species complex. *Molecular Ecology*, 15(1), 191–207. https://doi.org/10.1111/j.1365-294X.2005.02757.x

Lamphere, B.A. & Blum, M.J. (2012). Genetic estimates of population structure and dispersal in a benthic stream fish. *Ecology of Freshwater Fish*, 21(1), 75–86. https://doi.org/10.1111/j.1600-0633.2011.00525.x

Lane, T., Jones, J., Ostby, B. & Butler, R. (2021). Long-term monitoring of two endangered freshwater mussels (Bivalvia: Unionidae) reveals how demographic vital rates are influenced by species life history traits. *PLoS ONE*, 16(8), e0256279. https://doi.org/10.1371/journal.pone.0256279

Lischer, H.E.L. & Excoffier, L. (2012). PGDSpider: An automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics*, 28(2), 298–299. https://doi.org/10.1093/bioinformatics/btr642

Malinsky, M., Trucchi, E., Lawson, D.J. & Falush, D. (2018). RADpainter and fineRADstructure: Population inference from RADseq data. *Molecular Biology and Evolution*, 35(5), 1284–1290. https://doi.org/10.1093/molbev/msy023

Marandel, F., Lorance, P., Berthelé, O., Trenkel, V.M., Waples, R.S. & Lamy, J.-B. (2019). Estimating effective population size of large marine populations, is it feasible? *Fish and Fisheries*, 20(1), 189–198. https://doi.org/10.1111/faf.12338

McCleave, J.D. (1964). Movement and population of the Mottled Sculpin (*Cottus bairdi* Girard) in a small montana stream. *Copeia*, 1964(3), 506–513. https://doi.org/10.2307/1441514

McGregor, S.W. & Garner, J.T. (2003). Changes in the freshwater mussel (Bivalvia: Unionidae) fauna of the Bear Creek system of northwest Alabama and northeast Mississippi. *American Malacological Bulletin*, 18(1–2), 61–70.

McMurray, S.E. & Roe, K.J. (2017). Perspectives on the controlled propagation, augmentation, and reintroduction of freshwater mussels (Mollusca: Bivalvia: Unionidae). *Freshwater Mollusk Biology and Conservation*, 20(1), 1–12. https://doi.org/10.31931/fmbc.v20i1.2017.1-12

Meyer, S.C. (2021). Comparative conservation genomics of a suite of imperiled freshwater mussels. PhD Dissertation. Georgia Southern University.

National Native Mussel Conservation Committee. (1998). National strategy for the conservation of native freshwater mussels. *Journal of Shellfish Research*, 17, 1419–1428.

NatureServe. (2021). NatureServe web service. Available at: https://services.natureserve.org/

Noskova, E., Ulyantsev, V., Koepfli, K.-P., O'Brien, S.J. & Dobrynin, P. (2020). GADMA: Genetic algorithm for inferring demographic history of multiple populations from allele frequency spectrum data. *GigaScience*, 9(3), giaa005. https://doi.org/10.1093/gigascience/giaa005

Pasqualotto, A.C., Denning, D.W. & Anderson, M.J. (2007). A cautionary tale: Lack of consistency in allele sizes between two laboratories for a published multilocus microsatellite typing system. *Journal of Clinical Microbiology*, 45(2), 522–528. https://doi.org/10.1128/JCM.02136-06

Peacock, E., Haag, W.R. & Warren, M.L. (2005). Prehistoric decline in freshwater mussels coincident with the advent of maize agriculture. *Conservation Biology*, 19(2), 547–551. https://doi.org/10.1111/j.1523-1739.2005.00036.x

Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J. et al. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, 94(3), 849–873. https://doi.org/10.1111/brv.12480

Roberts, J.H., Angermeier, P.L. & Hallerman, E.M. (2016). Extensive dispersal of Roanoke logperch (*Percina rex*) inferred from genetic marker data. *Ecology of Freshwater Fish*, 25(1), 1–16. https://doi.org/10.1111/eff.12177

Rochette, N.C., Rivera-Colón, A.G. & Catchen, J.M. (2019). Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. *Molecular Ecology*, 28(21), 4737–4754. https://doi.org/10.1111/mec.15253

Ross, R.D. (1971). The drainage history of the Tennessee River. In: P.C. Holt, R.A. Paterson, J. Hubbard (Eds.) *The Distributional History of the Biota of the Southern Appalachians, Part III, Vertebrates*. Blacksburg, VA: Virginia Polytechnic Institute, Research Division Monograph I.

Ross, R.D. (1972). Drainage evolution and fish distribution problems in the southern Appalachians of Virginia. In: P.C. Holt (Ed.) *The Distributional History of the Biota of the Southern Appalachians. III. Vertebrates*. Blacksburg, VA: Virginia Polytechnic Institute, Research Division Monograph 4.

Schwalb, A.N., Poos, M.S. & Ackerman, J.D. (2011). Movement of logperch – the obligate host fish for endangered snuffbox mussels: Implications for mussel dispersal. *Aquatic Sciences*, 73(2), 223–231. https://doi.org/10.1007/s00027-010-0171-6

Starnes, W.C. & Etnier, D.A. (1986). Drainage evolution and fish biogeography of the Tennessee and Cumberland rivers drainage realms. In: C.O. Hocutt, E.O. Wiley (Eds.) *The zoogeography of the North American freshwater fishes*. New York, NY: John Wiley & Sons, pp. 325–361.

Strayer, D.L. & Dudgeon, D. (2010). Freshwater biodiversity conservation: Recent progress and future challenges. *Journal of the North American Benthological Society*, 29(1), 344–358. https://doi.org/10.1899/08-171.1

Strayer, D.L., Geist, J., Haag, W.R., Jackson, J.K. & Newbold, J.D. (2019). Essay: Making the most of recent advances in freshwater mussel propagation and restoration. *Conservation Science and Practice*, 1(7), e53. https://doi.org/10.1111/csp2.53

U.S. Fish and Wildlife Service (USFWS). (2004). *Recovery plan for Cumberland Elktoe, oyster mussel, Cumberlandian Combshell, Purple Bean, and Rough Rabbitsfoot*. Atlanta, GA: U.S. Fish and Wildlife Service.

U.S. Fish and Wildlife Service (USFWS). (2006). Endangered and threatened wildlife and plants; establishment of nonessential experimental population status for 15 freshwater mussels, 1 freshwater snail, and 5 fishes in the lower French Broad River and in the lower Holston River, Tennessee; Proposed Rule. *Federal Register*, 71(113), 34195–34230.

U.S. Fish and Wildlife Service (USFWS). (2019). *Cumberlandian Combshell* Epioblasma brevidens *5-year review: Summary and evaluation*. Cookeville, TN: U.S. Fish and Wildlife Service.

Vaughn, C.C. (2018). Ecosystem services provided by freshwater mussels. *Hydrobiologia*, 810(1), 15–27. https://doi.org/10.1007/s10750-017-3139-x

Waples, R.K., Larson, W.A. & Waples, R.S. (2016). Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. *Heredity*, 117(4), 233–240. https://doi.org/10.1038/hdy.2016.60

Williams, J.D., Warren, M.L., Cummings, K.S., Harris, J.L. & Neves, R.J. (1993). Conservation status of freshwater mussels of the United States and Canada. *Fisheries*, 18(9), 6–22. https://doi.org/10.1577/1548-8446(1993)018<0006:CSOFMO>2.0.CO;2

Yeager, B.L. & Saylor, C.F. (1995). Fish hosts for four species of freshwater mussels (Pelecypoda: Unionidae) in the upper Tennessee River drainage. *The American Midland Naturalist*, 133(1), 1–6. https://doi.org/10.2307/2426342

Zipper, C.E., Beaty, B., Johnson, G.C., Jones, J.W., Krstolic, J.L., Ostby, B.J.K. et al. (2014). Freshwater mussel population status and habitat quality in the Clinch River, Virginia and Tennessee, USA: A featured collection. *JAWRA Journal of the American Water Resources Association*, 50(4), 807–819. https://doi.org/10.1111/jawr.12220

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Gladstone, N.S., Garrison, N.L., Lane, T., Johnson, P.D., Garner, J. & Whelan, N.V. (2022). Population genomics reveal low differentiation and complex demographic histories in a highly fragmented and endangered freshwater mussel. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 1–14. https://doi.org/10.1002/aqc.3849