Co-varying patterns of genetic diversity and structure with life-history traits of freshwater mussel species (Bivalvia:Unionidae) in the Poyang Lake drainage, China

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Abstract: Freshwater mussels are one of the most diverse groups of freshwater organisms as well as one of the most endangered groups of organisms on Earth. Freshwater mussels have a life history that greatly influences their geographical distribution, genetic structure, and demographic characteristics. Here, we describe and compare the spatial genetic structure and diversity of 5 freshwater mussel species with different brooding periods and uses of larval parasitism. These 5 species co-occur in tributaries in Poyang Lake in south central China, and we studied their populations in the Gan and Fuhe Rivers, which are both large tributaries of Poyang Lake. Cytochrome c oxidase subunit-I (COI) haplotype richness and diversity of species with a spring or summer glochidia brooding period (*Lamprotula caveata*, *Nodularia douglasiae*, and *Solenaia oleivora*) were greater than richness and diversity in species with a winter brooding period (*Anemina arcaeformis*, *S. carinata*). In addition, the COI haplotype richness and diversity of *A. arcaeformis*, which does not have a parasitic portion of its life cycle, was lower than 3 species that require a host fish to complete their life cycles (*L. caveata*, *N. douglasiae*, and *S. oleivora*) and was higher than *S. carinata*. We also used the COI sequences and microsatellite datasets to determine whether the populations of each of the 5 species in the Gan and Fuhe Rivers are admixed or genetically distinct. Genetic differentiation was evident among collection populations of *L. caveata*, *N. douglasiae*, and *S. carinata* and largely absent in *A. arcaeformis* and *S. oleivora*. We conclude that differences in genetic diversity and patterns of genetic structure in these sympatric species could result from the different life-history attributes of these species, particularly timing and length of the brooding period, nonparasitic vs parasitic life cycles, and different host fish requirements. Therefore, we suggest increased emphasis on life history and reproductive biology research and urge managers to consider that these traits differ among freshwater mussels when making management decisions for the conservation of genetic and species diversity.
Key words: China, freshwater mussels, genetic diversity, genetic structure, host fish, life history, Poyang Lake, Yangtze River drainage

Loss of biodiversity is a major global concern, especially for freshwater species (Dudgeon et al. 2006). The number of endangered species rises as a result of numerous threats, such as water pollution, habitat loss, and habitat fragmentation, which can act from genetic scales to entire ecosystems. Thus, it is imperative to recognize the appropriate scale at which to focus conservation efforts (Vaughn 2010).

Freshwater mussels (Unionida) often comprise a large proportion of the benthic biomass in freshwater ecosystems (Strayer et al. 2004). Freshwater mussels are an important

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indicator group for overall environmental health (Vaughn and Hakenkamp 2001, Vaughn 2018). These mussels provide important ecosystem services, such as water purification, and are prey for many commercial fishes and other wildlife (Haag 2012, Lopes-Lima et al. 2016, Sun et al. 2018, Vaughn 2018). Freshwater mussels also have economic and cultural value because they are a source of protein for humans and of valuable materials such as shells and pearls (Haag 2012). However, freshwater mussel diversity and abundances have declined rapidly across the globe in recent decades (Lydeard et al. 2004, Strayer et al. 2008, Zieritz et al. 2018). According to the International Union for Conservation of Nature's Red List of Threatened Species (IUCN 2019), 246 freshwater mussels have been identified as extinct, endangered, threatened, or near threatened. Freshwater mussels are attracting considerable attention from a global audience that includes international nonprofits, national governments, and local conservation groups as a result of the number of highly-imperiled species and the important ecosystem services they provide (Vaughn 2018, Zieritz et al. 2018).

The life cycles of most freshwater mussel species include a parasitic larval stage and a free-living, filter-feeding, and relatively-sessile adult stage. The parasitic stage occurs after maturation, when larval mussels are discharged from their mother and become parasitic on the gills or fins of a host fish. Mussel reliance on host fish is critical for both larval development and long-distance dispersal (Vaughn 2012) and can influence their geographic distribution, genetic structure, and demographic characteristics (Watters 1992, Zanatta and Wilson 2011, Mock et al. 2013). This close relationship with a host increases mussel extirpation risk because it makes the larval and juvenile life stages susceptible to both the direct effects of anthropogenic disturbances on their populations and the indirect effects of anthropogenic disturbances on host fish populations (Spooner et al. 2011).

Poyang Lake is the largest freshwater lake in China (Fig. 1), and this lake and its tributaries are an important biodiversity hotspot for Asian freshwater mussels. Fifty-six species of freshwater mussels have been reported in this lake, including ~75% of the freshwater mussels endemic to China (Wu et al. 2000, Xiong et al. 2012, Sun et al. 2018, Zieritz et al. 2018). These endemic freshwater mussels vary in numerous traits, including whether they are nonparasitic or parasitic and the timing of their brooding period (Table 1), but because of natural factors and human activities, their populations have declined markedly in this lake. Their different traits provide a unique opportunity to assess the influence and relationship of life history on the genetic structure and diversity of sympatric freshwater mussel genera.

The geographic patterns of genetic diversity of freshwater mussels can influence their conservation and recovery (Geist 2010). To ensure the maintenance of diversity and adaptive potential, it is necessary to define conservation units and evaluate current genetic diversity before initiating any recovery measures, such as augmenting natural populations with captive-grown individuals (Jones et al. 2006, Hoftyzer et al. 2008). Population genetics can be used to understand the basic ecology of freshwater mussels and define distinct populations (Liu et al. 2017, Sun et al. 2018, Zanatta et al. 2018).

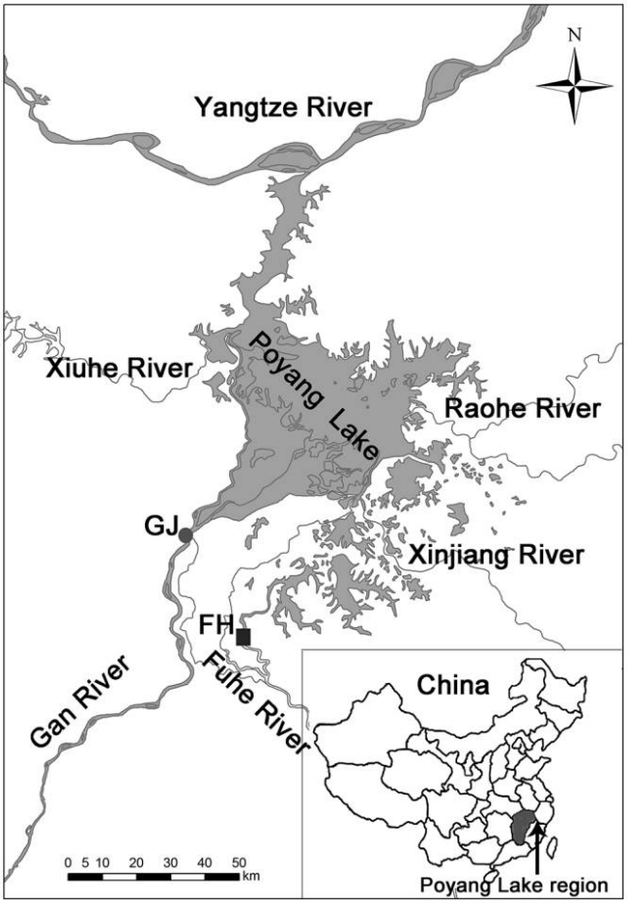

Figure 1. Map showing the collection locations of *Anemina arcaeformis*, *Lamprotula caveata*, *Nodularia douglasiae*, *Solenaia carinata*, and *S. oleivora* in the Poyang Lake region of China. We collected freshwater mussels from the Fuhe River (FH, gray square) and the Gan River (GJ, gray circle).

Here we describe and compare patterns of spatial genetic structure and diversity of freshwater mussels that have different brooding periods and parasitism characteristics and that live in the Poyang Lake region. Many factors can cause changes in genetic diversity, including internal factors such as life-history traits, host fish populations, and geomorphic changes as well as external factors such as anthropogenic disturbances (Geist 2010, Mock et al. 2013, Zanatta et al. 2018). We hypothesized that species with different brooding periods and larval parasitism characteristics will vary in their spatial genetic structure and diversity. We predicted

Table 1. Distribution, International Union for Conservation of Nature (IUCN) status, brooding period, host fish and nonparasitic/parasitic of 5 freshwater mussels. LC = least concern, NE = not evaluated.

	Anemina arcaeformis	*Lamprotula caveata*	*Nodularia douglasiae*	*Solenaia carinata*	*Solenaia oleivora*	References
Distribution	Eastern and southeastern Asia, Russia	Endemic to China	Eastern and southeastern Asia, Russia	Endemic to Yangtze basin	Endemic to China	Liu et al. 1979, Sun et al. 2018
IUCN status	LC	LC	LC	NE	NE	IUCN 2019
Brooding period	Dec–Mar	Apr–Aug	Feb–Jul	Nov–Feb	Feb–May	Cao et al. 2018, Wu et al. 2018
Nonparasitic or parasitic	Nonparasitic	Parasitic	Parasitic	Parasitic	Parasitic	Shu and Ouyang 2004, Wu 1998, Cao et al. 2018, Wu et al. 2018
Host fish	—	Unknown	Unknown	Unknown	Unknown	—

that nonparasitic species and species with winter brooding periods have lower genetic diversity than parasitic species or species with other brooding periods. This study will provide important information for the management and conservation of freshwater mussels.

METHODS

Mitochondrial DNA (mtDNA) analysis provides estimates of the phylogenetic relationships and population evolution in freshwater mussels (Gissi et al. 2008, Stoger and Schrodl 2013). Microsatellites are demonstrably-useful markers for studying fine-scale patterns of genetic diversity in freshwater mussels (e.g., Geist et al. 2010, Galbraith et al. 2015, Zanatta et al. 2018). Combining analyses of mtDNA sequence data and microsatellite genotypes can help to reveal both the course-scale and fine-scale evolutionary history and genetic structure of a species (e.g., Liu et al. 2017, Mathias et al. 2018). This study describes and compares patterns of spatial genetic structure and diversity of 5 freshwater mussel species that have different brooding periods and parasitism characteristics based on microsatellite DNA genotypes and mtDNA sequences.

The 5 mussel species we studied have different brooding periods and parasitism characteristics (Table 1). The brooding periods of *N. douglasiae* (February–July), *L. caveata* (April–August), and *S. oleivora* (February–May) are in spring and summer, whereas those of *A. arcaeformis* (December–March) and *Solenaia carinata* (November–February) are in winter (Cao et al. 2018, Wu et al. 2018). In addition, *A. arcaeformis* is a nonparasitic species whose larvae do not need to attach to a host fish (Shu and Ouyang 2004). In contrast, *L. caveata*, *N. douglasiae*, *S. carinata*, and *S. oleivora* larvae are all obligate parasites of the gills or fins of a host fish (Wu 1998, Cao et al. 2018, Wu et al. 2018). These differences provide a unique opportunity to assess the influence and relationship of life history on the genetic structure and diversity of sympatric freshwater mussel genera.

Sample collection and DNA extraction

We collected a total of 42 *A. arcaeformis*, 60 *L. caveata*, 60 *N. douglasiae*, 64 *S. carinata*, and 60 *S. oleivora* individuals from a site on both the Gan and Fuhe Rivers, which are major tributaries to Poyang Lake, China (Table 2, Fig. 1). These collections included 30 *N. douglasiae* individuals previously taken from the Gan River (Liu et al. 2017) and 64 *S. carinata* individuals previously taken from the Gan and Fuhe Rivers (n = 41 and 23, respectively; Sun et al. 2018). Specimens were collected by hand in shallow, wadeable water. Tissues were preserved in 95% ethanol and stored at –20°C until DNA extraction.

We used the TINAamp Marine Animals DNA Kit (TianGen®, Beijing, China) to extract the genomic DNA from mantle tissue. We then used a NanoDrop™ 2000 (Thermo Fisher Scientific, Waltham, Massachusetts) and agarose gel electrophoresis to estimate the concentration and quality of DNA.

mtDNA sequence generation

We amplified and sequenced a fragment of the cytochrome c oxidase subunit-I (COI) mtDNA gene from each collected individual. We used the forward primer sequence LCO22me2 (5′-GGTCAACAAAYCATAARGATATTGG-3′) and the reverse primer sequence HCO700DY2 (5′-TCAGGGTGACCAAAAAAYCA-3′) (Walker et al. 2007). We screened each primer for reliable amplification in each mussel species. The polymerase chain reaction (PCR) we used to amplify DNA was 25 μL in volume and contained 12.5 μL of 2x Taq PCR MasterMix (TianGen), 8.5 μL of ddH_2O, 1.0 μL of 10 μM forward primer, 1.0 μL of 10 μM reverse primer, and 2 μL of genomic DNA (~100 ng/μL). PCR amplifications were done with the following touchdown thermal cycling program: an initial denaturation at 94°C for 2 min, followed by 35 cycles at 94°C for 1 min, an annealing temperature of 50°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 7 min.

Table 2. Collection location and genetic diversity of 5 freshwater mussels based on microsatellite loci and mitochondrial DNA COI sequences. n = sample size, n_A = number of alleles, n_E = effective number of alleles, H_E = expected heterozygosity, H_O = observed heterozygosity, H = number of haplotypes, H_d = haplotype diversity, π = nucleotide diversity, FH = Fuhe River, GJ = Gan River.

		Microsatellites					COI sequences			
Species	Collection location	n	n_A	n_E	H_E	H_O	n	H	H_d	π
Anemina arcaeformis	FH	22	3.50	2.19	0.58	0.53	14	5	0.79	0.002
	GJ	20	3.20	2.09	0.59	0.50	15	9	0.88	0.004
	Total	42	3.60	2.16	0.58	0.51	29	10	0.84	0.003
Lamprotula caveata	FH	30	3.25	2.38	0.67	0.54	20	20	1.00	0.012
	GJ	30	3.13	2.29	0.62	0.53	29	19	0.94	0.008
	Total	60	3.38	2.40	0.64	0.54	49	38	0.98	0.010
Nodularia douglasiae	FH	30	2.50	1.92	0.60	0.48	30	18	0.94	0.043
	GJ	30	4.10	1.93	0.56	0.48	26	16	0.95	0.035
	Total	60	4.60	2.02	0.58	0.50	56	28	0.95	0.044
Solenaia carinata	FH	23	3.30	2.04	0.45	0.49	16	8	0.70	0.003
	GJ	41	3.90	2.46	0.51	0.57	37	13	0.71	0.002
	Total	64	4.10	2.37	0.49	0.55	53	16	0.70	0.002
Solenaia oleivora	FH	30	8.38	5.33	0.65	0.75	28	23	0.98	0.032
	GJ	30	8.00	4.91	0.70	0.72	29	25	0.99	0.012
	Total	60	9.38	5.38	0.68	0.74	57	44	0.99	0.023

We used a 1% agarose gel to electrophorese the PCR products to confirm successful amplification, then purified the PCR products with an EZ-10 Spin Column PCR Product Purification Kit (Promega™, Madison, Wisconsin). The purified DNA was then sequenced on an ABI 3730XL DNA Analyzer (Applied Biosystems®, Carlsbad, California).

Microsatellite DNA genotyping

We also genotyped between 8 and 10 microsatellite loci for each species (Table S1). The PCR amplifications for *A. arcaeformis* and *L. caveata* were done in a 20-μL volume that contained 12.5 μL of 2x Taq PCR MasterMix (TianGen), 5.2 μL of ddH_2O, 1.0 μL of 10 μM forward primer with an M13 tag on the 5′ end, 1.0 μL of 10 μM reverse primer, 0.8 μL of 10 μMHEX- or 6-FAM-labeled M13 universal primer, and 2 μL of genomic DNA (~100 ng/μL). *Lamprotula caveata* PCR amplification used the following touchdown thermal cycling program: an initial denaturation at 94°C for 5 min, followed by 33 cycles of 94°C for 1 min, annealing temperature for 30 s, 72°C for 45 s, and a final extension at 72°C for 10 min. *Anemina arcaeformis* PCR amplification used the following touchdown thermal cycling program: an initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, annealing temperature for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min. PCR amplification conditions for *N. douglasiae*, *S. carinata*, and *S. oleivora* are described in Liu et al. (2017), Sun et al. (2018), and Xu (2014), respectively. An ABI 3730 automated sequencer was used to analyze amplification products. We used GENEMAPPER v3.7 (Applied Biosystems®) with a TAMRA-labeled size standard. We also used this software to score alleles for each locus.

Statistical analysis

mtDNA sequences We used the mtDNA sequences to calculate the number of haplotypes, haplotype diversity, and mean nucleotide diversity for each species in each collection location with DNASP v5.10 (Librado and Rozas 2009). We then used NETWORK 4.5 to construct a network of COI haplotypes for each of the 5 freshwater mussel species, assuming a 95% connection limit (Leigh and Bryant 2015).

We used 3 different approaches to search for a signature of population expansion: neutrality tests, mismatch distribution, and Bayesian skyline plots. We examined deviations from neutrality with Tajima's D and Fu's FS tests in ARLEQUIN 3.5 (Excoffier and Lischer 2010). We used DNASP 5.0 (Librado and Rozas 2009) for mismatch distribution analysis (MDA). We used BEAST 1.4.7 for Bayesian skyline plot analysis (Drummond et al. 2005, Drummond and Rambaut 2007).

We then used a Bayesian skyline plot to reconstruct the effective population size fluctuations since the time of the most recent common ancestor for each species. For this analysis, we used the (HKY+G) substitution model from JMODELTEST 0.1.1, mutation rates of 2.0×10^{-8}, and a strict molecular clock (Brown et al. 1979). We then ran Markov Chain Monte Carlo for 500 million iterations, with sampling every 1000 iterations after a burn-in of the initial

10% of iterations. We used TRACER 1.5 to construct the Bayesian skyline plot (Rambaut and Drummond 2007).

We used ARLEQUIN 3.5 to run an analysis of molecular variance (AMOVA) to test the statistical significance of genetic divergence within and among populations (Excoffier et al. 1992, Excoffier and Lischer 2010). We did 1000 permutations of this analysis to test whether each pair of collection sites of the same species differed.

Microsatellite genotypes For each species, possible null alleles for each population were detected with MICROCHECKER v2.2.3 (van Oosterhout et al. 2004). We used POPGENE v1.32 (Yeh et al. 2000) to calculate the number of alleles, the effective number of alleles (n_E), inbreeding index, observed heterozygosity, and expected heterozygosity, and then tested for Hardy–Weinberg Equilibrium for all alleles at each collection location. We used Mann–Whitney U-tests to compare genetic diversity metrics between the Gan River and Fuhe River collection sites of each species. We used BOTTLENECK v1.2.02 to detect any recent genetic bottlenecks (within $2n_E - 4n_E$ generations) in each of the rivers using 3 models (the infinite alleles model, 2-phase model, and stepwise mutation model) and a mode-shift test (Piry et al. 1999).

We used STRUCTURE v2.3.3 to assess genetic structure based on the microsatellite loci datasets of each species in the entire study area. This analysis allowed for admixture among genetic populations (K) and assumed that allele frequencies were correlated. To allow detection of substructure across the region, the maximum value of K was set as 5 (i.e., number of collection sites + 3). We did 10 iterations of this analysis for each value of K. For each iteration we used an initial burn-in period of 200,000 replicates to assure stationarity and an additional 200,000 replicates to evaluate K. We used both the ΔK method (following Evanno et al. 2005) and the log-likelihood method to evaluate each value of K with STRUCTURE HARVESTER v0.6.8 (Earl and von Holdt 2012). To further analyze the pattern of genetic structure among collection populations, we used GenAlEx v6.5 to conduct principal coordinate analyses. These analyses ordinated genetic distance estimates that had been calculated from the genotypic data of individuals (Nei 1972, Peakall and Smouse 2012).

We tested the statistical significance of genetic divergence within and among populations of each species with an AMOVA in GENALEX 6.5 (Excoffier et al. 1992, Excoffier and Lischer 2010). One-thousand permutations were performed to test the significance of each pairwise comparison among collection populations.

RESULTS

mtDNA sequences

The genetic diversity of some species differed based on mtDNA sequences. The average length of the aligned mtDNA sequences of *A. arcaeformis*, *L. caveata*, *N. douglasiae*, *S. carinata*, and *S. oleivora* were 534, 604, 524, 581, and 581 bp, respectively. Species varied considerably in their number of COI haplotypes. *Lamprotula caveata* (38, GenBank: MK911894–MK911931), *N. douglasiae* (28, MK912002–MK912029), and *S. oleivora* (44, MK911932–MK911975) had considerably more haplotypes than *A. arcaeformis* (10, MK911976–MK911985) and *S. carinata* (16, MK911986–MK912001) (Table 2). The haplotype diversity and nucleotide diversity of *S. oleivora* (0.99 and 0.023), *N. douglasiae* (0.95 and 0.044), and *L. caveata* (0.98 and 0.010) were greater than those of *S. carinata* (0.70 and 0.002) and *A. arcaeformis* (0.84 and 0.003) (Table 2). COI diversity metrics were somewhat higher in the Gan River than in the Fuhe River for 3 of the 5 species (*A. arcaeformis*, *S. carinata*, and *S. oleivora*).

The haplotype network analyses showed a general lack of geographic resolution, and many haplotypes were shared between the sampling locations for all species (Fig. 2A–E). The most frequent haplotype was found in 10 specimens of *A. arcaeformis* (Hap2), 6 specimens of *L. caveata* (Hap23), 10 specimens of *N. douglasiae* (Hap1), 27 specimens of *S. carinata* (Hap3), and 4 specimens of *S. oleivora* (Hap9) (Fig. 2A–E). Some haplotypes generated were rare (e.g., only found in a single specimen): 5 haplotypes from *A. arcaeformis*, 34 from *L. caveata*, 15 from *N. douglasiae*, 10 from *S. carinata*, and 36 from *S. oleivora*.

The genetic structure of some species differed between the 2 collection sites. AMOVAs of COI haplotypes showed that 6.5, 18.9, and 1.1% of the total genetic variance occurred between the *L. caveata*, *N. douglasiae*, and *S. carinata* collection populations, respectively. F-statistical (F_{st}) values also differed among the populations of these species (F_{st} = 0.06, 0.19, 0.01, respectively; $p < 0.0001$; Table 3). In contrast, *A. arcaeformis* and *S. oleivora* individuals collected from the 2 rivers were not genetically different based on F_{st} values (Table 3).

MDA of pairwise differences were not different for *L. caveata*, *N. douglasiae*, and *S. oleivora* (Fig. 3B, C, E). In contrast, MDA tests for *A. arcaeformis* and *S. carinata* indicated a recent population expansion (Fig. 3A, D). Neutrality tests (Tajima's D test and Fu's FS test) suggest that *S. carinata* populations in the Fuhe and Gan Rivers experienced a recent population expansion (Table 4). Additionally, the Bayesian skyline plots showed evidence that *L. caveata* and *S. oleivora* had a small expansion event occurring between 200,000 to 300,000 years before present (ypb) and 10,000 to 50,000 ybp, respectively. However, this result may instead indicate that the population size was constant, which would suggest a lack of support for the recent expansion trend (Fig. 4A–E).

Microsatellite genotypes

The quality of the microsatellite dataset was assessed using MICROCHECKER v2.2.3. Null alleles were found only

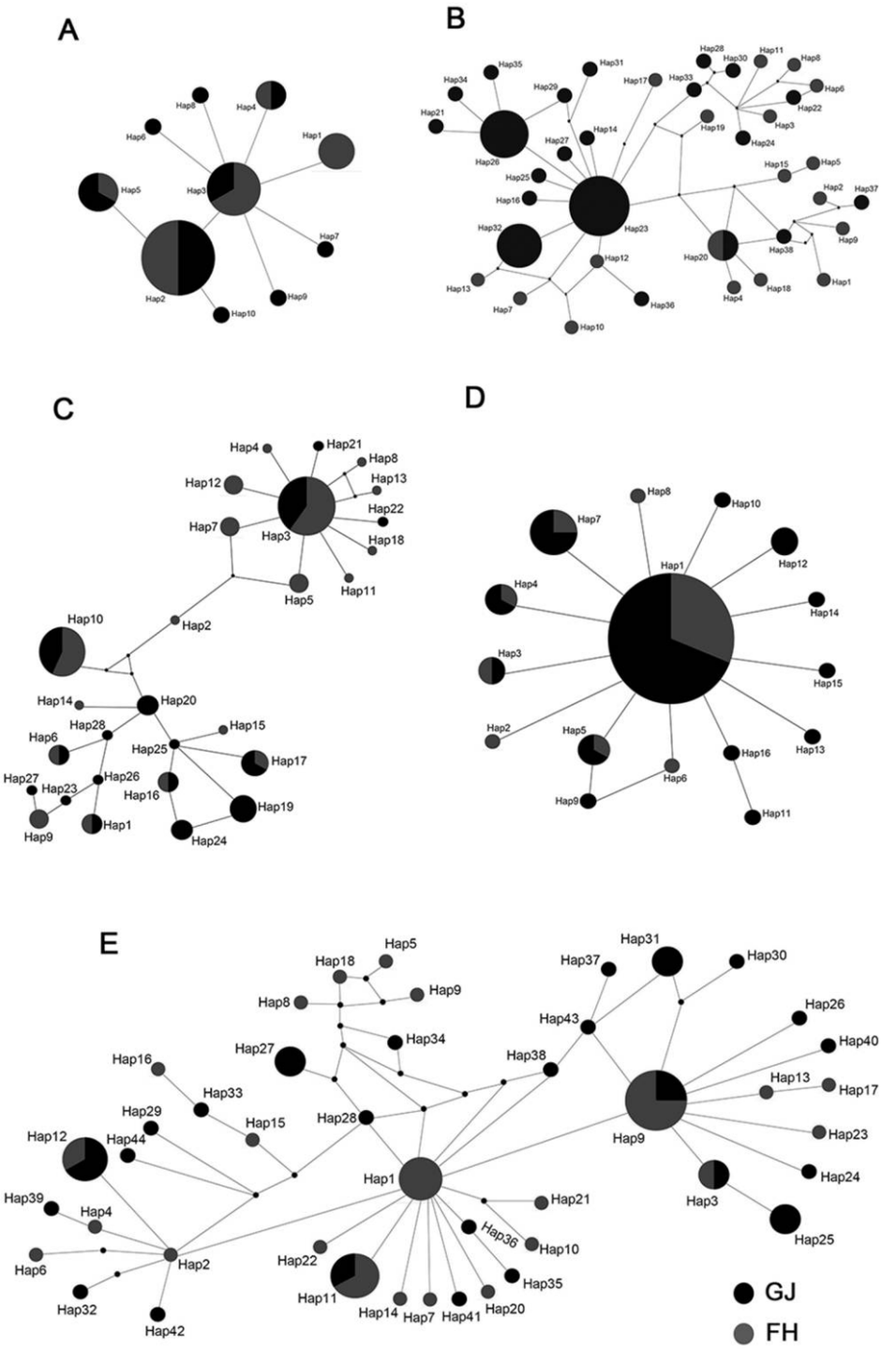

Figure 2. Haplotype network for *Anemina arcaeformis* (A), *Lamprotula caveata* (B), *Nodularia douglasiae* (C), *Solenaia carinata* (D), and *S. oleivora* (E) populations in the Fuhe River (FH, gray) and the Gan River (GJ, black). Black dots are inferred missing haplotypes. Haplotype frequency within each network is relative to the size of the circle.

at 6 loci (Sol06, Scastt2, Scastt5, Scastt10, Sol10, and Sol11) with estimated null allele frequencies ranging from 0.00 to 0.26 at any given population–locus combination (Table S2). These generally-low predicted null allele frequencies are below the threshold (mean < 0.20) that could affect the interpretations of population-level analyses (Dakin and Avise 2004, Carlsson 2008). All loci were, therefore, included in this study.

Table 3. Analysis of molecular variation calculated with microsatellite loci and mitochondrial DNA COI sequences for 5 freshwater mussels in 2 collection locations, the Fuhe and Gan Rivers. Bold values of F-statistics (F_{st}) indicate evidence of genetic differentiation ($p < 0.01$). df = degrees of freedom, SS = Sum of squares, VC = variance components, PoV = percentage of variation.

	Analysis of molecular variation					
	Source of variation	df	SS	VC	PoV	F_{st}
Anemina arcaeformis						
COI sequences	Among populations	1	0.76	−0.01	−1.3	
	Within populations	27	25.11	0.93	101.3	−0.01
	Total	28	25.87	0.92	100	
Microsatellite markers	Among populations	1	1.5	−0.02	−0.7	−0.01
	Among ind within populations	40	89.6	−0.34	−13.2	−0.13
	Within ind	42	122.5	2.92	113.9	−0.14
	Total	83	213.6	2.56		
Lamprotula caveata						
COI sequences	Among populations	1	7.7	0.20	6.5	
	Within populations	47	136.9	2.91	93.5	**0.06**
	Total	48	144.6	3.11		
Microsatellite markers	Among populations	1	4.4	0.04	2.1	**0.02**
	Among ind within populations	58	97.7	−0.44	−20.3	−0.21
	Within ind	60	154.0	2.57	118.2	−0.18
	Total	119	256.1	2.17		
Nodularia douglasiae						
COI sequences	Among populations	1	85.5	2.66	18.93	
	Within populations	54	614.9	11.39	81.07	**0.19**
	Total	55	700.4	14.05		
Microsatellite markers	Among populations	1	16.4	0.24	9.2	**0.09**
	Among ind within populations	58	107.2	−0.53	−20.3	−0.22
	Within ind	60	175.0	2.92	111.1	−0.11
	Total	119	298.6	2.63		
Solenaia carinata						
COI sequences	Among populations	1	0.54	−0.01	−1.1	
	Within populations	51	35.95	0.70	101.1	**0.01**
	Total	52	36.49	0.69	100	
Microsatellite markers	Among populations	1	7.8	0.08	2.9	**0.03**
	Among ind within populations	62	187.6	0.29	10.3	0.11
	Within ind	64	156.5	2.45	86.8	0.13
	Total	127	351.8	2.82		
Solenaia oleivora						
COI sequences	Among populations	1	18.2	0.41	6.0	
	Within populations	55	357.1	6.49	94.0	0.06
	Total	56	375.3	6.90		
Microsatellite markers	Among populations	1	4.5	0.02	0.74	0.01
	Among ind within populations	58	184.5	0.23	7.81	0.08
	Within ind	60	163.0	2.71	91.44	0.09
	Total	119	352.0	2.97		

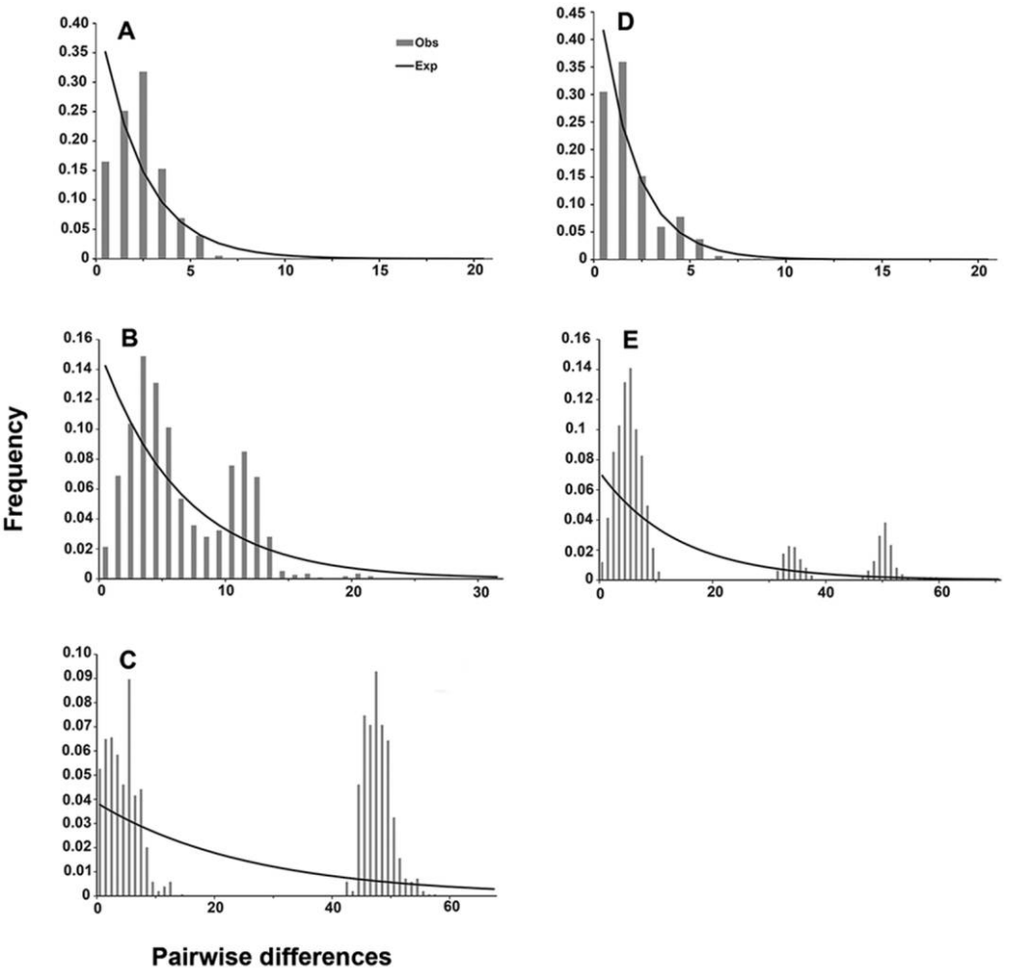

Figure 3. Mismatch distribution analysis for *Anemina arcaeformis* (A), *Lamprotula caveata* (B), *Nodularia douglasiae* (C), *Solenaia carinata* (D), and *S. oleivora* (E) populations in the Fuhe and Gan Rivers. Obs = observed, Exp = expected.

Genetic diversity metrics varied somewhat among the species. The mean number of alleles was greatest for *S. oleivora* (9.38) and least for *L. caveata* (3.38). The mean number of effective alleles was greatest for *S. oleivora* (5.38) and least for *N. douglasiae* (2.02). Observed heterozygosity was greatest for *S. oleivora* (0.68) and least for *S. carinata* (0.49). Expected heterozygosity was greatest for *S. oleivora* (0.74) and least for *S. carinata* (0.55) and *N. douglasiae* (0.50) (Table 2). Diversity metrics between the rivers were not different for any species (Mann–Whitney U-tests, $p > 0.05$). No evidence of a genetic bottleneck for any mussel species was found at either collection location (Table S3).

The microsatellite data indicated that *N. douglasiae* and *S. carinata* were 2 distinct populations (Fig. 5A, B), whereas *A. arcaeformis*, *L. caveata*, and *S. oleivora* were most likely single genetic populations based on STRUCTURE analysis using the log-likelihood and the ΔK methods from Evanno et al. (2005) (Fig. S1). The principal coordinate analyses (Fig. 6A–E) showed similar patterns of genetic structure to those resolved with STRUCTURE.

The microsatellite datasets showed that 2.1, 2.9, and 9.2% of the total genetic variance occurred between the 2 rivers for *L. caveata*, *N. douglasiae*, and *S. carinata*, respectively (AMOVA; $F_{st} = 0.02$ [*L. caveata*], $F_{st} = 0.03$ [*N. douglasiae*], $F_{st} = 0.09$ [*S. carinata*], $p < 0.0001$; Table 3). Populations of *A. arcaeformis* and *S. oleivora* did not differ between the 2 rivers (Table 3).

DISCUSSION

Effects of life history on genetic diversity and structure

The life history of many freshwater mussels involves an obligate parasitic stage that requires an intermediate host fish for their larvae (Vaughn 2012). This requirement can influence the genetic diversity and structure of freshwater mussels (Watters 1992, Mock et al. 2013). In this study, the genetic diversity of *A. arcaeformis*, which is nonparasitic,

Table 4. Neutrality tests and mismatch distribution for 5 freshwater mussels in 2 collection locations, the Fuhe River (FH) and Gan River (GJ). Bold type indicates $p < 0.01$. P_{SSD} = Sum of squared deviation p-value, P_{RAG} = Raggedness p-value.

		Neutrality tests			Mismatch distribution	
Species	Collection location	π (%)	Tajima's D	Fu's FS	P_{SSD}	P_{RAG}
Anemina arcaeformis	FH	1.31	−0.56	**−22.3**	0.40	0.36
	GJ	2.36	−1.61	**−18.6**	0.47	0.47
Lamprotula caveata	FH	7.32	−0.52	**−15.3**	0.20	0.03
	GJ	4.81	−1.57	**−25.6**	0.01	1.00
Nodularia douglasiae	FH	24.89	1.78	**−13.0**	0.50	0.37
	GJ	20.31	0.74	**−11.8**	0.81	0.71
Solenaia carinata	FH	1.88	**−2.29**	**−23.1**	**0**	1.00
	GJ	1.22	**−2.22**	**−28.6**	0.60	0.34
Solenaia oleivora	FH	18.90	−0.63	**−14.2**	**0**	0.98
	GJ	7.28	**−1.94**	**−25.0**	0.40	0.69

was lower (Mann–Whitney U-tests, $p < 0.05$) than the genetic diversity of obligate parasitic species *L. caveata*, *N. douglasiae*, and *S. oleivora*. However, the parasitic species *S. carinata* had the lowest genetic diversity of all 5 species (Table 1). Nonparasitic larval metamorphosis occurs in a few species of freshwater mussels and may partially explain the large geographic distribution of species with this trait (Wei et al. 1993, Shu and Ouyang 2004, Dickinson and Sietman 2008, Wu et al. 2018). However, this mechanism phenomenon (why parasitism influences genetic diversity) needs further research.

In addition, freshwater mussels that use hosts with different dispersal capabilities can influence their population divergence (Haag 2012, Ferguson et al. 2013). For example, some freshwater mussels with hosts having limited abilities show high levels of population divergence (Zanatta and Wilson 2011). In contrast, mussels with highly-vagile hosts show lower levels of population divergence (Berg et al. 1998, Mathias et al. 2018, Pfeiffer et al. 2018). In our study, genetic differentiation was evident only among collection locations for *N. douglasiae* and *S. carinata* in both the microsatellite and mtDNA COI datasets. Thus, the host fishes of *N. douglasiae* and *S. carinata* may not travel long distances or may not travel through both rivers and lakes (Zanatta et al. 2011, 2018, Liu et al. 2017). However, even in *N. douglasiae* and *S. carinata* there was considerable evidence of admixture between the collection locations, possibly because the collection locations were close together.

The COI mtDNA diversity of species with spring or summer glochidial brooding periods (*L. caveata*, *N. douglasiae*, *S. oleivora*) were higher (Mann–Whitney U-tests, $p < 0.05$) than species with a winter brooding period (*A. arcaeformis* and *S. carinata*) (Table 1). Winter brooders may have lower fecundity than mussels that brood at other times of the year (Wu et al. 2018; Table S1), which could cause differing amounts of genetic diversity for these species. Fecundity is an important trait for freshwater mussels as the survival rate of glochidia and juvenile mussels is relatively low (Bauer and Wächtler 2001, Wu et al. 2018). A small population is more likely to lose genetic diversity than a huge population (Frankham et al. 2010, Allendorf et al. 2012). Therefore, mussels with high fecundity are likely to have higher genetic diversity.

Comparison of genetic diversity in rare, threatened, and common freshwater mussels

Information on the genetic diversity of freshwater mussels is critically important for guiding conservation and management decisions of rare, threatened, and endangered species. Higher levels of genetic diversity among populations could improve evolutionary potential for dealing with habitat change, effects of pathogen infection, and other selective forces (Freeland et al. 2011, Liu and Yao 2012, Wu et al. 2013, Liu et al. 2017). Compared to common species, endangered species often have relatively-low genetic diversity (Meffe 1986). Restoration of these endangered species is difficult without basic information on historical distributions and connectivity because of already-reduced genetic diversity in small populations (Galbraith et al. 2015). Comparative analyses between endangered and common species may be a means of circumventing this problem because common species with similar life histories to imperiled species can serve as surrogates, eliminating the need to sample rare or sensitive populations (Edwards and Wyatt 1994, Maki et al. 2002). Furthermore, comparisons of genetic diversity and structure among threatened and endangered species may provide insight into mechanisms of species decline and loss of diversity and insight into potential recovery strategies (Galbraith et al. 2015). In our study, the genetic diversity of narrow-range endemic and potentially-imperiled *S. carinata* and *S. oleivora* had considerably-different levels of diversity (COI and microsatellites), with little genetic

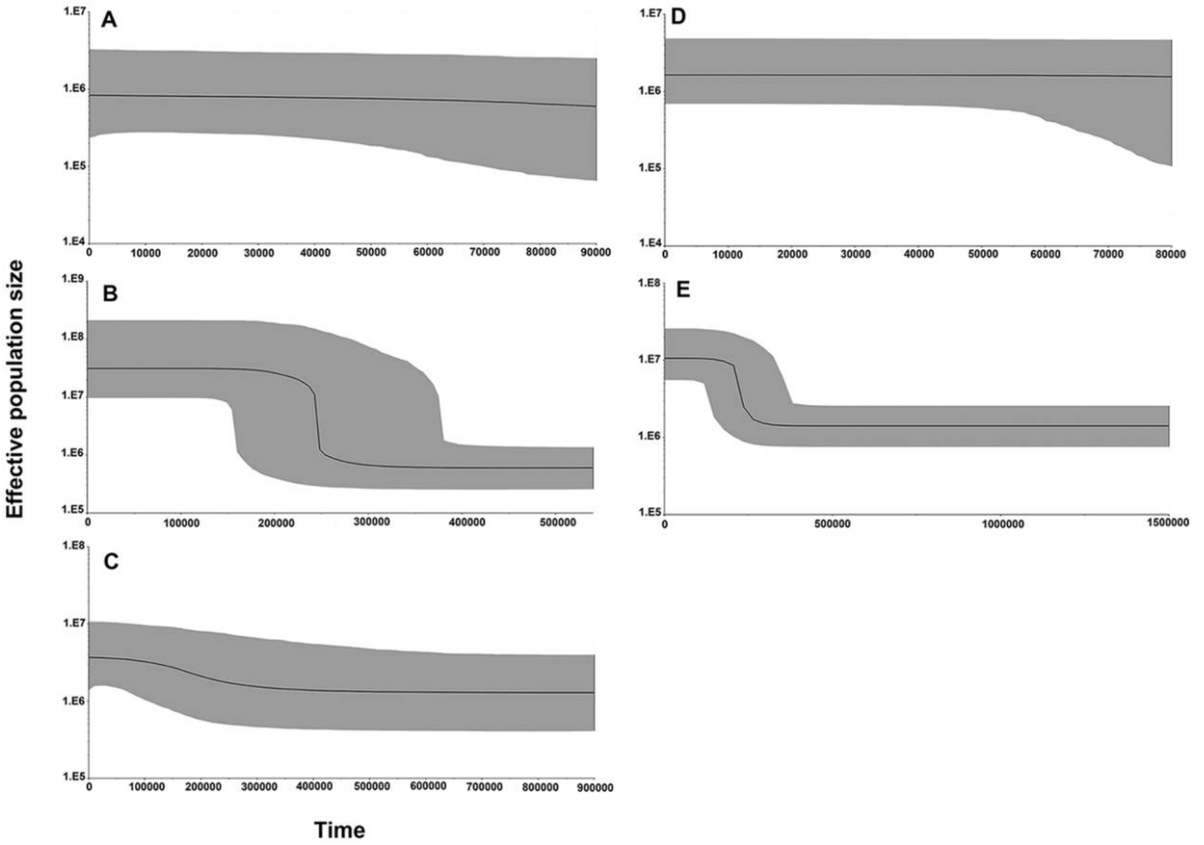

Figure 4. Bayesian skyline plot for *Anemina arcaeformis* (A), *Lamprotula caveata* (B), *Nodularia douglasiae* (C), *Solenaia carinata* (D), and *S. oleivora* (E) populations in the Fuhe and Gan Rivers. These analyses reconstruct the population size history assuming an evolutionary rate of 2.0×10^{-8} substitutions/site/year. The black line represents estimates of mean effective population size and the gray-lined section delineates the 95% highest posterior density limits.

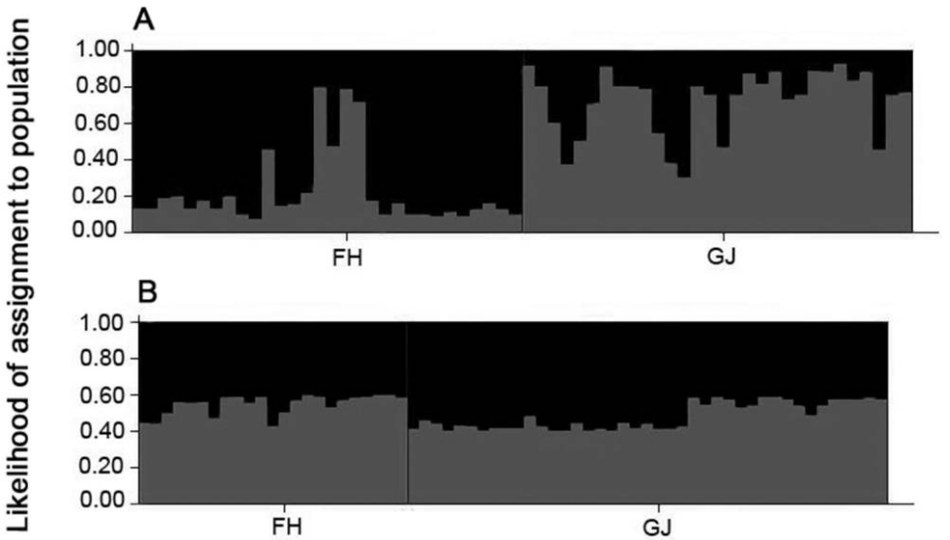

Figure 5. STRUCTURE bar plots for *Nodularia douglasiae* (A), and *Solenaia carinata* (B) populations in the Fuhe River (FH) and the Gan River (GJ). Both of these species had genetic populations (K) = 2. Results for species with $K = 1$ (*Anemina arcaeformis*, *Lamprotula caveata*, and *S. oleivora*) are not shown. STRUCTURE runs were completed without a priori populations assigned, and we assumed admixture and correlated alleles existed.

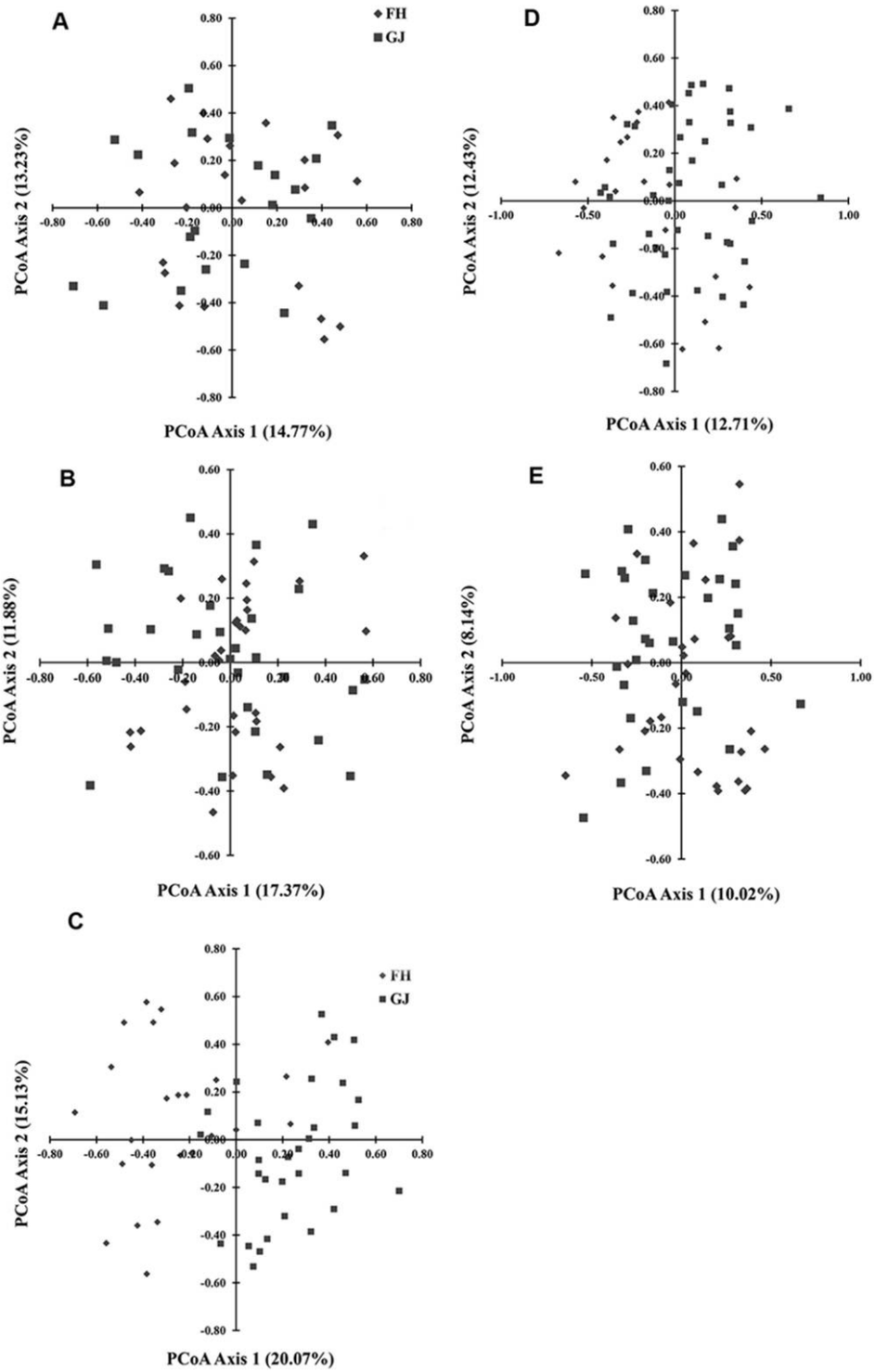

Figure 6. Principal coordinates analyses (PCoA) based on multi-locus microsatellite genotypes of individuals of *Anemina arcaeformis* (A), *Lamprotula caveata* (B), *Nodularia douglasiae* (C), *Solenaia carinata* (D), and *S. oleivora* (E) from the Fuhe River (FH, squares) and the Gan River (GJ, diamonds).

diversity found in *S. carinata* and much higher diversity found in *S. oleivora*. This may be because *S. carinata* is less common than *S. oleivora* (Cao et al. 2018). Although both have similar morphology and life styles, *S. carinata* population size, density, and distribution were much smaller than that of *S. oleivora*, and the ratio of *S. carinata* and *S. oleivora* from the catch of local fishermen was 1:37.5 (Cao et al. 2018). The genetic diversity of *S. oleivora* was the highest of all the species analyzed, possibly because this species has not experienced recent demographic declines (Xu, 2014, Cao et al. 2018, Wu et al. 2018). Not only did the genetic diversity of imperiled species differ from species that were more widespread and common, there were considerable differences in genetic diversity among the IUCN's least-concern species, including *A. arcaeformis*, *L, caveata*, and *N. douglasiae*. This indicates that there may not be a single general pattern of genetic diversity for mussels in this region and that using common species as surrogates for investigating patterns of genetic diversity for imperiled species may not be possible (Galbraith et al. 2015).

Effects of human disturbance on genetic structure

Geographic isolation is an important factor that affects distribution patterns and genetic structure of species (Hayes et al. 2008, Lv et al. 2013). The effects of dams, sand mining, water pollution, overfishing, and other human disturbances have the potential to reduce gene flow and dispersal (of sperm and host fishes) which results in more rapid loss of genetic diversity in isolated populations (Haag 2012, Ferguson et al. 2013). In our study, the haplotype networks and AMOVA of the COI dataset showed a general lack of geographic resolution among the haplotypes for *A. arcaeformis*, *L. caveata*, *S. carinata*, and *S. oleivora*. Only *N. douglasiae* had groups of haplotypes that were strongly segregated between the Fuhe and Gan Rivers. Based on the microsatellite dataset, *L. caveata*, *N. douglasiae*, and *S. carinata* populations differed between the 2 collection locations, but only *N. douglasiae* had F_{st} values that would be considered moderate differentiation ($0.05 < F_{st} < 0.15$; Wright 1965). Even for species with $F_{st} > 0.05$ showed evidence of admixture, possibly because collection sites were close together. Detecting changes in genetic structure because of population fragmentation associated with anthropogenic habitat alteration (i.e., construction of dams) in species with long generation times and large effective populations may not be possible when the alterations to habitat or barrier construction are recent (Hoffman et al. 2017).

The MDA showed that populations of *L. caveata*, *N. douglasiae*, and *S. oleivora* have been historically stable and have not experienced any population expansions. In contrast, *A. arcaeformis* and *S. carinata* both showed evidence of a population expansion. These expansions could be related to human population expansion and watercourse and habitat alterations following the advent of intensification of agriculture (Zhao et al. 2013). Alternatively, long-term ecological or climatic changes unrelated to human activities could be attributed to these population expansions (Zhao et al. 2013). Although the Bayesian skyline plots show a population size that is stable over time, it is characterized by a small, weakly-supported, ancient increase in population size ~200,000 to 300,000 ybp and 10,000 to 50,000 ybp for *L. caveata* and *S. oleivora*, respectively.

Conservation implications

Freshwater mussels are simultaneously among the most diverse and endangered faunal groups in the world (Strayer et al. 2004, Bogan 2008). The life history of freshwater mussels can influence their geographical distribution, genetic structure, and demographic characteristics because of their reliance on host fishes for larval development and dispersal (Watters 1992, Mock et al. 2013). The numerous, potentially synergistic, and ongoing threats to the diversity of Chinese freshwater mussels (summarized above and in Zieritz et al. 2018) have the potential to cause rapid declines of both freshwater mussels and their host fishes in the Yangtze River drainage (Xiong et al. 2012). The generation of baseline genetic diversity data will help guide species conservation and recovery programs. Our study reinforces that there is considerable variation in both the spatial patterns of genetic diversity and genetic structure among freshwater mussels, which may be related brooding periods and parasitism characteristics (e.g., Mock et al. 2013, Lopes-Lima et al. 2015, Zanatta et al. 2018), even at relatively-small geographic scales (Galbraith et al. 2015). However, a general lack of information on life history, reproductive traits and timing, and host fish identities are major limiting factors for making conservation assessments and recovery for freshwater mussels in China. Due to the critical nature of the parasitic stage of freshwater mussels to their continued survival (Haag 2012), increased emphasis on life history and reproductive biology research for Chinese taxa is needed (e.g., Wu et al. 2018). Thus, management practices that seek to conserve genetic diversity cannot necessarily be broadly applied to all co-occurring species.

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