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A Comparative Phylogeographic Approach to Facilitate Recovery of an Imperiled Freshwater Mussel (Bivalvia: Unionida: *Potamilus inflatus*)

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Abstract: North American freshwaters are among the world's most threatened ecosystems, and freshwater mussels are among the most imperiled inhabiting these systems. A critical aspect of conservation biology is delineating patterns of genetic diversity, which can be difficult when a taxon has been extirpated from a significant portion of its historical range. In such cases, evaluating conservation and recovery options may benefit by using surrogate species as proxies when assessing overall patterns of genetic diversity. Here, we integrate the premise of surrogate species into a comparative phylogeographic framework to hypothesize genetic relationships between extant and extirpated populations of *Potamilus inflatus* by characterizing genetic structure in co-distributed congeners with similar life histories and dispersal capabilities. Our mitochondrial and nuclear sequence data exhibited variable patterns of genetic divergence between *Potamilus* spp. native to the Mobile and Pascagoula + Pearl + Pontchartrain (PPP) provinces. However, hierarchical Approximate Bayesian Computation indicated that the diversification between Mobile and PPP clades was synchronous and represents a genetic signature of a common history of vicariance. Recent fluctuations in sea-level appear to have caused *Potamilus* spp. in the PPP to form a single genetic cluster, providing justification for using individuals from the Amite River as a source of brood stock to re-establish extirpated populations of *P. inflatus*. Future studies utilizing eDNA and genome-wide molecular data are essential to better understand the distribution of *P. inflatus* and establish robust recovery plans. Given the imperilment status of freshwater mussels globally, our study represents a useful methodology for predicting relationships among extant and extirpated populations of imperiled species.

Keywords: surrogate species; phylogenetics; synchronous diversification; endangered species; genetic management; conservation

1. Introduction

Due to anthropogenic alterations to the environment, the world is losing species at rates comparable to mass extinctions during major transitions of geological time periods [1,2]. North American freshwaters are among the world's most threatened ecosystems [3], and freshwater mussels (Bivalvia: Unionida) are among the most imperiled groups of organisms inhabiting these systems with 65% of all recognized species considered to be of conservation concern [4–6]. Several inherent biological characters (e.g., limited locomotive capabilities in many species, extreme sensitivity to pollutants, obligate parasitism, and filter feeding) have disproportionately impacted mussels in anthropogenically dominated landscapes [7–9], leading to extensive population decline of both common and rare species [4,6,10]. Given these declines, establishing robust species-specific status assessments is essential toward future implementation of effective conservation and recovery strategies for these highly imperiled organisms [6,11].

One critical aspect of conservation biology is delineating patterns of genetic diversity across geographic ranges of species [12]. In general, freshwater organisms have unique biogeographic constraints as they are restricted by both terrestrial and marine barriers. Thus, dispersal between watersheds is primarily limited to connectivity of freshwaters during rare geologic events and often leaves unique genetic signatures [13,14]. Comparative phylogeographic approaches offer options for understanding the effects of geological processes on observed genetic diversity in co-distributed taxa with similar dispersal capabilities and life histories [15,16]. Multiple studies have used comparative phylogeography to resolve the evolutionary history of aquatic taxa in the southeastern United States and showed concordance in phylogeographic clustering across co-distributed taxa [17–19]. However, these examples have concentrated on relatively common species, and determining relationships among populations of imperiled species can be problematic when taxa have been extirpated from a significant portion of their historical range. The use of surrogate species (e.g., common species with similar life history characteristics used as proxies for imperiled species) is increasingly being used in conservation practices [20], but this methodology has not been explored in many freshwater taxa [21]. Studies focused on freshwater mussels have compared genetic structure among common and rare species [22–26]; however, no study has used resolved phylogeographic patterns among co-distributed congeners to make inferences regarding the hypothetical relationship between extant and extirpated populations. Here, we explore the use of comparative phylogeography for hypothesizing relationships among extant and extirpated populations of an imperiled freshwater mussel species by characterizing genetic structure in co-distributed taxa with analogous dispersal capabilities and life histories.

The genus *Potamilus* is a highly specialized group of freshwater mussels consisting of ten currently recognized species [27,28], including *P. fragilis* and *P. leptodon* (formerly *Leptodea*), which were added to the genus in a recent phylogenomic study [28]. All species in this genus have similar life history characteristics, including brooding phenology and reproductive timing, early maturation, high fecundity, parasitic growth during encystment, and specialized infection of *Aplodinotus grunniens* [28,29]. One species in this genus, *Potamilus inflatus*, is listed as threatened under the United States Endangered Species Act (ESA) [30] and was historically distributed throughout the Mobile, Pearl, and Lake Pontchartrain drainages [31,32]. Systematic habitat destruction has extirpated the species from much of its historical range and extant populations are restricted to the Tombigbee and Black Warrior rivers in the Mobile Basin, and a 40 km-long stretch of the Amite River in the Lake Pontchartrain drainage [33,34]. Concomitant to extirpation throughout large portions of the Lake Pontchartrain drainage, *P. inflatus* is believed to be extirpated from the entire Pearl River system [33,35]. Only two live individuals (MMNS13211; [36]) and three dead shells have ever been collected in the Pearl River system [37] despite extensive surveys throughout the basin [38,39]. Further, a mill spill in 2011 led to extensive fish and mussel kills (estimated total of 591,561 fish and mussels) throughout the presumptive range of *P. inflatus* in the Pearl River, however, no specimens of *P. inflatus* were salvaged [40,41].

Understanding genetic diversity across populations of *P. inflatus* is critical to determine threats to extant populations and establish effective recovery strategies to re-establish the species throughout its historical range, especially considering a previous assessment identified significant intra-specific variation between extant populations [42]. This problem is of the upmost importance given the threatened status of *P. inflatus* under the ESA and the possibility of recovery if viable populations are re-established in historically occupied areas where the species has been considered extirpated [35]. To facilitate conservation and recovery, we use phylogeographic techniques to evaluate range-wide genetic diversity within *P. inflatus* and sympatric congeners *P. fragilis* and *P. purpuratus* using multi-locus sequence data. Next, we integrate the premise of surrogate species into a comparative phylogeographic framework to hypothesize genetic relationships between extant and extirpated populations of *P. inflatus* by characterizing genetic structure in co-distributed congeners with similar life histories to better inform conservation and recovery planning.

2. Materials and Methods

2.1. Taxon Sampling

We examined genetic diversity from co-distributed members of *Potamilus* native to the Mobile, Pascagoula, Pearl, and Pontchartrain drainages (Table 1; Figure 1). A total of 103 individuals were examined in this study (Table 1), and more details on the specimens and collections are available on ScienceBase (https://doi.org/10.5066/P9Q3CFL5) and Johnson and Smith (Under Review). Mantle tissue clips from vouchered individuals in public museums were used to extract genomic DNA using the Qiagen PureGene DNA extraction kit with the standard extraction protocol (Qiagen, Hilden, Germany). We amplified and sequenced two mitochondrial (mtDNA) loci commonly used in freshwater mussel phylogenetic studies: a partial portion of *cytochrome c oxidase subunit 1* (CO1) and *NADH dehydrogenase subunit 1* (ND1). For all *P. inflatus* and a subset of *P. fragilis* and *P. purpuratus*, we sequenced three nuclear (nDNA) loci: the commonly used *internal transcribed spacer 1* (ITS1), and two protein-coding loci *fem-1 homolog C* (FEM1) and *UbiA prenyltransferase domain-containing protein 1* (UbiA). A subset of individuals representing the geographic range of *P. fragilis* and *P. purpuratus* were chosen for the additional nDNA loci due to the high prevalence of multiple copies at ITS1 and low genetic diversity at FEM1 and UbiA (Table 1; Figure 1). We utilized two recently developed primer sets from Johnson and Smith (Under Review) to amplify FEM1 and UbiA based on data generated in phylogenetic studies using the recently developed anchored hybrid enrichment probe set Unioverse [28,43,44]. Primers for all loci and thermal cycling conditions are reported in Table 2.

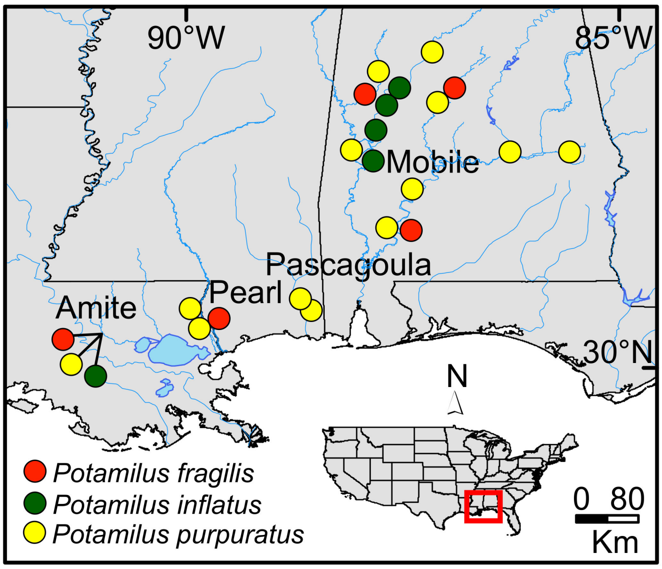

Figure 1. Collection locations for *Potamilus fragilis* (red), *P. inflatus* (green), and *P. purpuratus* (yellow) in the Amite (Pontchartrain), Mobile, Pascagoula, and Pearl River drainages.

Table 1. Molecular material examined in this study. Museum abbreviations are as follows: UA—Alabama Museum of Natural History; UF—Florida Museum. GenBank or SRA accession numbers are provided for each locus, and missing values were not used in phylogenetic analyses.

Taxon	ID	Drainage	Source	CO1	ND1	ITS1	FEM1	UbiA
Potamilus fragilis	LfraAla001	Mobile	UF438237	MT662019	MT669665	MT661766	MT669798	MT669771
Potamilus fragilis	LfraAmi040	Pontchartrain	UF439330	MT662020	MT669666	MT661773	MT669778	MT669751
Potamilus fragilis	LfraAmi041	Pontchartrain	UF439352	MT662021	MT669667			
Potamilus fragilis	LfraAmi042	Pontchartrain	UF439352	MT662022	MT669668			
Potamilus fragilis	LfraPrl043	Pearl	UF439332	MT662023	MT669669			
Potamilus fragilis	LfraPrl044	Pearl	UF439332	MT662024	MT669670	MT661780	MT669785	MT669758
Potamilus fragilis	LfraPrl045	Pearl	UF439365	MT662025	MT669671			
Potamilus fragilis	LfraPrl046	Pearl	UF439343	MT662026	MT669672			
Potamilus fragilis	LfraPrl047	Pearl	UF439343	MT662027	MT669673			
Potamilus fragilis	LfraPrl048	Pearl	UF439343	MT662028	MT669674			
Potamilus fragilis	LfraAmi057	Pontchartrain	UF439529	MT662029	MT669675			
Potamilus fragilis	LfraAmi058	Pontchartrain	UF439529	MT662030	MT669676			
Potamilus fragilis	LfraAmi059	Pontchartrain	UF439529	MT662031	MT669677			
Potamilus fragilis	LfraMob063	Mobile	UF439528	MT662033	MT669679			
Potamilus fragilis	LfraMob064	Mobile	UF439528	MT662032	MT669678			
Potamilus fragilis	LfraMob065	Mobile	Uncatologed	MT662034	MT669680	MT661792	MT669797	MT669770
Potamilus inflatus	PinfMob001	Mobile	UF439131	MT662002	MT669647	MT661768	MT669773	MT669746
Potamilus inflatus	PinfMob002	Mobile	UF439131	MK044952	MK045103	MK036203	MT669774	MT669747
Potamilus inflatus	PinfMob003	Mobile	UF439131	MT662003	MT669648	MT661769	MT669775	MT669748
Potamilus inflatus	PinfMob004	Mobile	UF439131	MK044953	MK045104	MK036204	SRR10579071	SRR10579071
Potamilus inflatus	PinfMob005	Mobile	UF439131	MT662004	MT669649	MT661770	MT669776	MT669749
Potamilus inflatus	PinfMob006	Mobile	UF439131	MT662005	MT669650	MT661771	MT669777	MT669750

Table 1. *Cont.*

Taxon	ID	Drainage	Source	CO1	ND1	ITS1	FEM1	UbiA
Potamilus inflatus	PinfAmi010	Pontchartrain	UF439530	MT662006	MT669651	MT661774	MT669779	MT669752
Potamilus inflatus	PinfAmi011	Pontchartrain	UF439530	MT662007	MT669652	MT661775	MT669780	MT669753
Potamilus inflatus	PinfAmi012	Pontchartrain	UF439531	MT662008	MT669653	MT661776	MT669781	MT669754
Potamilus inflatus	PinfAmi013	Pontchartrain	UF439532	MT662009	MT669654	MT661777	MT669782	MT669755
Potamilus inflatus	PinfAmi014	Pontchartrain	UF439532	MT662010	MT669655	MT661778	MT669783	MT669756
Potamilus inflatus	PinfAmi015	Pontchartrain	UF439533	MT662011	MT669656	MT661779	MT669784	MT669757
Potamilus inflatus	PinfMob019	Mobile	UF439514	MT662012	MT669657	MT661783	MT669788	MT669761
Potamilus inflatus	PinfMob020	Mobile	UF439514	MT662013	MT669658	MT661784	MT669789	MT669762
Potamilus inflatus	PinfMob021	Mobile	UF439514	MT662014	MT669659	MT661785	MT669790	MT669763
Potamilus inflatus	PinfMob022	Mobile	UF439514	MT662015	MT669660	MT661786	MT669791	MT669764
Potamilus inflatus	PinfMob023	Mobile	UF439514	MT662016	MT669661	MT661787	MT669792	MT669765
Potamilus inflatus	PinfMob017	Mobile	UF439513	MT662017	MT669662	MT661788	MT669793	MT669766
Potamilus inflatus	PinfMob018	Mobile	UF439513	MT662018	MT669663	MT661789	MT669794	MT669767
Potamilus inflatus	PinfMob016	Mobile	UA2696		MT669664	MT661781	MT669786	MT669759
Potamilus purpuratus	PpurPas001	Pascagoula	UF438434	MT662035	MT669681			
Potamilus purpuratus	PpurPrl022	Pearl	UF439145	MT662036	MT669682			
Potamilus purpuratus	PpurPrl023	Pearl	UF439145	MK044960	MK045111	MK036211	MT669799	MT669772
Potamilus purpuratus	PpurPrl024	Pearl	UF439145	MK044961	MK045112	MK036212		
Potamilus purpuratus	PpurPrl025	Pearl	UF439145	MT662037	MT669683			
Potamilus purpuratus	PpurPrl026	Pearl	UF439145	MT662038	MT669684	MT661767		
Potamilus purpuratus	PpurAmi038	Pontchartrain	UF439452	MT662039	MT669685			
Potamilus purpuratus	PpurAmi039	Pontchartrain	UF439452	MT662040	MT669686			
Potamilus purpuratus	PpurAmi040	Pontchartrain	UF439452	MT662041	MT669687			

Table 1. *Cont.*

Taxon	ID	Drainage	Source	CO1	ND1	ITS1	FEM1	UbiA
Potamilus purpuratus	PpurAmi041	Pontchartrain	UF439452	MT662042	MT669688			
Potamilus purpuratus	PpurAmi042	Pontchartrain	UF439452	MT662043	MT669689			
Potamilus purpuratus	PpurAmi043	Pontchartrain	UF439453	MT662044	MT669690			
Potamilus purpuratus	PpurAmi044	Pontchartrain	UF439453	MT662045	MT669691			
Potamilus purpuratus	PpurAmi045	Pontchartrain	UF439453	MT662046	MT669692	MT661772	SRR10579081	SRR10579081
Potamilus purpuratus	PpurAmi046	Pontchartrain	UF439453	MT662047	MT669693			
Potamilus purpuratus	PpurAmi047	Pontchartrain	UF439453	MT662048	MT669694			
Potamilus purpuratus	PpurAmi048	Pontchartrain	UF439454	MT662049	MT669695			
Potamilus purpuratus	PpurAmi049	Pontchartrain	UF439454	MT662050	MT669696			
Potamilus purpuratus	PpurAmi050	Pontchartrain	UF439454	MT662051	MT669697			
Potamilus purpuratus	PpurAmi051	Pontchartrain	UF439454	MT662052	MT669698			
Potamilus purpuratus	PpurPrl052	Pearl	UF439456	MT662053	MT669699			
Potamilus purpuratus	PpurPrl053	Pearl	UF439456	MT662054	MT669700			
Potamilus purpuratus	PpurPrl054	Pearl	UF439457	MT662055	MT669701			
Potamilus purpuratus	PpurPrl055	Pearl	UF439457	MT662056	MT669702			
Potamilus purpuratus	PpurPrl056	Pearl	UF439457	MT662057	MT669703			
Potamilus purpuratus	PpurPrl057	Pearl	UF439457	MT662058	MT669704			
Potamilus purpuratus	PpurPrl058	Pearl	UF439457	MT662059	MT669705			
Potamilus purpuratus	PpurPrl059	Pearl	UF439456	MT662060	MT669706			
Potamilus purpuratus	PpurPrl060	Pearl	UF439456	MT662061	MT669707			
Potamilus purpuratus	PpurPrl061	Pearl	UF439456	MT662062	MT669708			
Potamilus purpuratus	PpurPrl062	Pearl	UF439456	MT662063	MT669709			
Potamilus purpuratus	PpurPrl063	Pearl	UF439456	MT662064	MT669710			
Potamilus purpuratus	PpurPrl064	Pearl	UF439458	MT662065	MT669711			

Table 1. *Cont.*

Taxon	ID	Drainage	Source	CO1	ND1	ITS1	FEM1	UbiA
Potamilus purpuratus	PpurPrl065	Pearl	UF439459	MT662066	MT669712			
Potamilus purpuratus	PpurPrl066	Pearl	UF439459	MT662067	MT669713			
Potamilus purpuratus	PpurPrl067	Pearl	UF439459	MT662068	MT669714			
Potamilus purpuratus	PpurPrl068	Pearl	UF439459	MT662069	MT669715			
Potamilus purpuratus	PpurPrl069	Pearl	UF439459	MT662070	MT669716			
Potamilus purpuratus	PpurMob081	Mobile	UA62	MT662071	MT669717			
Potamilus purpuratus	PpurMob082	Mobile	UA2469	MT662072	MT669718			
Potamilus purpuratus	PpurMob083	Mobile	UA2510	MT662073	MT669719			
Potamilus purpuratus	PpurMob084	Mobile	UA2562	MT662074	MT669720			
Potamilus purpuratus	PpurMob085	Mobile	UA2740	MT662075	MT669721	MT661782	MT669787	MT669760
Potamilus purpuratus	PpurMob086	Mobile	UA3100	MT662076	MT669722			
Potamilus purpuratus	PpurMob087	Mobile	UA3123	MT662077	MT669723			
Potamilus purpuratus	PpurMob088	Mobile	UA3205	MT662078	MT669724			
Potamilus purpuratus	PpurMob089	Mobile	UA3417	MT662079	MT669725			
Potamilus purpuratus	PpurMob090	Mobile	UA3482	MT662080	MT669726			
Potamilus purpuratus	PpurPas097	Pascagoula	UF439510	MT662081	MT669727			
Potamilus purpuratus	PpurPas098	Pascagoula	UF439510	MT662082	MT669728			
Potamilus purpuratus	PpurPas099	Pascagoula	UF439510	MT662083	MT669729			
Potamilus purpuratus	PpurPas100	Pascagoula	UF439510	MT662084	MT669730			
Potamilus purpuratus	PpurPas101	Pascagoula	UF439510	MT662085	MT669731	MT661790	MT669795	MT669768
Potamilus purpuratus	PpurPas102	Pascagoula	UF439510	MT662086	MT669732			
Potamilus purpuratus	PpurPas103	Pascagoula	UF439510	MT662087	MT669733			
Potamilus purpuratus	PpurMob107	Mobile	UF439527	MT662088	MT669734	MT661791	MT669796	MT669769
Potamilus purpuratus	PpurMob108	Mobile	UF439527	MT662089	MT669735			

Table 1. *Cont.*

Taxon	ID	Drainage	Source	CO1	ND1	ITS1	FEM1	UbiA
Potamilus purpuratus	PpurMob109	Mobile	UF439527	MT662090	MT669736			
Potamilus purpuratus	PpurMob110	Mobile	UF439527	MT662091	MT669737			
Potamilus purpuratus	PpurMob111	Mobile	UF439527	MT662092	MT669738			
Potamilus purpuratus	PpurMob112	Mobile	UF439527	MT662093	MT669739			
Potamilus purpuratus	PpurMob113	Mobile	UF439527	MT662094	MT669740			
Potamilus purpuratus	PpurMob114	Mobile	UF439527	MT662095	MT669741			
Potamilus purpuratus	PpurMob115	Mobile	UF439527	MT662096	MT669742			
Potamilus purpuratus	PpurMob116	Mobile	UF439527	MT662097	MT669743			
Potamilus purpuratus	PpurMob117	Mobile	UF439527	MT662098	MT669744			
Potamilus purpuratus	PpurMob118	Mobile	UF439527	MT662099	MT669745			

PCRs were conducted using a 25 μL mixture of the following: molecular grade water (9.5 μL), MyTaq^{TM} Red Mix (12.5 μL; Bioline), primers (1.0 μL each) and DNA template (100 ng). Products were sent to Molecular Cloning Laboratories (McLAB, South San Francisco, CA, USA) for bi-directional sequencing on an ABI 3730. Geneious v 10.2.3 was used to assemble contigs and edit chromatograms [45], and sequences were aligned in Mesquite v 3.31 [46] using MAFFT v 7.311 [47]. Loci were aligned independently using the L-INS-i method in MAFFT and translated into amino acids to ensure absence of stop codons and gaps. Incomplete codons at each terminal end were removed. The total number of individuals included for each locus are as follows: CO1-102, ND1-103, FEM1-29, UBiA-29, and ITS1-31. Novel GenBank accessions for this study were as follows: CO1: MT662002–MT662099; FEM1: MT669773–MT669799; ITS1: MT661766–MT661792; ND1: MT669647–MT669745; and UbiA: MT669746–MT669772 (Table 1).

2.2. Phylogenetic Analyses

Phylogenetic reconstruction was performed on our five-locus molecular matrix consisting of 28 individuals and 3368 bp (CO1 = 657 bp; ND1 = 900 bp; FEM1 = 501 bp; UbiA = 765 bp; ITS1 = 545 bp) using IQ-TREE v 2.0-rc1 [48,49]. Both mtDNA and nDNA protein coding genes were partitioned by codon position. Partitions and substitution models for the analysis were determined by ModelFinder [50] using Bayesian inference criteria. We used 10 independent runs of an initial tree search and 1000 ultrafast bootstrap replicates (BS) for nodal support [51].

Coalescent-based approaches have been repeatably criticized to delimit populations and not species [52], including in freshwater mussels [53–55]. Here, we use the Bayesian coalescent-based model STACEY [56] in BEAST v 2.6.2 [57] to define genetic clusters in our molecular dataset for downstream analysis. STACEY allows for the inclusion of individuals with missing data, so we included all available data for the 5 loci in the analysis. *Potamilus* spp. were binned by drainage of capture, and we allowed the model to freely assign drainages to appropriate clusters. A substitution model for each locus alignment was determined using ModelFinder, a strict molecular clock was set at 1.0 for CO1, and clock rates for the four additional loci were estimated by STACEY. The Epi Tree prior was used as the species tree prior with a collapse height of 0.0001. Our analyses executed 10^9 generations and logged every 5000 trees with an initial 10% burn-in. Effective sample size (ESS) was ensured using Tracer v 1.7 [57], and the most likely number of clusters was calculated by SpeciesDelimitationAnalyser (SpeciesDA) v 1.8.0 [56] with a collapse height of 0.0001, a 1.0 simcutoff, and an initial 10% burn-in (2000 trees).

To estimate divergence times among well-supported clusters, we used the Bayesian coalescent-based model *BEAST [58] in BEAST. We chose a coalescent approach to account for concatenation methods, which typically overestimate the divergence times across species trees [59,60]. Similar to STACEY, *BEAST allows for the inclusion of individuals with missing data and all available data for the five loci were included in the analysis. For each species, individuals were grouped based on the most likely clusters resolved by STACEY: (1) Mobile; and (2) Pascagoula + Pearl + Pontchartrain (herein referred to as PPP). A strict molecular clock and an HKY model of nucleotide evolution was fit to each locus to better match priors for comparative phylogeographic analyses (see below). The substitution rate for CO1 was set to $2.56 \times 10^{-9} \pm 0.6 \times 10^{-9}$ substitutions per site per year [61], and substitution rates were estimated for the four additional loci. Yule process was used as the species tree prior paired with a piecewise linear and constant root population size model. The analysis was run for $1.5*10^9$ MCMC generations sampling every 5000 generations and a 10% burn-in. Effective sample size (ESS) was ensured using Tracer v 1.7 [57], and a maximum clade credibility tree was created using TreeAnnotator v 2.5 [57].

2.3. Phylogeographic Analyses

To visualize genetic divergence with respect to geographic distribution, we created a median joining haplotype network [62] for each of the three *Potamilus* spp. independently in PopART 1.7 [63] with the default epsilon value set at 0. Additionally, an analysis of molecular variance (AMOVA) was conducted for each species independently in PopART to further evaluate the geographic distribution of genetic diversity. Each analysis was performed on a concatenated alignment of CO1 and ND1, and missing data in both PopART analyses was handled using complete deletion. To further assess genetic variation within *Potamilus* spp. with regard to geography, we calculated DNA sequence divergence between clusters of *Potamilus* spp. using uncorrected pairwise genetic distances in MEGAX [64]. Partial deletion was used to handle missing data in MEGAX calculations. For haplotype networks, species were grouped by drainage and groups for all other analyses were as follows: *P. fragilis* from the Mobile and Pearl + Pontchartrain, *P. inflatus* from the Mobile and Pontchartrain, and *P. purpuratus* from the Mobile and PPP.

2.4. Comparative Phylogeography

We tested for simultaneous divergence between clusters of *Potamilus* spp. defined by STACEY under a hierarchical Approximate Bayesian Computation (hABC) approach as implemented in the PyMsBayes package [65]. Specifically, we tested if divergence between Mobile and PPP clusters of *P. fragilis*, *P. inflatus*, and *P. purpuratus* was synchronous. PyMsBayes implements a modified version of *msBayes* [66] that specifies a Dirichlet-process prior (*dpp*) to compare fit of empirical data to simulated data under user-informed priors [14]. We used *dpp-msbayes* to test for synchronous divergence between Mobile and PPP clusters of *Potamilus* spp. using alignments from all available loci. We used results from our *BEAST divergence time analysis to guide prior selection for *dpp-msbayes* as follows: the concentration parameter [1000, 0.00141] in which there was prior probability for one, two, or three divergence events, population size (θ) [1, 0.0005], and divergence times (τ) [1, 0.01]. To allow *dpp-msbayes* to freely explore different divergence scenarios, we allowed the model to estimate independent parameters for each species (θ parameter = 012) and the number of divergence events (τ classes = 0). Transition-transversion rate of the HKY substitution model was estimated for each alignment independently using IQ-TREE. Our *dpp-msbayes* run performed a total of 10^7 simulations with 10,000 standardizing samples and reported every 20,000 simulations. We retained the 1000 simulations with the best fit to empirical data to estimate posterior probability (PP) values for each divergence scenario. To measure support for the number of divergence events, Bayes factors were measured using twice the difference of $-ln$ likelihood [67].

Table 2. Primers used for PCR and cycling conditions used in this study.

Locus	Primers	Source	Conditions
CO1	F: 5′-GTTCCACAAATCATAAGGATATTGG-3′ R: 5′-TACACCTCAGGGTGACCAAAAAACCA-3′	[68]	[69]
ND1	F: 5′-TGGCAGAAAAGTGCATCAGATTAAAGC-3′ R: 5′-CCTGCTTGGAAGGCAAGTGTACT-3′	[70]	[70]
ITS1	F: 5′-AAAAAGCTTCCGTAGGTGAACCTGCG-3′ R: 5′-AGCTTGCTGCGTTCTTCATCG-3′	[71]	[71]
FEM1	F: 5′- GTRATGGAGTATCGCAGTGT-3′ R: 5′-ACRCTYTTCCTGTTAACATC-3′	[44]	[44]
UbiA	F: 5′- TTTACTCCTGTTGCACTTGGGA-3′ R: 5′-AGCATCTGTCATGAAGACTCCAAC-3′	[44]	[44]

3. Results

Molecular Analyses

Five partitions and substitution models were determined by ModelFinder for phylogenetic reconstruction in IQ-TREE: TN + F + I for mtDNA codon 1 and nDNA codon 3, TN + F + I for mtDNA codon 2 and nDNA codon 2, K3Pu + F + G4 for mtDNA codon 3, F81 + F for nDNA codon 1, and K2P + I for ITS1. All species-level relationships had full support (BS = 100) and the only two major nodes that were not strongly supported (i.e., BS ≥ 95) were the PPP clade of *P. fragilis* (BS = 94) and the Mobile clade of *P. purpuratus* (BS = 92; Figure S1). All three taxa were resolved as monophyletic with *P. inflatus*, the sister to *P. fragilis* and *P. purpuratus*, aligning with findings in a previous phylogenetic study [28].

Substitution models determined by ModelFinder for locus alignments in the STACEY analysis were: HKY + I for CO1, HKY + I for ND1, JC for FEM1, F81 + I for UbiA, and K2P + I (=K80 + I) for ITS1. Convergence of the analysis was supported by all parameters having ESS values > 200, and all nodes were strongly supported (PP ≥ 95). SpeciesDA supported six clusters (54%): (1) *P. inflatus* from the Mobile, (2) *P. inflatus* from the Pontchartrain, (3) *P. fragilis* from the Mobile, (4) *P. fragilis* from the Pearl + Pontchartrain, (5) *P. purpuratus* from the Mobile, and (6) *P. purpuratus* from the PPP (Figure S2). The second most likely clustering scenario supported seven clusters (18.5%), with the Pearl population of *P. purpuratus* recognized as a distinct cluster.

The topological reconstruction from *BEAST was congruent with IQ-TREE and STACEY topologies, and all nodes were strongly supported (Figure 2A). Mobile and PPP clusters of *Potamilus* spp. were resolved as monophyletic with full support (PP = 100; Figure 2A). Convergence of the analysis was supported by all parameters having ESS values > 200. Divergence estimates differed slightly among Mobile and PPP clusters of *Potamilus* spp. The split between clusters of *P. inflatus* was estimated to have occurred ~2.13 Mya (95% CI 0.28–3.92 Mya; Figure 2A), and the splits between clusters within *P. fragilis* and *P. purpuratus* were estimated to have occurred more recently: ~1.35 Mya (95% CI 0.54–2.27 Mya) and ~0.72 Mya (95% CI 0.27–1.39 Mya), respectively (Figure 2A).

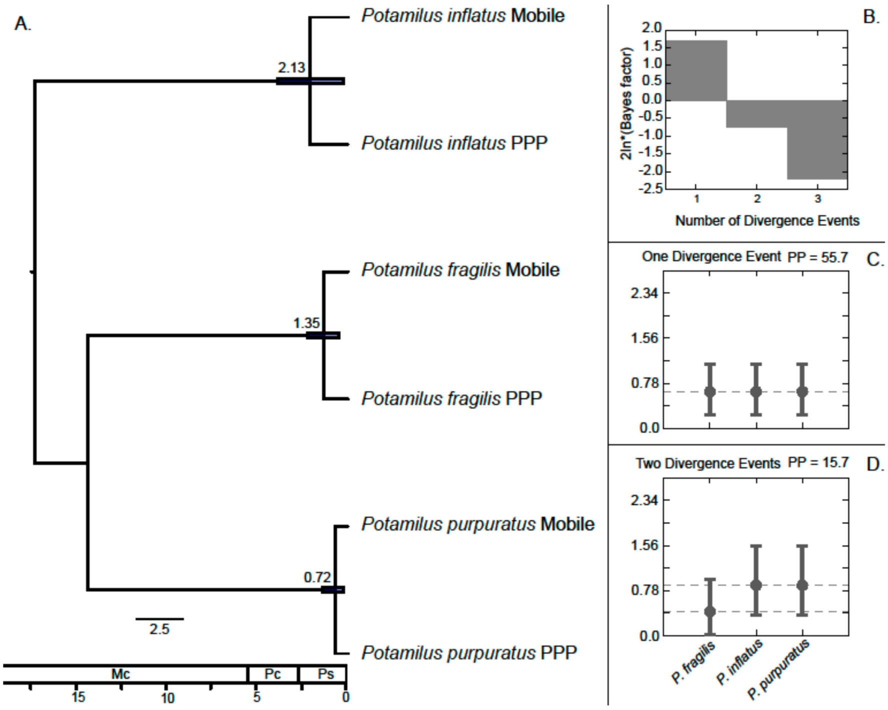

Figure 2. Summary of synchronous diversification analyses performed in *BEAST and *Dpp-msbayes*. (**A**) Phylogenetic reconstruction with divergence time scaled in million years before present; node bars represent the 95% confidence intervals. Values above branches represent estimated divergence time. Epoch abbreviations are as follows: Mc—Miocene, Pc—Pliocene, and Ps—Pleistocene. All nodes were strongly supported with posterior probability greater than 97. (**B**) Bayes Factor support for the number of divergence events generated by *Dpp-msbayes*. (**C**,**D**) The two most likely divergence scenarios between *Potamilus* species in the Mobile and PPP provinces resolved by *Dpp-msbayes* with posterior probability (PP) support of each scenario. Units on the *y*-axis are divergence times in million years before present.

Mean uncorrected p-distances between Mobile and PPP clusters for all species were larger than 1% and are reported in Table 3. Distance values were larger in *P. inflatus* (2.33%) when compared to *P. fragilis* (1.11%) and *P. purpuratus* (1.31%). Haplotype networks were concordant with phylogenetic analyses and showed clear separation between the Mobile and PPP groupings of all three *Potamilus* spp. (Figure 3). However, within the PPP province there was haplotype sharing between drainages in *P. fragilis* and *P. purpuratus* (Figure 3). AMOVAs indicated the majority of molecular variation was distributed between Mobile and PPP groups for all *Potamilus* spp. (Table 3). Molecular variance was higher within *P. fragilis* (19.1%) than *P. inflatus* (1.1%) and *P. purpuratus* (3.7%).

Table 3. Summary of analysis of molecular variance (AMOVA) analyses in PopArt. Sample sizes for each taxon from the Mobile drainage and Pascagoula + Pearl + Pontchartrain (PPP) are reported. All values for each comparison were found to be significant ($\alpha < 0.0001$).

Taxon	N Mobile	N PPP	AMOVA between	AMOVA within	Distance between (Uncorrected p)
Potamilus fragilis	4	12	80.9%	19.1%	1.11%
Potamilus inflatus	13	6	98.9%	1.1%	2.33%
Potamilus purpuratus	22	45	96.3%	3.7%	1.31%

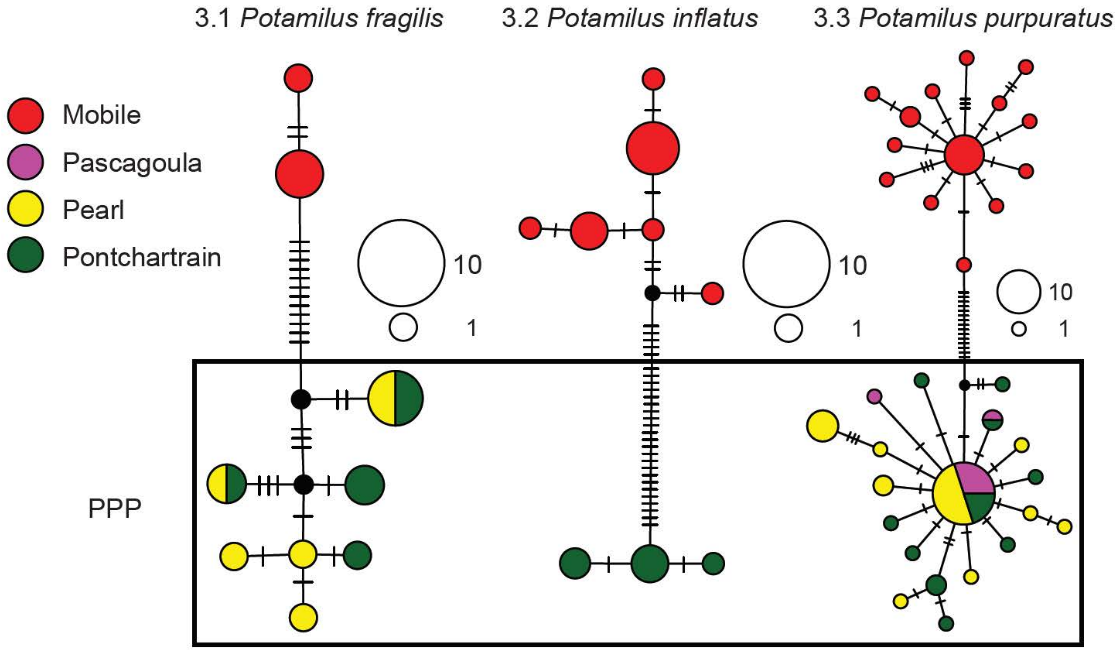

Figure 3. Haplotype networks based on a concatenated alignment of CO1 and ND1 for *Potamilus fragilis* (**A**), *P. inflatus* (**B**), and *P. purpuratus* (**C**). Each circle represents a unique haplotype and size is relative to the number of individuals. Black circles represent unsampled haplotypes and individual tick marks indicate nucleotide substitutions. Specimens are grouped by drainage of capture: Mobile, Pascagoula, Pearl, and Pontchartrain.

The *dpp-msbayes* analysis supported synchronous divergence between clusters of *Potamilus* spp. (Figure 2B–D). Support for a single divergence event was 55.7 PP, with the next best supported scenario of two divergence events (*P. inflatus* and *P. purpuratus* equal, and *P. fragilis* subsequently diverged independently) at 15.7 PP (Figure 2C,D). Similarly, Bayes factors indicated positive support for one divergence event ($2ln\text{BF} = 1.7$), and negative support for two ($2ln\text{BF} = -0.74$) and three ($2ln\text{BF} = -2.19$) divergence events (Figure 2B). The overlap of confidence intervals for divergence estimates in the *BEAST analysis and *dpp-msbayes* further supports evidence of synchronous divergence between *Potamilus* spp. (Figure 2A).

4. Discussion

Accurate evaluations of genetic diversity are a critical component in developing effective conservation and recovery strategies. The specific goal of our study was to characterize range-wide genetic variation of *P. inflatus*. Given the overall rarity of the species and plausible extirpation from multiple river systems, estimating genetic relationships across the historical range of *P. inflatus* is completely dependent on understanding the genetic composition of closely related and co-distributed species with similar dispersal capabilities and life histories. Our comparative phylogeographic approach integrated the premise of surrogate species to predict relationships among extant and extirpated populations. Although the use of comparative phylogeography has been used to characterize genetic diversity in common and rare species within freshwater mussels [22–26], the use of surrogate species within a comparative phylogeographic framework to hypothesize relationships among extant and extirpated populations of imperiled species is a novel approach. Below, we discuss the evolutionary forces driving congruent patterns of genetic divergence within *Potamilus* spp., and how our findings may impact future conservation and recovery efforts for *P. inflatus*.

4.1. Patterns of Genetic Variation in Potamilus Species

Large-scale environmental change has substantial effects on communities of species and associated microbiota [14,72,73]. This is certainly the case in mussels and their hosts, as biogeographical processes are a driver of faunal structure and genetic diversity [28,29,53,74–76]. Given biogeography is a critical driver of genetic variation, identifying faunal provinces is the first step toward understanding specific patterns of phylogeography [77]. Multiple attempts have been made to classify North American mussel fauna into biogeographic provinces [78–82], and understanding the processes that have driven faunal shifts across these regions has been integral toward understanding the evolution of the group [28,74,83]. In the case of the Mobile and PPP provinces, the drainages have been linked in hierarchical classifications of mussel diversity based on species composition [79]. Prior to our study, however, these relationships have not been tested in a molecular context. Our molecular analyses align with the hypothetical historical connection between the Mobile and PPP, as our phylogenetic and coalescent-based species delimitation analyses strongly supported *Potamilus* spp. in these biogeographic provinces as distinct clines. These results align with other mussel species showing genetic distinctiveness across these drainages [53,69,83,84], as well as other aquatic species [85–90].

The geological connection between the Mobile and PPP drainages has been hypothesized by numerous authors (reviewed by [91]) and a vicariance event between the two systems has likely driven the observed genetic differentiation in *Potamilus* spp. If a vicariance event was the causation of molecular diversification for all the species, we would expect to see similar patterns of divergence across *Potamilus* spp. Results from our molecular analyses, however, deviated from these expected patterns of genetic drift and showed varying levels of sequence divergence (Table 3). Specifically, genetic distance values between populations of *P. inflatus* were larger than those in *P. fragilis* and *P. purpuratus* (Table 3). However, it is an unrealistic expectation to assume that rates of evolution are identical between species, especially across geographically isolated populations [18,92,93]. Variable rates of molecular diversification within *Potamilus* spp. could be indicative of a variety of confounding variables, such as differing population demographics (e.g., population size, age structure), evolutionary processes (e.g., mutation rate, genetic drift, selection), or species-specific traits (e.g., habitat preferences) rather than multiple hypothetical vicariance events. To address this issue, we used a hABC approach to explicitly test whether divergence between Mobile and PPP populations of *Potamilus* spp. occurred synchronously. Our results suggest that the divergence between *Potamilus* spp. in the Mobile and PPP occurred simultaneously and further support previously described biogeographic provinces [79]. The causative event driving genetic differentiation between these groupings is uncertain, but additional molecular investigations in other freshwater mussels, as well as host fishes, may further elucidate the timing and patterns of faunal exchange between these two provinces.

Despite extensive geographic range within the PPP, our molecular data showed no diagnostic divergence between drainages within the province (Figure 3; Table 3). Limited genetic diversity was suspected within *P. inflatus* given there is only one extant population; however, the more common and wide-ranging species, *P. fragilis* and *P. purpuratus*, both showed haplotype sharing between drainages and no evidence of drainage specific structuring within the PPP (Figure 3A,C; Table 3). A signal for incomplete lineage sorting at nDNA loci is expected due to the effective population size being nearly four times that of mtDNA loci [94,95]; however, incomplete lineage sorting of mtDNA loci likely indicates relatively recent gene flow between populations. Approximately 18 Kya during the last glacial lowstand; geological evidence suggests the PPP drainages were connected [91,96], permitting gene flow to occur. Subsequent sea level rise from deglaciation began to form modern fluvial systems in the PPP [96], causing genetic isolation among contemporary populations of *Potamilus* spp. Given the hypothetical mtDNA mutation rates of freshwater mussels [61,97], it is an unrealistic expectation that mtDNA markers would become fixed across these drainages, and using more rapidly evolving markers (genotype-by-sequencing, whole genome resequencing) would be necessary to delineate fine-scale genomic differentiation among *Potamilus* spp. inhabiting these drainages or test for ongoing gene flow. However, only one extant population of *P. inflatus* occurs within the PPP (Amite River—Pontchartrain drainage) and it is a realistic expectation that the presumed extirpated populations of *P. inflatus* in the Pontchartrain and Pearl drainages would be most closely related to members of the Amite River given the patterns of genetic diversity seen in *P. fragilis* and *P. purpuratus*.

4.2. Implications on Conservation

Numerous practices have been proposed for delineating population units using genetic data for conservation and management [98–102], and in particular, ESA listed species have been partitioned into distinct population segments (DPSs), evolutionary significant units (ESUs), or management units (MUs) to facilitate recovery practices [101–104]. However, under the ESA, DPSs only apply to vertebrate species [103] and formal recognition of population units remains rare in freshwater mussels [105]. This is particularly concerning because information regarding population units is often required to facilitate conservation and recovery efforts [106]. In the case of *P. inflatus*, we observed high levels of molecular divergence at mtDNA loci. Formal recognition of ESUs are diagnosed based on reciprocal monophyly [104] and significant differences in allele frequencies at both mtDNA and nDNA loci [107]. Although individuals from the Amite River and Mobile drainage show evidence of fixation at mtDNA markers, we saw no evidence of fixation at nDNA loci, which would rule out the recognition of the two populations as ESUs. However, it is an unrealistic expectation that the nuclear loci used in this study would diagnose population units within species, and assessments with more rapidly evolving nuclear data (e.g., microsatellites, genome-wide SNPs) would facilitate delineation of ESUs. Nonetheless, molecular data from this study paired with available distributional information [34,35] provide ample evidence for the delineation of the Amite River and Mobile drainage populations of *P. inflatus* as two distinct MUs [102,104,107]. The designation of these MUs ensures the protection of irreplaceable genetic variation, and in particular, emphasizes conservation needs in the highly susceptible Amite River given its limited geographical distribution [34] and presumed extirpation of populations from adjacent systems that were hypothetically closely related based on our comparative phylogeographic approach. Future long-term monitoring efforts will be useful to identify specific population characteristics such as abundance, age-class structure, dispersal capabilities, and reproductive timing within these MUs and may lead to fine-scale delineation of population units.

Captive propagation of freshwater mussels is a critical component of recovery planning for many species [106,108] and likely the only viable option for re-establishing extirpated populations of *P. inflatus* [35], especially in the Pearl River drainage. Our assessment provides defensible justification for natural resource managers to use individuals from the Amite River rather than the Mobile drainage as a source of brood stock for recovery efforts for *P. inflatus* that include translocation or captive propagation in the Pearl and Pontchartrain drainages. Based on the likely scenario that extant populations of

P. inflatus are restricted to the Amite River and Mobile drainage, possible re-establishment sites include historically occupied reaches in the Bogue Chitto, Comite, Pearl, and Tangipahoa rivers.

5. Conclusions

Our study revealed congruent patterns of molecular diversification within a group of freshwater mussels with analogous life history traits and dispersal capabilities. Our findings suggest synchronous diversification between *Potamilus* spp. in the Mobile and PPP, which advances knowledge regarding the drivers of molecular diversification and biogeography of freshwater mussels in these provinces. Patterns of genetic variation in *Potamilus* spp. recovered by our comparative phylogeographic approach provided defensible justification for the use of the Amite River brood stock for re-establishing *P. inflatus* in PPP drainages. This finding provides direction for natural resource managers to develop appropriate recovery practices that may include captive propagation and translocation. Although a useful tool, without proper guidance and planning efforts, introduction of captive raised or translocated individuals has the potential to harm existing populations or nontarget species [109,110]. Recovery planning would greatly benefit from robust distributional information for *P. inflatus,* and future efforts utilizing both eDNA sampling and traditional surveys would help resolve whether the species is truly extirpated from select drainages. We also encourage further evaluations using fine-scale genomic markers and detailed genetic management planning to characterize genetic diversity of brood stock and captively bred individuals in efforts to maximize genetic diversity in augmented or re-establish populations.

Given the imperilment status of freshwater mussel species globally [111], our study represents a useful methodology for hypothesizing the genetic relationships of extant and extirpated populations of imperiled species to facilitate recovery planning. The use of mtDNA may be limited on a regional scale in most species; however, comparative phylogeographic approaches incorporating more rapidly evolving genome-wide markers introduces a more robust methodology for evaluating population dynamics within drainages and even at a local scale using surrogate species. As the understanding of phylogenetic relationships and life history characteristics continues to improve, utilizing comparative phylogeographic methodologies is a promising tool toward effective species recovery and long-term viability of freshwater mussels.

Supplementary Materials: The following figures are available online at http://www.mdpi.com/1424-2818/12/7/281/s1, Figure S1: Maximum likelihood (IQ-TREE) phylogenetic reconstruction based on a concatenated alignment of CO1, ND1, ITS1, FEM1, and UbiA. Values above branches represent ultrafast bootstrap support and information on taxon labels can be found in Table 1., Figure S2: Bayesian inference (STACEY) phylogenetic reconstruction based on a concatenated alignment of CO1, ND1, ITS1, FEM1, and UbiA. Terminals are collapsed to represent the best number of clusters ($n = 6$): Mobile and Pascagoula + Pearl + Pontchartrain (PPP) populations of *Potamilus fragilis*, *P. inflatus*, *P. purpuratus*. All branches were strongly supported by posterior probability being greater than or equal to 99.

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References

1. Butchart, S.H.M.; Walpole, M.; Collen, B.; van Strien, A.; Scharlemann, J.P.W.; Almond, R.E.A.; Baillie, J.E.M.; Bomhard, B.; Brown, C.; Bruno, J.; et al. Global biodiversity: Indicators of recent declines. *Science* **2010**, *328*, 1164–1168. [CrossRef]
2. Rands, M.R.W.; Adams, W.M.; Bennun, L.; Butchart, S.H.M.; Clements, A.; Coomes, D.; Entwistle, A.; Hodge, I.; Kapos, V.; Scharlemann, J.P.W.; et al. Biodiversity conservation: Challenges beyond 2010. *Science* **2010**, *329*, 1298–1303. [CrossRef]
3. Dudgeon, D.; Arthington, A.H.; Gessner, M.O.; Kawabata, Z.-I.; Knowler, D.J.; Lévêque, C.; Naiman, R.J.; Prieur-Richard, A.-H.; Soto, D.; Stiassny, M.L.J.; et al. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol. Rev.* **2006**, *81*, 163–182. [CrossRef]
4. Williams, J.D.; Warren, M.L.; Cummings, K.S.; Harris, J.L.; Neves, R.J. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* **1993**, *18*, 6–22. [CrossRef]
5. Strayer, D.L.; Downing, J.A.; Haag, W.R.; King, T.L.; Layer, J.B.; Newton, T.J.; Nichols, S.J. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience* **2004**, *54*, 429–439. [CrossRef]
6. Haag, W.R.; Williams, J.D. Biodiversity on the brink: An assessment of conservation strategies for North American freshwater mussels. *Hydrobiologia* **2014**, *735*, 45–60. [CrossRef]
7. Watters, G.T. Form and function of unionoidean shell sculpture and shape (Bivalvia). *Am. Malacol. Bull.* **1993**, *11*, 1–20.
8. Bringolf, R.B.; Cope, W.G.; Barnhart, M.C.; Mosher, S.; Lazaro, P.R.; Shea, D. Acute and chronic toxicity of pesticide formulations (atrazine, chlorpyrifos, and permethrin) to glochidia and juveniles of *Lampsilis siliquoidea*. *Environ. Toxicol. Chem.* **2007**, *26*, 2101–2107. [CrossRef]
9. Wang, N.; Ingersoll, C.G.; Greer, I.E.; Hardesty, D.K.; Ivey, C.D.; Kunz, J.L.; Brumbaugh, W.G.; Dwyer, F.J.; Roberts, A.D.; Augspurger, T.; et al. Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). *Environ. Toxicol. Chem.* **2007**, *26*, 2048–2056. [CrossRef]
10. Vaughn, C.C.; Taylor, C.M. Impoundments and the decline of freshwater mussels: A case study of an extinction gradient. *Conserv. Biol.* **1999**, *13*, 912–920. [CrossRef]
11. Ferreira-Rodríguez, N.; Akiyama, Y.B.; Aksenova, O.V.; Araujo, R.; Christopher Barnhart, M.; Bespalaya, Y.V.; Bogan, A.E.; Bolotov, I.N.; Budha, P.B.; Clavijo, C.; et al. Research priorities for freshwater mussel conservation assessment. *Biol. Conserv.* **2019**, *231*, 77–87. [CrossRef]
12. Allendorf, F.W.; Luikart, G.; Aitken, S.N. *Conservation and the Genetics of Populations*, 2nd ed.; Wiley-Blackwell: Hoboken, NJ, USA, 2013; ISBN 978-0-470-67145-0.
13. Unmack, P.J. Biogeography of Australian freshwater fishes. *J. Biogeogr.* **2001**, *28*, 1053–1089. [CrossRef]
14. Oaks, J.R. An improved approximate-Bayesian model-choice method for estimating shared evolutionary history. *BMC Evol. Biol.* **2014**, *14*, 150. [CrossRef] [PubMed]
15. Moritz, C.; Faith, D.P. Comparative phylogeography and the identification of genetically divergent areas for conservation. *Mol. Ecol.* **1998**, *7*, 419–429. [CrossRef]
16. Hickerson, M.J.; Carstens, B.C.; Cavender-Bares, J.; Crandall, K.A.; Graham, C.H.; Johnson, J.B.; Rissler, L.; Victoriano, P.F.; Yoder, A.D. Phylogeography's past, present, and future: 10 years after Avise, 2000. *Mol. Phylogenet. Evol.* **2010**, *54*, 291–301. [CrossRef]
17. Bermingham, E.; Avise, J.C. Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics* **1986**, *113*, 939–965.
18. Avise, J.C. Molecular population structure and the biogeographic history of a regional fauna: A case history with lessons for conservation biology. *Oikos* **1992**, *63*, 62–76. [CrossRef]
19. Walker, D.; Avise, J.C. Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. *Annu. Rev. Ecol. Syst.* **1998**, *29*, 23–58. [CrossRef]
20. Grantham, H.S.; Pressey, R.L.; Wells, J.A.; Beattie, A.J. Effectiveness of biodiversity surrogates for conservation planning: Different measures of effectiveness generate a kaleidoscope of variation. *PLoS ONE* **2010**, *5*, e11430. [CrossRef]

21. Stewart, D.R.; Underwood, Z.E.; Rahel, F.J.; Walters, A.W. The effectiveness of surrogate taxa to conserve freshwater biodiversity. *Conserv. Biol.* **2018**, *32*, 183–194. [CrossRef]
22. Galbraith, H.S.; Zanatta, D.T.; Wilson, C.C. Comparative analysis of riverscape genetic structure in rare, threatened and common freshwater mussels. *Conserv. Genet.* **2015**, *16*, 845–857. [CrossRef]
23. Elderkin, C.L.; Christian, A.D.; Metcalfe-Smith, J.L.; Berg, D.J. Population genetics and phylogeography of freshwater mussels in North America, *Elliptio dilatata* and *Actinonaias ligamentina* (Bivalvia: Unionidae). *Mol. Ecol.* **2008**, *17*, 2149–2163. [CrossRef]
24. Chong, J.P.; Roe, K.J. A comparison of genetic diversity and population structure of the endangered scaleshell mussel (*Leptodea leptodon*), the fragile papershell (*Leptodea fragilis*) and their host-fish the freshwater drum (*Aplodinotus grunniens*). *Conserv. Genet.* **2018**, *19*, 425–437. [CrossRef]
25. Mock, K.E.; Brim Box, J.C.; Chong, J.P.; Furnish, J.; Howard, J.K. Comparison of population genetic patterns in two widespread freshwater mussels with contrasting life histories in western North America. *Mol. Ecol.* **2013**, *22*, 6060–6073. [CrossRef]
26. Jones, J.W.; Neves, R.J.; Hallerman, E.M. Historical demography of freshwater mussels (Bivalvia: Unionidae): Genetic evidence for population expansion and contraction during the late Pleistocene and Holocene. *Biol. J. Linn. Soc.* **2015**, *114*, 376–397. [CrossRef]
27. Williams, J.D.; Bogan, A.E.; Butler, R.S.; Cummings, K.S.; Garner, J.T.; Harris, J.L.; Johnson, N.A.; Watters, G.T. A revised list of the freshwater mussels (Mollusca: Bivalvia: Unionida) of the United States and Canada. *Freshw. Mollusk Biol. Conserv.* **2017**, *20*, 33–58. [CrossRef]
28. Smith, C.H.; Pfeiffer, J.M.; Johnson, N.A. Comparative phylogenomics reveal complex evolution of life history strategies in a clade of bivalves with parasitic larvae (Bivalvia: Unionoida: Ambleminae). *Cladistics* **2020**, in press. [CrossRef]
29. Haag, W.R. *North American Freshwater Mussels: Natural History, Ecology, and Conservation*; Cambridge University Press: Cambridge, MA, USA, 2012; ISBN 978-0-521-19938-4.
30. USFWS. Endangered and threatened wildlife and plants; determination of threatened status for the Inflated Heelsplitter, *Potamilus inflatus*. *Fed. Regist.* **1990**, *55*, 39868–39872.
31. Williams, J.D.; Bogan, A.E.; Garner, J.T. *Freshwater Mussels of Alabama and the Mobile Basin in Georgia*; University of Alabama Press: Tuscaloosa, AL, USA, 2008; ISBN 978-0-8173-1613-6.
32. Jones, R.L.; Wagner, M.D.; Slack, W.T.; Peyton, J.S.; Hartfield, P.D. *Guide to the Identification and Distribution of Freshwater Mussels (Bivalvia: Unionidae) in Mississippi*; Mississippi Department of Wildlife, Fisheries, and Parks: Jackson, MS, USA, 2019.
33. Hartfield, P.D. *Status Survey for the Alabama Heelsplitter Mussel Potamilus Inflatus (Lea, 1831)*; U.S. Fish and Wildlife Service: Jackson, MS, USA, 1988.
34. Brown, K.M.; Daniel, W.M. The population ecology of the threatened Inflated Heelsplitter, *Potamilus inflatus*, in the Amite River, Louisiana. *Am. Midl. Nat.* **2014**, *171*, 328–339. [CrossRef]
35. USFWS. *Inflated Heelsplitter Mussel (Potamilus Inflatus) 5-Year Review: Summary and Evaluation*; US Fish and Wildlife Service: Daphne, AL, USA, 2014.
36. Frierson, L.S. A comparison of the Unionidae of the Pearl and Sabine rivers. *Nautilus* **1911**, *24*, 134–136.
37. George, S.G.; Reine, K.J. Rediscovery of the Inflated Heelsplitter mussel, *Potamilus inflatus*, from the Pearl River drainage. *J. Freshw. Ecol.* **1996**, *11*, 245–246. [CrossRef]
38. Brown, K.M.; Daniel, W.; George, G. The effect of Hurricane Katrina on the mussel assemblage of the Pearl River, Louisiana. *Aquat. Ecol.* **2010**, *44*, 223–231. [CrossRef]
39. Brown, K.M.; Banks, P.D. The conservation of unionid mussels in Louisiana rivers: Diversity, assemblage composition and substrate use. *Aquat. Conserv.* **2001**, *11*, 189–198. [CrossRef]
40. Brown, K.M.; Daniel, W.M. Mussel mortality from a toxic spill in the Pearl River, Louisiana. *Ellipsaria* **2012**, *14*, 28–31.
41. LDWF. *Investigation of a Fish and Mollusk Kill in the Lower Pearl River, Louisiana and Mississippi*; Louisiana Department of Wildlife and Fisheries: Baton Rouge, LA, USA, 2011.
42. Roe, K.J.; Lydeard, C. Molecular systematics of the freshwater mussel genus *Potamilus* (Bivalvia: Unionidae). *Malacologia* **1998**, *39*, 195–205.
43. Pfeiffer, J.M.; Breinholt, J.W.; Page, L.M. Unioverse: Phylogenomic resources for reconstructing the evolution of freshwater mussels (Unionoida). *Mol. Phylogenet. Evol.* **2019**, *137*, 114–126. [CrossRef] [PubMed]

44. Johnson, N.A.; Smith, C.H. Novel genetic resources to facilitate future molecular studies in freshwater mussels (Bivalvia: Unionidae). *Data*. under review.
45. Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; et al. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **2012**, *28*, 1647–1649. [CrossRef]
46. Maddison, W.P.; Maddison, D.R. Mesquite: A modular system for evolutionary analysis. Version 3.31. **2017**. Available online: https://www.mesquiteproject.org/ (accessed on 1 July 2020).
47. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef]
48. Minh, B.Q.; Schmidt, H.A.; Chernomor, O.; Schrempf, D.; Woodhams, M.D.; von Haeseler, A.; Lanfear, R. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* **2020**, *37*, 1530–1534. [CrossRef] [PubMed]
49. Chernomor, O.; von Haeseler, A.; Minh, B.Q. Terrace aware data structure for phylogenomic inference from supermatrices. *Syst. Biol.* **2016**, *65*, 997–1008. [CrossRef]
50. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.F.; von Haeseler, A.; Jermiin, L.S. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* **2017**, *14*, 587–589. [CrossRef]
51. Hoang, D.T.; Chernomor, O.; von Haeseler, A.; Quang Minh, B.; Sy Vinh, L. Ufboot2: Improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* **2018**, *35*, 518–522. [CrossRef] [PubMed]
52. Sukumaran, J.; Knowles, L.L. Multispecies coalescent delimits structure, not species. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 1607–1612. [CrossRef] [PubMed]
53. Smith, C.H.; Johnson, N.A.; Pfeiffer, J.M.; Gangloff, M.M. Molecular and morphological data reveal non-monophyly and speciation in imperiled freshwater mussels (*Anodontoides* and *Strophitus*). *Mol. Phylogenet. Evol.* **2018**, *119*, 50–62. [CrossRef]
54. Smith, C.H.; Johnson, N.A.; Inoue, K.; Doyle, R.D.; Randklev, C.R. Integrative taxonomy reveals a new species of freshwater mussel, *Potamilus streckersoni* sp. nov. (Bivalvia: Unionidae): Implications for conservation and management. *Syst. Biodivers.* **2019**, *17*, 331–348. [CrossRef]
55. Pfeiffer, J.M.; Johnson, N.A.; Randklev, C.R.; Howells, R.G.; Williams, J.D. Generic reclassification and species boundaries in the rediscovered freshwater mussel '*Quadrula*' *mitchelli* (Simpson in Dall, 1896). *Conserv. Genet.* **2016**, *17*, 279–292. [CrossRef]
56. Jones, G. Algorithmic improvements to species delimitation and phylogeny estimation under the multispecies coalescent. *J. Math. Biol.* **2017**, *74*, 447–467. [CrossRef] [PubMed]
57. Bouckaert, R.; Heled, J.; Kühnert, D.; Vaughan, T.; Wu, C.-H.; Xie, D.; Suchard, M.A.; Rambaut, A.; Drummond, A.J. BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* **2014**, *10*, e1003537. [CrossRef]
58. Heled, J.; Drummond, A.J. Bayesian inference of species trees from multilocus data. *Mol. Biol. Evol.* **2010**, *27*, 570–580. [CrossRef] [PubMed]
59. Arbogast, B.S.; Edwards, S.V.; Wakeley, J.; Beerli, P.; Slowinski, J.B. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. *Annu. Rev. Ecol. Syst.* **2002**, *33*, 707–740. [CrossRef]
60. Ogilvie, H.A.; Heled, J.; Xie, D.; Drummond, A.J. Computational performance and statistical accuracy of *BEAST and comparisons with other methods. *Syst. Biol.* **2016**, *65*, 381–396. [CrossRef] [PubMed]
61. Froufe, E.; Gonçalves, D.V.; Teixeira, A.; Sousa, R.; Varandas, S.; Ghamizi, M.; Zieritz, A.; Lopes-Lima, M. Who lives where? Molecular and morphometric analyses clarify which *Unio* species (Unionida, Mollusca) inhabit the southwestern Palearctic. *Org. Divers. Evol.* **2016**, *16*, 597–611. [CrossRef]
62. Bandelt, H.J.; Forster, P.; Rohl, A. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **1999**, *16*, 37–48. [CrossRef] [PubMed]
63. Leigh, J.W.; Bryant, D. POPART: full-feature software for haplotype network construction. *Methods Ecol. Evol.* **2015**, *6*, 1110–1116. [CrossRef]
64. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef]
65. Oaks, J.R.; Linkem, C.W.; Sukumaran, J. Implications of uniformly distributed, empirically informed priors for phylogeographical model selection: A reply to Hickerson et al. *Evolution* **2014**, *68*, 3607–3617. [CrossRef]

66. Huang, W.; Takebayashi, N.; Qi, Y.; Hickerson, M.J. MTML-msBayes: approximate Bayesian comparative phylogeographic inference from multiple taxa and multiple loci with rate heterogeneity. *Bioinformatics* **2011**, *12*, 1. [CrossRef]
67. Kass, R.E.; Raftery, A.E. Bayes factors. *J. Am. Stat. Assoc.* **1995**, *90*, 773–795. [CrossRef]
68. Campbell, D.C.; Serb, J.M.; Buhay, J.E.; Roe, K.J.; Minton, R.L.; Lydeard, C. Phylogeny of North American amblemines (Bivalvia, Unionoida): Prodigious polyphyly proves pervasive across genera. *Invertebr. Biol.* **2005**, *124*, 131–164. [CrossRef]
69. Johnson, N.A.; Smith, C.H.; Pfeiffer, J.M.; Randklev, C.R.; Williams, J.D.; Austin, J.D. Integrative taxonomy resolves taxonomic uncertainty for freshwater mussels being considered for protection under the U.S. Endangered Species Act. *Sci. Rep.* **2018**, *8*, 15892. [CrossRef] [PubMed]
70. Serb, J.M.; Buhay, J.E.; Lydeard, C. Molecular systematics of the North American freshwater bivalve genus *Quadrula* (Unionidae: Ambleminae) based on mitochondrial ND1 sequences. *Mol. Phylogenet. Evol.* **2003**, *28*, 1–11. [CrossRef]
71. King, T.L.; Eackles, M.S.; Gjetvaj, B.; Hoeh, W.R. Intraspecific phylogeography of *Lasmigona subviridis* (Bivalvia: Unionidae): Conservation implications of range discontinuity. *Mol. Ecol.* **1999**, *8*, S65–S78. [CrossRef]
72. Thompson, J.N. *The Geographic Mosaic of Coevolution*; University of Chicago Press: Chicago, IL, USA, 2005; ISBN 978-0-226-79762-5.
73. Hoberg, E.P. Phylogeny and historical reconstruction: Host-parasite systems as keystones in biogeography and ecology. In *Biodiversity II: Understanding and Protecting our Biological Resources*; John Henry Press: Washington, DC, USA, 1997; pp. 243–261.
74. Inoue, K.; Lang, B.K.; Berg, D.J. Past climate change drives current genetic structure of an endangered freshwater mussel species. *Mol. Ecol.* **2015**, *24*, 1910–1926. [CrossRef]
75. Beaver, C.E.; Woolnough, D.A.; Zanatta, D.T. Assessment of genetic diversity and structure among populations of *Epioblasma triquetra* in the Laurentian Great Lakes drainage. *Freshw. Sci.* **2019**, *38*, 527–542. [CrossRef]
76. Scott, M.W.; Morris, T.J.; Zanatta, D.T. Population structure, genetic diversity, and colonization history of the eastern pondmussel, *Sagittunio nasutus*, in the Great Lakes drainage. *Aquat. Conserv.* **2020**, *30*, 631–646. [CrossRef]
77. Whittaker, R.J.; Araújo, M.B.; Jepson, P.; Ladle, R.J.; Watson, J.E.M.; Willis, K.J. Conservation biogeography: assessment and prospect. *Divers. Distrib.* **2005**, *11*, 3–23. [CrossRef]
78. Burlakova, L.E.; Karatayev, A.Y.; Karatayev, V.A.; May, M.E.; Bennett, D.L.; Cook, M.J. Biogeography and conservation of freshwater mussels (Bivalvia: Unionidae) in Texas: Patterns of diversity and threats. *Divers. Distrib.* **2011**, *17*, 393–407. [CrossRef]
79. Haag, W.R. A hierarchical classification of freshwater mussel diversity in North America. *J. Biogeogr.* **2010**, *37*, 12–26. [CrossRef]
80. Johnson, R.I. The systematics and zoogeography of the Unionidae (Mollusca: Bivalvia) of the southern Atlantic slope region. *Harv. Univ. Mus. Comp. Zool. Bull.* **1970**, *140*, 263–450.
81. Neck, R.W. Preliminary analysis of the ecological zoogeography of the freshwater mussels of Texas. In *Proceedings of the Symposium on Recent Benthological Investigations in Texas and Adjacent States*; Texas Academy of Science: Austin, TX, USA, 1982; pp. 33–42.
82. Sepkosk, J., Jr.; Rex, M. Distribution of freshwater mussels: Coastal rivers as biogeographic islands. *Syst. Zool.* **1974**, *23*, 165–188. [CrossRef]
83. Lopes-Lima, M.; Burlakova, L.; Karatayev, A.; Gomes, A. Revisiting the North American freshwater mussel genus *Quadrula* sensu lato (Bivalvia Unionidae): Phylogeny, taxonomy and species delineation. *Zool. Scr.* **2019**, *48*, 313–336. [CrossRef]
84. Gangloff, M.M.; Hamstead, B.A.; Abernethy, E.F.; Hartfield, P.D. Genetic distinctiveness of *Ligumia recta*, the black sandshell, in the Mobile River basin and implications for its conservation. *Conserv. Genet.* **2013**, *14*, 913–916. [CrossRef]
85. Warren, M.L.; Burr, B.M.; Walsh, S.J.; Bart, H.L., Jr.; Cashner, R.C.; Etnier, D.A.; Freeman, B.J.; Kuhajda, B.R.; Mayden, R.L.; Robison, H.W.; et al. Diversity, distribution, and conservation status of the native freshwater fishes of the southern United States. *Fisheries* **2000**, *25*, 7–31. [CrossRef]
86. Ross, S.T. *The Inland Fishes of Mississippi*; University of Mississippi Press: Oxford, MA, USA, 2001; ISBN 978-1-57806-246-1.

87. Ennen, J.R.; Lovich, J.E.; Kreiser, B.R.; Selman, W.; Qualls, C.P. Genetic and morphological variation between populations of the Pascagoula map turtle (*Graptemys gibbonsi*) in the Pearl and Pascagoula rivers with description of a new species. *Chelonian. Conserv. Biol.* **2010**, *9*, 98–113. [CrossRef]
88. Halas, D.; Simons, A.M. Cryptic speciation reversal in the *Etheostoma zonale* (Teleostei: Percidae) species group, with an examination of the effect of recombination and introgression on species tree inference. *Mol. Phylogenet. Evol.* **2014**, *70*, 13–28. [CrossRef]
89. Egge, J.J.D.; Hagbo, T.J. Comparative phylogeography of Mississippi embayment fishes. *PLoS ONE* **2015**, *10*, e0116719. [CrossRef]
90. Wiley, E.O.; Mayden, R.L. Species and speciation in phylogenetic systematics, with examples from the North American fish fauna. *Ann. Mo. Bot. Gard.* **1985**, *72*, 596–635. [CrossRef]
91. Otvos, E.G. Coastal barriers, northern Gulf—Last Eustatic Cycle; genetic categories and development contrasts. A review. *Quat. Sci. Rev.* **2018**, *193*, 212–243. [CrossRef]
92. Laporte, V.; Charlesworth, B. Effective population size and population subdivision in demographically structured populations. *Genetics* **2002**, *162*, 501–519.
93. Charlesworth, B. Effective population size and patterns of molecular evolution and variation. *Nat. Rev. Genet.* **2009**, *10*, 195–205. [CrossRef]
94. Moore, W.S. Inferring phylogenies from mtDNA variation: Mitochondrial-gene trees versus nuclear-gene trees. *Evolution* **1995**, *49*, 718–726. [CrossRef]
95. Toews, D.P.L.; Brelsford, A. The biogeography of mitochondrial and nuclear discordance in animals. *Mol. Ecol.* **2012**, *21*, 3907–3930. [CrossRef]
96. Flocks, J.; Kulp, M.; Smith, J.; Williams, S.J. Review of the geologic history of the Pontchartrain basin, northern Gulf of Mexico. *J. Coast. Res.* **2009**, *54*, 12–22. [CrossRef]
97. Bolotov, I.N.; Vikhrev, I.V.; Bespalaya, Y.V.; Gofarov, M.Y.; Kondakov, A.V.; Konopleva, E.S.; Bolotov, N.N.; Lyubas, A.A. Multi-locus fossil-calibrated phylogeny, biogeography and a subgeneric revision of the Margaritiferidae (Mollusca: Bivalvia: Unionoida). *Mol. Phylogenet. Evol.* **2016**, *103*, 104–121. [CrossRef] [PubMed]
98. Funk, W.C.; McKay, J.K.; Hohenlohe, P.A.; Allendorf, F.W. Harnessing genomics for delineating conservation units. *Trends Ecol. Evol.* **2012**, *27*, 489–496. [CrossRef] [PubMed]
99. Coates, D.J.; Byrne, M.; Moritz, C. Genetic diversity and conservation units: Dealing with the species-population continuum in the age of genomics. *Front. Ecol. Evol.* **2018**, *6*, 165. [CrossRef]
100. Mee, J.A.; Bernatchez, L.; Reist, J.D.; Rogers, S.M.; Taylor, E.B. Identifying designatable units for intraspecific conservation prioritization: A hierarchical approach applied to the lake whitefish species complex (*Coregonus* spp.). *Evol. Appl.* **2015**, *8*, 423–441. [CrossRef]
101. Waples, R.S. Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. *Mar. Fish Rev.* **1991**, *53*, 11–22.
102. Palsbøll, P.J.; Bérubé, M.; Allendorf, F.W. Identification of management units using population genetic data. *Trends Ecol. Evol.* **2007**, *22*, 11–16. [CrossRef] [PubMed]
103. Waples, R.S.; Nammack, M.; Cochrane, J.F.; Hutchings, J.A. A tale of two acts: endangered species listing practices in Canada and the United States. *BioScience* **2013**, *63*, 723–734. [CrossRef]
104. Moritz, C. Defining 'Evolutionarily Significant Units' for conservation. *Trends Ecol. Evol.* **1994**, *9*, 373–375. [CrossRef]
105. Grobler, P.J.; Jones, J.W.; Johnson, N.A.; Beaty, B.; Struthers, J.; Neves, R.J.; Hallerman, E.M. Patterns of genetic differentiation and conservation of the Slabside Pearlymussel, *Lexingtonia dolabelloides* (Lea, 1840) in the Tennessee River drainage. *J. Mollus Stud.* **2006**, *72*, 65–75. [CrossRef]
106. McMurray, S.E.; Roe, K.J. Perspectives on the controlled propagation, augmentation, and introduction of freshwater mussels (Mollusca: Bivalvia: Unionoida). *Freshw. Mollusk Biol. Conserv.* **2017**, *20*, 1–12. [CrossRef]
107. Moritz, C. Strategies to protect biological diversity and the evolutionary processes that sustain it. *Syst. Biol.* **2002**, *51*, 238–254. [CrossRef]
108. Neves, R.J. Propagation of endangered freshwater mussels in North America. *J. Conchol. Spec. Publ.* **2004**, *3*, 69–80.
109. Snyder, N.F.R.; Derrickson, S.R.; Beissinger, S.R.; Wiley, J.W.; Smith, T.B.; Toone, W.D.; Miller, B. Limitations of captive breeding in endangered species recovery. *Conserv. Biol.* **1996**, *10*, 338–348. [CrossRef]

110. Olden, J.D.; Kennard, M.J.; Lawler, J.J.; Poff, N.L. Challenges and opportunities in implementing managed relocation for conservation of freshwater species. *Conserv. Biol.* **2011**, *25*, 40–47. [CrossRef]

111. Lopes-Lima, M.; Burlakova, L.E.; Karatayev, A.Y.; Mehler, K.; Seddon, M.; Sousa, R. Conservation of freshwater bivalves at the global scale: Diversity, threats and research needs. *Hydrobiologia* **2018**, *810*, 1–14. [CrossRef]