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REGULAR ARTICLE

GENETIC VARIATION IN AN INVASIVE CLAM (FORM B: *CORBICULA LARGILLIERTII*) ACROSS ILLINOIS, USA

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ABSTRACT

Population genetics is an important tool for understanding the source, establishment, and subsequent spread of invasive species. It is particularly pertinent for invasive species that go unrecognized or are understudied due to their limited detection, cryptic diversity, and/or overall lack of interest. One genus of invasive clams, *Corbicula*, has been a particularly successful invader, both globally and in the USA, but has received less attention than other aquatic invaders (e.g., invasive carp or dreissenid mussels). Herein, we address the genetic diversity of one of the three invasive *Corbicula* species—Form B: *Corbicula largillierti*—by using the 28S nuclear and cytochrome c oxidase subunit 1 (COI) mitochondrial gene in 74 individuals from six localities across multiple watersheds in Illinois, USA. We identified spatial structure in both genes and revealed an overall high level of genetic diversity and differentiation among localities. This information can be useful for addressing questions pertaining to propagule pressure, local spread, and overall genetic variation of this otherwise understudied invasive species.

KEY WORDS: invasive freshwater mollusk, basket clam, aquatic invasive species, biogeography, Mississippi River watershed, nonnative species

INTRODUCTION

Conventional wisdom holds that successful invaders establish and spread despite substantial genetic bottlenecks during founder events (i.e., founder effect) that result in low overall genetic diversity (Tsutsui et al. 2000). But this theoretical paradox quickly breaks down when empirical investigations reveal that successful invasions are, in fact, often the result of multiple introductions of numerous individuals (Roman and Darling 2007). Subsequently, high propagule pressure promotes high genetic diversity that in turn allows for species to thrive in a wider range of environmental and ecological conditions (Lockwood et al. 2005; Simberloff 2009). Once in a new environment, however, successful individuals may experience strong selection resulting in only a few genotypes persisting and propagating (Comeault et al. 2020). Understanding patterns of genetic diversity across a species' invaded range is pertinent to effective management of the species, because it provides information regarding the evolutionary potential or the efficiency of potential biological control (Sakai et al. 2001).

Species of the bivalve genus *Corbicula*, commonly known as the basket clam, are native to freshwater environments in eastern Asia as well as to regions of Africa and Australia. However, historic invasions into nonindigenous watersheds worldwide, mainly facilitated by human transport (likely via ballast water exchange and purposeful transport as a food item), have rendered it a particularly prolific aquatic invasive species (AIS) for global freshwater ecosystems (Sousa et al. 2008a). Invasive *Corbicula* populations have been reported in North America for a century, first documented in the 1920s on Vancouver Island, British Columbia, Canada, as empty shells (McMahon 1982). Within a few decades, *Corbicula* spread along the Western Slope in Oregon, Washington, and California (Pennak 1953), USA, and, after secondary introductions, breached the Continental Divide by 1957 into the Tennessee (Sinclair and Isom 1961), Ohio (Stein 1962; Keup et al. 1963), and Pearl rivers, USA (Gunning and Suttkus 1966). Little is known regarding factors promoting the rapid North American expansion. Nevertheless,

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through a combination of dispersal and continual introductions, *Corbicula* reached eastern North America watersheds in the 1950s (McMahon 1982). Additionally, *Corbicula* is hypothesized to have reached South America in the 1960s (Reyna et al. 2013) and was first observed in Western Europe in the 1980s (Mouthon 1981). Because basket clams frequently go undetected, a comprehensive understanding of their invasion history is nonexistent. Even recently, new occurrences have been identified in North America (Tiemann et al. 2017) and elsewhere, although it is likely they had long been established in those places (Kamburska et al. 2013; Reyna et al. 2013; Azevêdo et al. 2014; Beghelli et al. 2014; Tiemann et al. 2017). *Corbicula* has been particularly problematic because it is difficult to identify and can outcompete native bivalve species on many fronts, including rapid growth, early maturity, and high fecundity (Sousa et al. 2008a, 2008b; Haag et al. 2021).

Corbicula's asexual mode of reproduction may contribute to its successful establishment and spread. Although sexual reproduction occurs in native *Corbicula* populations, invasive populations seem to exclusively reproduce asexually, resulting in populations of androgenic clonal lineages that are genetically similar when examined via mitochondrial DNA (mtDNA), but different when nuclear DNA (nDNA) is compared, save any chance mutations (Pigneur et al. 2014; Tiemann et al. 2017; Haponski and Foighil 2019). Molecular and morphological analyses have identified three distinct species (formerly listed as forms) of *Corbicula*: *C. fluminea* (Form A) (Müller, 1774), *C. largillierti* (Form B) (Philippi, 1844), and *C. squalida* (Form D) (Deshayes, 1854) in the USA, including in Illinois (Tiemann et al. 2017; Haponski and Foighil 2019). Although true genetic distinction is unclear, these morphotypes do not necessarily correspond to named species and may be subject to mutations or different selective pressures in novel environments (Tiemann et al. 2017; Haponski and Foighil 2019).

The documented spread of *Corbicula* across North America has continued contemporarily (Siripattrawan et al. 2000; Lee et al. 2005; Tiemann et al. 2017, 2018, 2024; Haponski and Foighil 2019; Modesto et al. 2023). Several studies have used molecular tools to address questions pertaining to taxonomy and phylogenetic relationships (Pigneur et al. 2011, 2014; Tiemann et al. 2017; Haponski and Foighil 2019), global biogeography (Lee et al. 2005; Pigneur et al. 2014), and detection (Blackman et al. 2022) of *Corbicula.* Fewer studies, however, have assessed population genetics and diversity on smaller scales, because the focus is typically on identifying source populations or large-scale levels of variation and less often on genetic variation between local populations.

In *C. largillierti* specifically, molecular tools seldom have been incorporated to study population genetics; thus, little is known about the genetic diversity, or lack thereof, within *C. largillierti*. Genetic analysis of other species of *Corbicula* suggest that invasive populations show a trend of low genetic diversity (Pigneur et al. 2014). These results can be attributed in part to the lack of sexual reproduction in these invasive populations in both North America and Europe (Hedtke et al. 2008; Pigneur et al. 2011). This low genetic diversity also is replicated in North and South America: only four mitochondrial COI haplotypes were found from 212 individuals across 12 populations, each associated with one of the three Forms, with Form D containing 1 individual with a second haplotype (Lee et al. 2005). Herein, we assess aspects of the genetic diversity of *C. largillierti* across multiple watersheds in Illinois, a midwestern state that connects the Great Lakes watershed to the Mississippi River watershed. Specifically, we address the following questions: (1) Is there genetic variation across the invasive range of *C. largillierti* in Illinois? (2) If so, does it conform to any geographic pattern? and (3) Do mtDNA and nDNA recover similar spatial patterns? Addressing these questions will help clarify the invasion's history and potential future directionality of the invasion of *C. largillierti*.

Table 1. Sampling locations and collection codes of the six *Corbicula largillierti* (Form B) populations used in study. Specimens are organized in an upstream to downstream manner within the Mississippi River basin. Numbers after river names indicate multiple localities within the same county.

INHS catalog no.	Sample size (*N*)	Illinois county	River locality ID	Longitude	Latitude
87279	12	Will	DP1	41.64084	−88.0131
87195	12	Will	DP2	41.38366	−88.2405
86178	5	Kankakee	KR1	41.124	−87.8796
87281	16	Kankakee	KR2	41.19975	−87.9786
87245	14	LaSalle	IR	41.31511	−88.6909
45684	15	Randolph	MR	37.8808	−89.7837

INHS = Illinois Natural History Survey; DP = Des Plaines River; KR = Kankakee River; IR = Illinois River; MR = Mississippi River.

METHODS

We used 74 specimens of *C. largillierti* from the Illinois Natural History Survey Mollusk Collections at the University of Illinois Champaign-Urbana. All specimens were collected between 2013 and 2017 from six sites within the upper Mississippi River basin (Table 1) and preserved in 95% ethanol. These sites represented multiple watersheds from the Des Plaines (DP1 and DP2) and Kankakee (KR1 and KR2) rivers, through their confluence at the Illinois River (IR), and downstream into the mainstem Mississippi River (MR) (Fig. 1).

We extracted and isolated DNA from all *Corbicula* samples by using a chloroform:isoamyl alcohol extraction method. After soft tissue samples were separated from the shells and soaked in 100% alcohol for at least 2 d, they were individually crushed and submerged in 400 µL tris-EDTA (TE) buffer. Samples were vortexed at 400 rpm for 1 hr with a stainless steel bead and then resubmerged in 400 µL of 60°C cetyltrimethylammonium bromide (CTAB) buffer (10 mL of CTAB solution, 0.2 g of polyvinyl pyrrolidone, and 20 µL of β-mercaptoethanol mixed with mechanical shaker at 3,000 rpm for 10 min) and 20 µL of Proteinase K and incubated overnight with mild

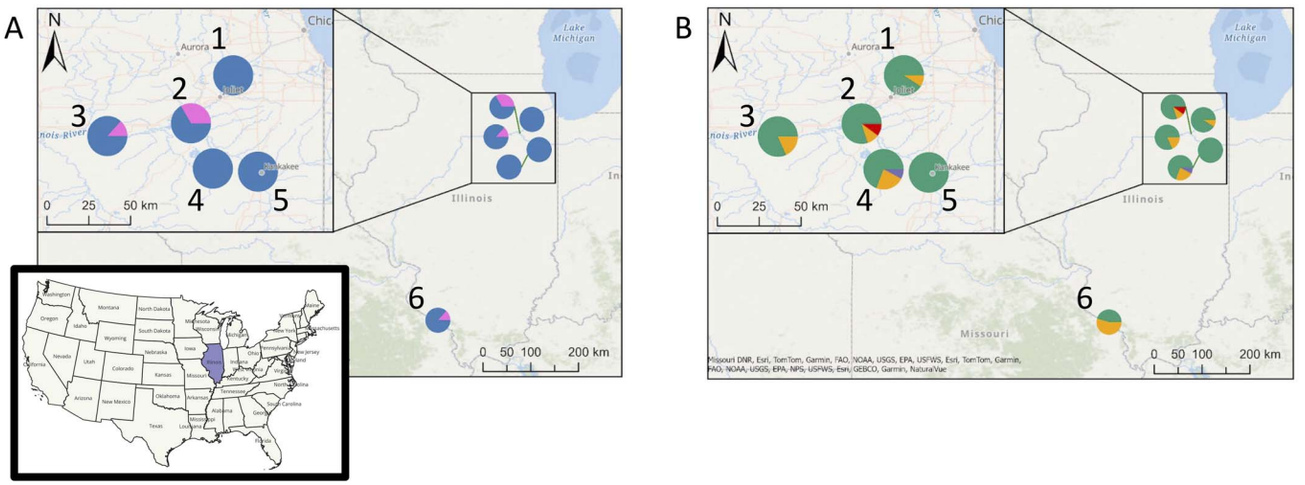

Figure 1. Sampling locations across the Mississippi and Illinois river basins, Illinois, USA. Inset in part A highlights the relative location of Illinois within the USA. Pie charts represent percentage of *Corbicula largillierti* (Form B) exhibiting each haplotype within the (A) mitochondrial and (B) nuclear gene. Mitochondrial haplotypes are separated into haplotypes A (Blue) and B (Pink). Nuclear haplotypes are separated and designated as haplotypes C (green), D (orange), E (red), and F (purple). Populations include 1 = Des Plaines River (DP)1, 2 = DP2, 3 = Illinois River (IR), 4 = Kankakee River (KR)2, 5 = KR1, and 6 = Mississippi River (MR).

mixing. Samples were extracted the next day with 400 µL of chloroform: isoamyl alcohol (24:1) three times (removing the stainless steel bead after the first extraction), with a 5-min centrifugation at 12,000 rpm after each extraction. Afterward, the remaining pellet was washed with 800 µL of 100% ethanol and 40 µL of sodium acetate; centrifuged at 12,000 rpm for 10 min at 4°C; washed again with 200 µL of 70% ethanol; centrifuged again at 12,000 rpm for 10 min; and dried in a vacufuge for 10 min (1,725 rpm at 43°C). Concentrated DNA was then rehydrated in 100 µL of TE buffer and subsequently stored at −20°C.

We used PCR to amplify our targeted DNA. To amplify the mitochondrial COI gene, we used universal primers LCO1490 and HCO2198 (Folmer et al. 1994). Our PCR chemistry consisted of 10 µL of GoTaq™ G2 Colorless MasterMix, 4.5 µL of water, 1.5 µL of LCO1490 primer, 1.5 µL of HCO2198 primer, and 2.5 µL of template DNA. Thermocycler settings for the PCR were set at 1 round of 95°C for 120 s, 35 rounds of 95°C for 60 s, 1 round of 40°C for 60 s, and 1 round of 72°C for 90 s, followed by a final extension round of 72°C for 420 s. For samples that had no amplification or contained multiple amplified bands, annealing temperature was increased to 41, 42, 43, 44, or 48°C to increase specificity of primer bonding. To amplify the 28S nuclear ribosomal gene, we used D23F and D6R primers (Park and O'Foighil 2000). Our PCR chemistry consisted of 10 µL of MasterMix, 4.5 µL of water, 1.5 µL of D23F primer, 1.5 µL of D6R primer, and 2.5 µL of template DNA. Thermocycler settings were as follows: 1 round of 95°C for 240 s, 32 rounds of 95°C for 45 s, 1 round of 57°C for 45 s, and 1 round of 72°C for 105 s, followed by a final extension round of 72°C for 600 s. We used a 2% agarose gel to test amplification quality. If multiple nontarget bands were present, we conducted a cleanup with AMPure XP bead-based reagent (Beckman Coulter, Brea, CA, USA) at a bead/sample ratio of 0.65× that binds to the target fragments, allowing for removal of additional amplicons. We then used ExoSAP-IT™ Express Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and protocol to clean up all PCR products. Samples were sequenced at the Roy J. Carver Biotechnology Center at University of Illinois at Urbana-Champaign on an ABI 3730xl DNA analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

We used the program Geneious Prime v2019.0.4 (Kearse et al. 2012) to trim, align, compare, and produce consensus sequences of each gene from all samples. Sequences derived via the reverse primer were first reverse complemented within the program. Forward and reverse complemented sequences of each specimen were pairwise aligned using the MUSCLE Alignment option (Edgar 2004). Primer sequences were then removed. Finally, all sequences were then aligned using the Consensus Align option within Geneious to produce a consensus among all specimens' mtDNA and nDNA sequences.

We mapped haplotype frequencies across each of the six localities and manually created distance-based haplotype networks for each gene by using the minimum spanning tree as described in Kruskal (1956). In brief, this approach relies on minimizing the length of branches and restricts the number of branches to n-1 based on the number of nodes (n). Given that these networks exhibited limited complexity with only four haplotypes, they could readily be constructed manually. Pairwise fixation index (F_{ST}) values and Nei's genetic distance between each pair of localities were calculated using GenAlEX v6.5 (Peakall and Smouse 2006). Pairwise F_{ST} assesses the level of genetic differentiation between populations on a scale from 0 (identical) to 1 (complete differentiation). Additionally, we conducted an analysis of molecular variance (AMOVA) to assess genetic variation at three levels: (1)

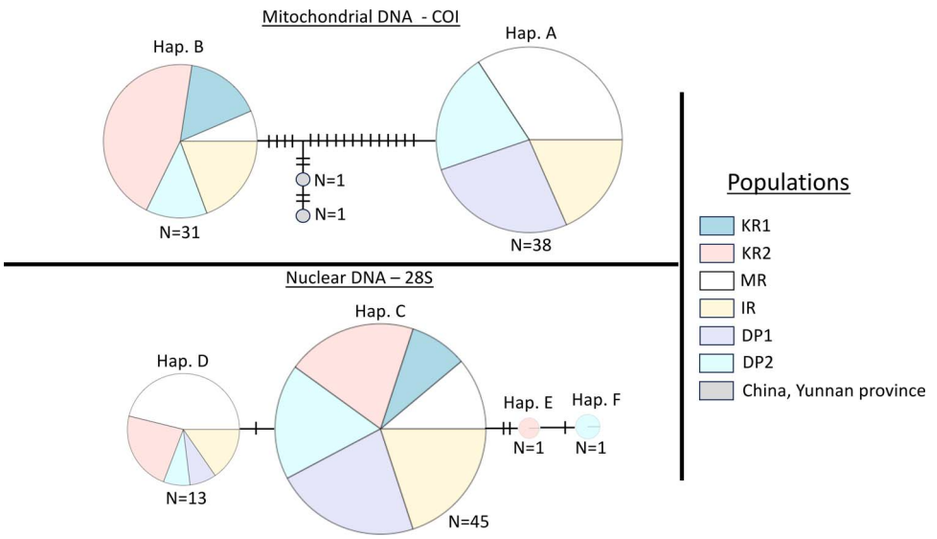

Figure 2. Haplotype network for mitochondrial DNA (top) and nuclear DNA (bottom) of *Corbicula largillierti* (Form B). Mitochondrial DNA network includes two samples of *C. largillierti* from China (GenBank KC211250.1 and KC211251.1). No additional samples for nuclear DNA were available. Size of pie charts is proportional to the number of individuals expressing each haplotype. Pie charts are divided based on localities. Each tick mark on the connecting lines represents a single single-nucleotide polymorphism (SNP) difference among haplotypes.

within-region variation (grouping KR1 and KR2 as well as DP1 and DP2), (2) between-location variation (KK1, KR2, DP1, DP2, IL, and MR), and (3) within-population variation.

RESULTS

We identified two distinct mtDNA haplotypes and four nDNA haplotypes (Fig. 2). For the COI region, we successfully sequenced 69 of 74 individuals. Both Kankakee River localities (KR1 and KR2) exhibited 100% haplotype A. One of the Des Plaines River localities (DP2) exhibited a ratio of 4:8, whereas the other (DP1) exhibited 100% haplotype B. The IR locality exhibited a ratio of 6:7, whereas the furthest downstream locality, the MR, exhibited a ratio of 2:13. Overall, we saw a haplotype distribution of 31:38 among all six localities. Both haplotypes exhibited single-nucleotide polymorphisms (SNPs) at 17 distinct locations across the 658-bp sequence (Table 2).

We successfully sequenced 60 of 74 specimens at the nuclear 28S gene. Of these 60, we were able to interpret only the reverse complement sequence of 48 specimens. For the other 12, we were able to interpret both the forward and reverse complement sequences, producing a full alignment of these 12. Overall, we saw four haplotypes (labeled as C, D, E, and F). Each of the four haplotypes was observed in at least one individual with both forward and reverse complement sequences. In one Kankakee River locality (KR1), we saw a ratio of 4:0:0:0 (C:D:E:F), but in the other locality (KR2), we observed a haplotype ratio of 9:3:1:0. In one Des Plaines River locality (DP2), we saw a ratio of 8:1:0:1, whereas in the other locality (DP1), our haplotype ratio was 10:1:0:0. In the IR, our haplotype ratio was 9:2:0:0, and in the MR, we observed a haplotype ratio of 5:6:0:0. Across the 697-bp sequence, we observed four distinct locations where SNPs occur (Table 3).

The F_{ST} values ranged from 0.022 between Des Plaines River (DP2) and IR localities to 0.947 between Kankakee River (KR1) and Des Plaines (DP1) localities. Likewise, Nei's genetic distance ranged from 0.025 between Des Plaines River (DP2) and IR localities to 1.754 between Kankakee River (KR1) and Des Plaines (DP1) localities (Table 4). Kankakee River (KR1) and Des Plaines River (DP1) shared no mtDNA haplotypes, even though these sites are separated

Table 2. Sequence location (bp) of SNPs found in the COI mitochondrial gene between haplotypes A ($n = 31$ individuals) and B ($n = 38$) and the associated nucleotides in *Corbicula largillierti* (Form B) samples.

	SNP location																
	bp 10	bp 59	bp 95	bp 110	bp 163	bp 175	bp 241	bp 289	bp 298	bp 349	bp 397	bp 478	bp 484	bp 490	bp 556	bp 580	bp 625
A	T	G	T	C	G	A	G	G	A	C	C	T	A	C	A	A	C
B	C	A	C	T	A	G	A	A	G	T	T	G	G	T	G	G	T

SNP = single-nucleotide polymorphism; COI = cytochrome c oxidase subunit.

Table 3. Sequence location (bp) of SNPs found in the nuclear 28S gene between haplotypes C, D, E, and F and the associated nucleotides in *Corbicula largillierti* (Form B) samples.

Haplotype	bp 86	bp 87	bp 261	bp 328
C (N = 45)	C	A	A	T
D (N = 13)	*	*	*	C
E (N = 1)	G	C	*	*
F (N = 1)	G	C	T	*

SNP = single-nucleotide polymorphism.
Asterisk (*) indicates the same base as haplotype C.

Table 5. Analysis of molecular variance among *Corbicula largillierti* localities.

Source of variation	df	Sum of squares	Estimated variance	% variation
Among regions	3	285.570	2.269	37
Among populations	2	20.160	0.331	5
Within populations	142	509.217	3.586	58
Total	147	814.946	6.186	100

F_{ST} = 0.4203 (P = 0.001).

by <100 km of river. All F_{ST} values above 0.36 included Kankakee River localities, which only contained individuals with haplotype B. This result is exemplified by the high level of differentiation compared with the Des Plaines River (DP1) location, for which all individuals were haplotype A (Table 4). Results from the AMOVA show that most of the variation (58%) occurs within populations. Following that, 37% of the variation occurs between among regions and only 5% is explained by among-population variation (Table 5).

DISCUSSION

There was greater genetic variation within *C. largillierti* across Illinois than in the initial analysis that found only one mtDNA haplotype and two nDNA haplotypes (Tiemann et al. 2017). We found two distinct mtDNA haplotypes distinguished by 17 SNPs as well as four distinct nuclear DNA haplotypes distinguished by 4 SNPs. Individuals containing both mtDNA haplotypes A and B contained both nuclear haplotypes C and D (i.e., A-C, A-D, B-C, and B-D individuals). The high amount of variation between the two mtDNA haplotypes, coupled with no intermediate haplotypes, suggests that these individuals may have come from different sources, although low sample sizes decreased the probability of capturing intermediate genotypes. High pairwise F_{ST} and Nei's genetic distance values were the result of spatial variation in mtDNA haplotypes driven by the lack of haplotype A in the two Kankakee River localities and, to a lesser extent, the lack of nuclear haplotype D at KR1. This high level of genetic variation was unexpected based on previous studies that showed less variation on smaller spatial scales (Lee et al. 2005; Tiemann et al. 2017; Haponski and Foighil 2019). Within the IR, some studies on bivalves show little genetic variation across the watershed (Sanfilippo et al. 2025), whereas other studies show levels of population structure in bivalves (Mathias et al. 2018) and fish (Corush et al. 2025). Despite the observed biogeographical variation, the AMOVA suggests that most of the genetic variation occurs within populations, but a large amount of variation also occurs at the watershed level.

Table 4. Nei's genetic distance (below diagonal) and pairwise F_{ST} values (above diagonal) for combined COI and 28S gene SNPs for the six *Corbicula largillierti* localities used in the study. Highlighted values represent within-river variation.

	DP1	DP2	KR1	KR2	IR	MR
DP1		0.091	1.677	1.754	0.213	0.021
DP2	0.169		0.568	0.586	0.025	0.055
KR1	0.947	0.415		0.003	0.319	1.182
KR2	0.856	0.409	0.070		0.329	1.190
IR	0.284	0.022	0.354	0.317		0.135
MR	0.081	0.064	0.743	0.659	0.130	

F_{ST} = fixation index; SNP = single-nucleotide polymorphism; COI = cytochrome c oxidase subunit; DR = Des Plaines; KR = Kankakee; IR = Illinois River; MR = Mississippi River.

The haplotype distribution patterns we observed might result from a wide variety of past and ongoing postinvasion effects driven by the interplay of introduction bottlenecks, multiple founder events, gene flow, and genetic drift (Jaspers et al. 2021). Multiple founder effects at a broad spatial scale can result in distinct haplotypes being differentially observed across the species' range (Zalewski et al. 2010; Jaspers et al. 2021). Our observations might be the result of separate introductions in both the Mississippi and Kankakee rivers, each with subsequent dispersal into the IR and its tributaries. Additionally, selection of specific genotypes in different rivers can increase differentiation between sites and lower genetic diversity within sites (Schulte et al. 2013), although these patterns can be difficult to detect in invasive species (Fitzpatrick et al. 2012). A hypothesis based on collections and occurrence data suggests that the species is moving upstream from the southern USA (Tiemann et al. 2022). Although this hypothesized upstream directionality has been observed in the IR in multiple species (Silver Carp, *Hypophthalmichthys molitrix*; Bighead Carp, *Hypophthalmichthys nobilis*; and Black Carp, *Mylopharyngodon piecus*; Chick and Pegg 2001; Kroboth et al. 2019), downstream invasions are also common. Examples include the Round Goby, *Neogobius melanostomus*; Eastern Banded Killifish, *Fundulus diaphanus*; Quagga Mussel, *Dreissena bugensis*; and Zebra Mussel, *Dreissena polymorpha*, all of which first invaded the Great Lakes and dispersed downstream (Willink et al. 2018; Tiemann et al. 2022, Harris et al. 2024; Hartman et al. 2024). The numerous examples of invasion in both directions removes assumptions based on generalities.

Although no definitive point of introduction or patterns of spread for either haplotype can be deduced from our data, there are some trends worth noting. MR is the only location to be comprised of primarily haplotype D individuals, whereas the northern locations are comprised primarily of haplotype C individuals. Single instances of haplotype E and F found in KR2 and DP2, respectively, suggest that increased sampling may reveal additional haplotypes. Both passive downstream and, albeit slow, active upstream dispersal (via humans, fish and bird digestive tracts, or actively moving pediveligers) are possible in this species (Prezant and Chalermwat 1984; Isom 1986; Voelz et al. 1998; Beran 2006; Gatlin et al. 2013; Rosa et al. 2014; Pernecker et al. 2021). However, unlike unionid bivalves, *Corbicula* species do not have glochidia (Hoggarth and Gaunt 1988).

Multiple introduction events may explain the presence of multiple haplotypes (barring the possibility of postinvasion mutations), but reasons for current distributions are less clear. The idea of location-specific haplotype competition has been hypothesized such as "boom-and-bust" in which one haplotype may temporarily, substantially outcompete others in one area before crashing, with concomitant opportunity for subsequent diversification (Doebeli et al. 2021). A boom-and-bust dynamic may explain higher or lower rates of certain haplotypes than we would expect to normally find in an area, as abrupt, temporary proliferation of one haplotype may persist or disappear over time. Mixing of gametes to create crossed, sexual lineages also may explain the discordance between COI patterns and 28S patterns, although this explanation contradicts previous studies of invasive *Corbicula* (Pigneur et al. 2014).

Further exploration of population structure of *C. largillierti* as well as other *Corbicula* lineages is warranted based on the occurrence of rare haplotypes and the patterns of fixation in mtDNA haplotypes at such a fine geographic scale. Specifically, expansion of the geographic scope with particular emphasis on sampling between current locations is imperative to gain a finer-grained perspective, particularly with respect to the differential haplotype composition among disparate sampling localities. Further exploration will facilitate a more nuanced understanding of how haplotype proportions are changing at a watershed scale and facilitate testing hypotheses related to passive and active dispersal, propagule pressure, and other factors related to establishment and spread of this invasive species. Combining a finer-grained perspective with biological and ecological covariates should yield an opportunity to understand how patterns of genetic diversity may be shaped via selection and/or are driving local adaptation, ultimately shaping our understanding of the evolution of this invasive species in novel waters. From a more technical standpoint, leveraging modern population genomic approaches (e.g., restriction site–associated DNA sequencing) and next-generation sequencing would provide a much more fulsome and nuanced perspective on invasion genetics by increasing the number of variable loci by orders of magnitude. On balance, these research lines may provide insight into the spread of this AIS and inform management strategies aimed at more efficient and effective mitigation.

AUTHOR CONTRIBUTION

BM, JBW, NJI, and JBC designed the study with input from all authors. BM, JBW, NJI, and JBC conducted the molecular lab work and data analysis. BM, JBW, and JBC wrote the original draft. All authors contributed to the final draft of the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY

All sequence data is available via GenBank (accession numbers PZ228526–PZ228594 and PZ229305–PZ229363).

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