

Investigations

Coevolution With Host Fishes Shapes Parasitic Life Histories in a Group of Freshwater Mussels (Unionidae: Quadrulini).

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Abstract

Ecological interactions among species often lead to parasitic lineages coevolving with host resources, which is often suggested as the primary driver of parasite diversification. Freshwater mussels are bivalves that possess a parasitic life cycle requiring larval encystment on freshwater vertebrates to complete metamorphosis. The North American freshwater mussel tribe Quadrulini has a suite of life history adaptations including highly specialized patterns of host use, infection strategies, and variable larval morphologies. However, the evolution of life histories has yet to be explored using phylogenetic comparative methods. In this study, we use a holistic approach incorporating biogeographical, ecological, molecular, and morphological datasets to reconstruct the evolution of Quadrulini. Comparative phylogenetic analyses suggested the diversification of Quadrulini has been driven, at least in part, by codiversification with their primary host fishes in Ictaluridae. Major diversification events in both ictalurids and quadrulines were estimated to have occurred in the Mississippi River basin throughout the Miocene. Life history characteristics associated with parasitism were supported to have coevolved with host repertoires, supporting the hypothesis that ecological interactions with host fishes have shaped the evolution of highly specialized traits in this group. Our findings demonstrate the importance of ecological interactions with host resources in shaping the evolutionary history of freshwater mussels.

Introduction

Ecological interactions among species over time and space often lead to parasitic lineages coevolving with host resources (Klassen, 1992). Co-speciation with host lineages has therefore been hypothesized to be the primary driver of parasite diversification, which has led to the assumption that phylogenies of parasites are congruent with their hosts (Hoberg & Brooks, 2008). However, many events (e.g., extinction, host-switching, vicariance) can lead to changes in a species host repertoire (i.e., suitable hosts) and incongruencies between host and parasite phylogenies. Thus, assessing coevolutionary hypotheses often requires a thorough understanding of the evolutionary histories of both parasites and their hosts (e.g., Brooks & Ferrao, 2005; Dowling et al., 2003).

Focal system

Freshwater mussels (Bivalvia: Unionida) are a diverse group of bivalves represented by 958 species globally (Graf & Cummings, 2021). The radiation of this group is hypothesized to be significantly influenced by an obligate parasitic life cycle that requires temporary larval encystment on the gills, fins, or epidermis of vertebrate hosts to complete metamorphosis (Barnhart et al., 2008). Here, we focus on the freshwater mussel tribe Quadrulini Ihering, 1901 (Fig. 1), which is a strictly North American taxon represented by 25 species (Graf & Cummings, 2021) that have highly specialized patterns of host use and associated life history traits. Species in *Cyclonaias* Pilsbry in Ortmann and Walker, 1922; *Quadrula* Rafinesque, 1820; and *Tritogonia* Agassiz, 1852 have been documented to primarily parasitize benthic ictalurids (Actinopterygii: Ictaluridae) (Haggerty et al., 1995; Hove et al., 2011, 2012), while species in *Theliderma* Swainson, 1840 use pelagic minnows in the family Leuciscidae as primary hosts (Fritts et al., 2012; Yeager & Neves,



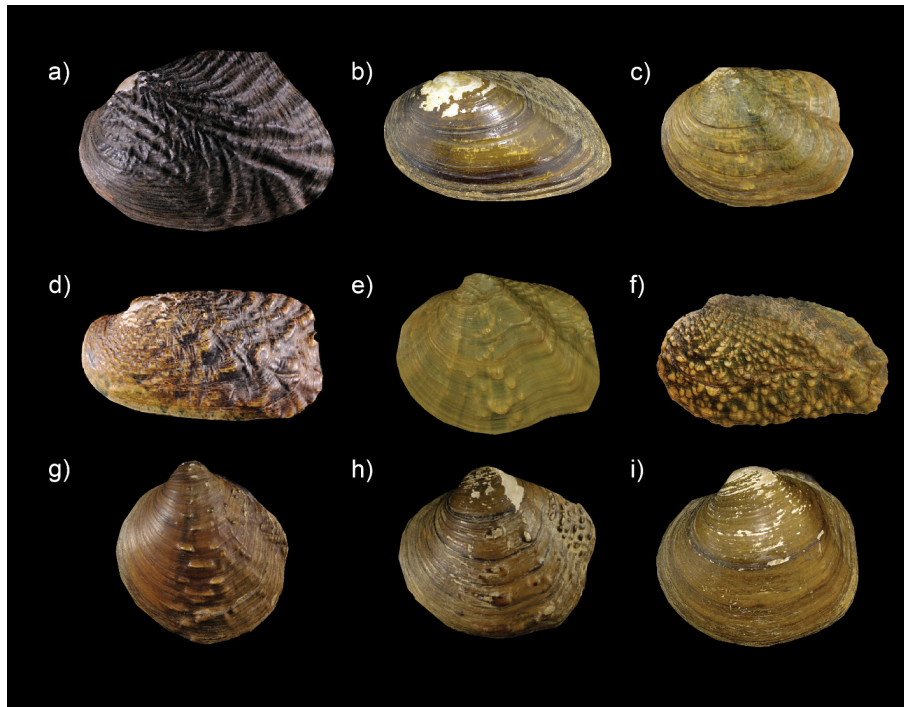


Figure 1. Select photographs of specimens representing taxa in Quadrulini.

a) *Megaloniaias nervosa* (USNM 83903), b) *Unio merus tetralasmus* (USNM 85604), c) *Theliderma metanevra* (USNM 84237), d) *Theliderma cylindrica* (USNM 151737), e) *Quadrula quadrula* (USNM 84165), f) *Quadrula verrucosa* (USNM 149358), g) *Cycloniaias tuberculata* (USNM 84293), h) *Pustulosa nodulata* (USNM 84200), and i) *Pustulosa pustulosa* (USNM 85768).

1986). Parasitism of ecologically and evolutionarily divergent host groups appears to have also led to divergent host attraction mechanisms ostensibly adapted to exploiting dietary preferences of their hosts. For example, *Theliderma* exhibit small, colorful mantle lures to attract sight-feeding minnows (Barnhart et al., 2008), while *Cycloniaias*, *Quadrula*, and *Tritogonia* exhibit various pale colored mantle lures that may resemble moribund fish or unionids (Sietman et al., 2012). However, host repertoire evolution in Quadrulini has yet to be explored using phylogenetic comparative methods, which is critical toward understanding how ecological interactions have influenced the origin of specialized life history traits (Braga et al., 2020).

Host repertoires in freshwater mussels are often characterized at the family level, which has been useful in understanding generalized patterns of host use (Hewitt et al., 2019, 2021; Smith et al., 2020), but greater taxonomic resolution of host use is necessary to understand more nuanced evolutionary patterns (e.g., codiversification, host switching, host extinction). Although species in *Cycloniaias*, *Quadrula*, and *Tritogonia* parasitize Ictaluridae, there are variable patterns of host use within the family. For example, some *Cycloniaias* species use madtoms (*Noturus* Rafinesque, 1818) as secondary hosts in addition to bullheads (*Ameiurus* Rafinesque, 1820), blue and channel catfishes (*Ictalurus* Rafinesque, 1820), and flathead catfish (*Pylodictis* Rafinesque, 1819) (Dudding et al., 2020; Haag & Leann Staton, 2003; Sietman et al., 2010). Reconstruction of ancestral host repertoires below the family level is necessary to understand the evolutionary ecology of Quadrulini more precisely. The biogeographic and evolutionary histories of Ictaluridae have been well characterized (Arce-H. et

al., 2016), which provides an opportunity to test if codiversification with ictalurids has contributed to the evolution of Quadrulini.

Objectives

In this study, we use a holistic approach incorporating biogeographical, ecological, molecular, and morphological datasets to reconstruct the evolution of Quadrulini. Our specific objectives were to: a) estimate a phylogenomic reconstruction of Quadrulini, b) reconstruct the evolution of host repertoires, c) test for codiversification between quadrulines and their host fishes, d) trace the evolution of life history characters (i.e., brooding and larval morphology, host infection strategy), and e) synthesize our findings in terms of their ecological and evolutionary significance. Our holistic analysis also provided clarity on the taxonomy of Quadrulini, and we make multiple taxonomic revisions to accurately reflect its evolutionary history (refer to *Systematics in Quadrulini* for additional details). We recognize 6 genera and 25 recent species in Quadrulini: *Cycloniaias* (*C. tuberculata* (Rafinesque, 1820)); *Megaloniaias* Utterback, 1915 (*M. nervosa* (Rafinesque, 1820)); *Pustulosa* stat. res. Frierson, 1927 (*P. infucata* comb. nov. (Conrad, 1834); *P. kieneriana* (Lea, 1852); *P. kleiniana* comb. nov. (Lea, 1852); *P. necki* comb. nov. (Burlakova, Karatayev, Lopes-Lima & Bogan in Burlakova et al., 2018); *P. nodulata* (Rafinesque, 1820); *P. petrina* (Gould, 1855); *P. pustulosa* (Lea, 1831); *P. succissa* comb. nov. (Lea, 1852)); *Quadrula* (*Q. couchiana* (Lea, 1860); *Q. fragosa* (Conrad, 1835); *Q. nobilis* (Conrad, 1854); *Q. quadrula* (Rafinesque, 1820); and *Q. verrucosa* (Rafinesque, 1820)); *Theliderma* (*T. cylindrica* (Say, 1817);

T. intermedia (Conrad, 1836); *T. johnsoni* (Bogan & Lopes-Lima in Lopes-Lima & Bogan, 2019); *T. metanevra* (Rafinesque, 1820); *T. sparsa* (Lea, 1841); *T. stapes* (Lea, 1831)); and *Unio* Conrad, 1853 (*U. carolinianus* (Bosc, 1801); *U. columbensis* (Lea, 1857); *U. declivis* (Say, 1831); *U. tetralasmus* (Say, 1831)).

Materials and methods

Taxon sampling and anchored hybrid enrichment data generation

We compiled or generated AHE data for 27 species from the subfamily Ambleminae, including all but 1 extant member of Quadrulini (*T. intermedia* – sample failed, low capture efficiency). Representative taxa from the subfamily Gonideinae (*Gonidea angulata*) and the amblemine tribes Amblemini Rafinesque, 1820 (*Amblema plicata* (Say, 1817)), Lampsilini Ihering, 1901 (*Lampsilis cardium* Rafinesque, 1820), Pleurobemini Hannibal, 1912 (*Pleurobema strodeanum* (Wright, 1898)), and Popenaiadini Heard & Guckert, 1970 (*Popenaias popeii* (Lea, 1857)) were selected as an outgroup. Information regarding taxon sampling, including catalog numbers and SRA accessions, can be found in [Table 1](#). For novel data, genomic DNA was extracted using the Qiagen PureGene DNA extraction kit (Qiagen, Hilden, Germany), and high molecular weight was ensured by visualizing isolations on a 1% agarose gel stained with GelGreen nucleic acid stain (Biotium, Hayward, CA, USA).

The Unioverse probe set (Pfeiffer et al., 2019) was used to capture orthologous nuclear protein-coding loci. Target capture, library construction, and sequencing was performed by RAPiD Genomics (Gainesville, FL, USA) following similar methodologies as outlined in Smith et al. (2020). TRIM GALORE! v0.4.0 (www.bioinformatics.babraham.ac.uk/projects/trim_galore/) was used to quality trim reads (Phred score < 20) and filter reads less than 30 nt. Individual loci were assembled using an iterative bait assembly (Breinholt et al., 2017) with Unioverse reference sequences as baits following parameters outlined in Pfeiffer et al. (2019). Individual loci were screened with single hit and orthology location mapping to the *Bathymodiolus platifrons* genome following Breinholt et al. (2017) with a BLASTN (Altschul et al., 1990) e-value of 0.00001 and a bit score cut off for a single hit set to 0.7. If more than one sequence was still present at a locus, we retained the sequence with the highest coverage (Pfeiffer et al., 2021). Loci with gene occupancy $\geq 70\%$ across Ambleminae and parsimony informative sites were retained for dataset generation.

We followed similar methods as Smith et al. (2020) to create a molecular supermatrix consisting of probe and both flanking regions for each AHE locus. Loci were initially aligned in MAFFT using the commands “–allowshift –alignlevel 0.8 –reorder –leavegappyregion”. Problematic flanking regions were then removed following Breinholt et al. (2017), and loci were trimmed using a 50% gap threshold in Trimal v 1.2 (Capella-Gutiérrez et al., 2009). Loci were concatenated into a super-matrix using FASconCAT-G v 1.0.5 (Kück & Longo, 2014).

Phylogenetic analyses

We used concatenation and coalescent-based species tree methods to perform phylogenetic reconstruction. For concatenation analyses, partitions were determined using ModelFinder (Kalyaanamoorthy et al., 2017) with an rcluster of 10. Using the best partitioning scheme, phylogenetic inference was performed under Maximum Likelihood (ML) and Maximum Parsimony (MP) in IQ-TREE v 2.1.2 (Minh et al., 2020) and PAUP* v 4.0a (Swofford, 2003), respectively. IQ-TREE2 analyses consisted of 10 independent runs and 1000 ultrafast bootstrap (ufBS) replicates for nodal support (Hoang et al., 2017). PAUP* analyses were performed using heuristic searches with 100 random sequence addition replicates conducted with tree-bisection-reconnection branch-swapping and 1000 bootstrap (BS) replicates for nodal support. We enforced two topological constraints to test previous taxonomic hypotheses in Quadrulini under ML and MP: 1) *Tritogonia* as monophyletic (*Q. nobilis* + *Q. verrucosa*) and 2) *Cyclonaias* (as previously recognized: *C. tuberculata* + *Pustulosa*) as monophyletic. For all comparisons, we used an AU test (Shimodaira, 2002) implementing 10,000 RELL (Kishino et al., 1990) replicates in ML analyses and Templeton (Templeton, 1983) and winning sites tests (Prager & Wilson, 1988) in MP analyses to test topologies. A significance level of $\alpha = 0.05$ was used when assessing the statistical significance between topologies.

The coalescent-based approach ASTRAL-III v 5.7.7 (Zhang et al., 2018) was used to generate a species tree from individual locus trees under ML. Locus trees were reconstructed using IQ-TREE2 with the best available nucleotide substitution model determined by ModelFinder and 1000 ufBS replicates for nodal support. Bipartitions with < 10 ufBS were removed using Newick Utilities v 1.6 (Junier & Zdobnov, 2010), per developers recommendation for accuracy. We also used the coalescent-based approach SVDquartets (Chifman & Kubatko, 2014) in PAUP* to infer the species tree using 1000 BS replicates for nodal support.

A calibrated phylogenetic analysis was performed on the concatenated dataset under Bayesian inference in BEAST v 2.6.5 (Bouckaert et al., 2019). The best partitioning scheme and models of nucleotide substitution used in ML were used for the analysis. To estimate divergence times, we used two calibrations based on fossils and geographic evidence of drainage evolution. First, we used a wide exponential prior that aged the crown of Ambleminae near the Cretaceous–Paleogene boundary while encompassing previous estimates based on calibrations from distantly related taxa (up to 120 Mya) (Huang et al., 2019; Zieritz et al., 2020) within the 95% quantile (mean = 20, offset = 60, 95% quantile = 120). We chose to set the lower limit of the crown of Ambleminae near the Cretaceous–Paleogene boundary given previous studies have documented a putative mass extinction event of North American freshwater mussels at the Cretaceous–Paleogene boundary (Hartman, 1998; Scholz & Hartman, 2007). Cretaceous–Paleogene extinction events have been documented to coincide with the crowns of many other taxonomic groups (Alfaro et al., 2018; Feng et al., 2017; Friedman, 2010; Longrich et al.,

Table 1. Taxa and voucher material used in the phylogenetic analyses.

Subfamily	Tribe	Taxa	Catalog number	Accession	Source
Ambleminae	Amblemini	<i>Amblema plicata</i>	UF 438572	SRR8473047	Pfeiffer et al., 2019
Ambleminae	Lampsilini	<i>Lampsilis cardium</i>	UMMZ 304654	SRR8473035	Pfeiffer et al., 2019
Ambleminae	Pleurobemini	<i>Pleurobema strodeanum</i>	UF 441317	SRR8473051	Pfeiffer et al., 2019
Ambleminae	Popenaiadini	<i>Popenaias popeii</i>	UF 438742	SRR8473050	Pfeiffer et al., 2019
Ambleminae	Quadrulini	<i>Cyclonaias tuberculata</i>	UF 438245	SRR17818386	This study
Ambleminae	Quadrulini	<i>Megalonaias nervosa</i>	NCSM 35123	SRR17818385	This study
Ambleminae	Quadrulini	<i>Pustulosa infucata</i>	UF 440951	SRR17818404	This study
Ambleminae	Quadrulini	<i>Pustulosa kieneriana</i>	UF 438660	SRR17818403	This study
Ambleminae	Quadrulini	<i>Pustulosa kleiniana</i>	UF 438023	SRR17818392	This study
Ambleminae	Quadrulini	<i>Pustulosa necki</i>	BSGLC 1672	SRR17818391	This study
Ambleminae	Quadrulini	<i>Pustulosa nodulata</i>	UF 439202	SRR17818390	This study
Ambleminae	Quadrulini	<i>Pustulosa petrina</i>	JBFMC 9504.1	SRR17818389	This study
Ambleminae	Quadrulini	<i>Pustulosa pustulosa</i>	UA 416	SRR17818388	This study
Ambleminae	Quadrulini	<i>Pustulosa succissa</i>	UF 438538	SRR17818387	This study
Ambleminae	Quadrulini	<i>Quadrula fragosa</i>	N/A (Swab)	SRR17818402	This study
Ambleminae	Quadrulini	<i>Quadrula nobilis</i>	BSGLC 1856	SRR17818397	This study
Ambleminae	Quadrulini	<i>Quadrula quadrula</i>	UF 441088	SRR8473075	Pfeiffer et al., 2019
Ambleminae	Quadrulini	<i>Quadrula verrucosa</i>	UA 1350	SRR17818396	This study
Ambleminae	Quadrulini	<i>Theliderma cylindrica</i>	UF 439167	SRR17818401	This study
Ambleminae	Quadrulini	<i>Theliderma johnsoni</i>	NCSM 30474	SRR17818400	This study
Ambleminae	Quadrulini	<i>Theliderma metanevra</i>	UF 439158	SRR17818399	This study
Ambleminae	Quadrulini	<i>Theliderma sparsa</i>	N/A (Swab)	SRR17818398	This study
Ambleminae	Quadrulini	<i>Uniomerus carolinianus</i>	UF 438215	SRR17818395	This study
Ambleminae	Quadrulini	<i>Uniomerus columbensis</i>	UF 441227	SRR17818394	This study
Ambleminae	Quadrulini	<i>Uniomerus declivis</i>	JBFMC 8586.2	SRR17818393	This study
Ambleminae	Quadrulini	<i>Uniomerus tetralasmus</i>	N/A (Transcriptome)	SRR910418	Luo et al., 2014
Gonideinae	Gonideini	<i>Gonidea angulata</i>	NCSM 41055.202	SRR8473064	Pfeiffer et al., 2019

Acronyms for collections are as follows: BSGLC – Buffalo State Great Lakes Center, JBFMC – Joseph Britton Freshwater Mussel Collection, NCSM – North Carolina Museum of Natural Sciences, UA – Alabama Museum of Natural History, UF – Florida Museum, and UMMZ – University of Michigan Museum of Zoology.

2012; Wible et al., 2007), which suggests, along with fossil evidence (Hartman, 1998; Scholz & Hartman, 2007), the possibility that the crown of Ambleminae may be at or near the Cretaceous–Paleogene boundary. We chose to not use an individual fossil to calibrate the phylogeny given we consider defensible placement of fossils difficult due to morphological convergence (Watters, 2001). Second, we set a MRCA prior on *P. necki*, *P. nodulata*, and *P. petrina* to coincide with the split of Mega-Brazos and Mega-Colombia basins in the late Miocene (Blum & Hattier-Womack, 2009). Briefly, climatic changes in the late Miocene led to the creation of two mega-drainages: 1) Mega-Brazos (Brazos, Calcasieu, Sabine, and Trinity drainages) and 2) Mega-Colombia (Colorado and Guadalupe drainages). These two mega-drainages separated in the late Miocene, which has been demonstrated to align with diversification in other freshwater mussels in central (*Fusconaia iheringi* and *F. mitchelli*) and east Texas (*F. askewi*) (Pieri et al., 2018; Smith et al., 2021). Based on this, we used a lognormal prior (Mean = 2.1, Standard Deviation = 0.2, 95% CI = 5.52–12.1) to age the split between central (*P. necki* and *P. petrina*) and east Texas (*P. nodulata*) lineages in *Pustulosa*. The analysis used an optimized relaxed clock (Douglas et al., 2021), a Yule model as the tree prior, and was run for 3×10^8 million MCMC generations sampling every 5000 trees. Effective sample size (ESS) as determined by Tracer v 1.7 (Rambaut et al., 2018) was used to ensure convergence of all parameters (ESS > 200), and a maximum clade credibility tree was created using TREEANNOTATOR v 2.6 (Bouckaert et al., 2019).

Host-parasite coevolution and host repertoire evolution

We compiled host use information for taxa in Quadrulini included in phylogenetic analyses from the Freshwater Mussel Host Database (Illinois Natural History Survey & Ohio State University Museum of Biological Diversity, 2017) and those available in the literature (Dudding et al., 2020; Haag & Leann Staton, 2003; Hove et al., 2011; Howard & Anson, 1922; Howells, 1997; Lane et al., 2021; Sietman et al., 2012). We only considered records based on laboratory transformations as hosts. Records were removed if they were based on unpublished data, were taxa not native to North America, or identified as unsuitable hosts from the literature (e.g., low transformation efficiency, inconsistent transformation across replicates, universal hosts).

We performed Procrustean Approach to Cophylogeny (PACo) (Hutchinson et al., 2017) in the R package *paco* on a time-calibrated freshwater fish phylogeny, the time-calibrated freshwater mussel phylogeny resolved by BEAST, and a species-level host use matrix to test for evidence of codiversification among freshwater mussels and their host fishes. Specifically, we tested if freshwater mussel diversification was dependent on freshwater fish diversification. We downloaded the time-calibrated ray-finned fish tree of life (Chang et al., 2019; Rabosky et al., 2018) and pruned the tree to host fish species confirmed by lab trials ($N = 59$; Table S1). We also pruned the time-calibrated mussel tree to taxa with host information ($N = 18$). Before the analysis, we used a Cailliez correction for negative eigenvalues (Cailliez, 1983), per developers recommendation. We used a r0 null model, and 1000 permutations were used to assess statistical significance using a critical value of 0.05.

We used a comparative phylogenetic approach as described in Braga et al. (2020) to model the host repertoire evolution in RevBayes v 1.1.1 (Höhna et al., 2016). To include a phylogeny of host fish, we downloaded the ray-finned fish tree of life and pruned the tree to include a representative from all fish families confirmed as hosts. We only included a single representative for fish families outside Ictaluridae to reduce computational demand. To further explore the evolution of host use within Ictaluridae, we included a representative from the genera *Ameiurus*, *Ictalurus*, *Noturus*, and *Pylodictis*. We did not include *Prietella* Carranza, 1954; *Satan* Hubbs & Bailey, 1947; and *Trogloglanis* Eigenmann, 1919 in our analyses given these taxa are not known to serve as hosts. Details about fish taxa included in the analysis can be found in Table S2. We created an interaction matrix between each freshwater mussel species and known host taxa included in phylogenetic reconstructions. Using the resulting interaction matrix, time-calibrated fish phylogeny, and time-calibrated mussel phylogeny, we set up a 2-state model similar to Braga et al. (2021), which included host and non-host states to infer host repertoire evolution. The model does not allow missing data, so we took a conservative approach and coded all missing interactions as non-host states. The analysis was run for 5×10^5 MCMC generations sampling every 50 cycles with a 10% burn-in. Tracer v 1.7 was used to ensure convergence of all parameters (ESS > 200).

We used four biogeographic regions for historical biogeography analyses based on Haag (2009): Atlantic, Eastern Gulf, Mississippian, and Pacific. We compiled current distributional data for taxa included in phylogenetic analyses from the literature (Haag, 2009; Johnson et al., 2018; Lopes-Lima et al., 2019; Williams et al., 2014). We then investigated historical biogeographical patterns of ictalurids by collapsing the ray-finned fish tree of life to freshwater fish in the genera *Ameiurus*, *Ictalurus*, *Noturus*, and *Pylodictis*. We ran this analysis to compare historical biogeography of freshwater mussels and ictalurids during specialization and host gain events. Distributional information for ictalurid taxa was compiled from Page and Burr (2011). Biogeographic analyses were performed on time-calibrated phylogenies using BioGeoBEARS (Matzke, 2013), and the DEC+J model (Matzke, 2014) was used to infer the most likely biogeographic history.

Historical biogeography

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Ancestral character reconstruction

We compiled available information for brooding morphology (i.e., ectobranchus or tetragenous) and larval measurements (i.e., larval height and larval length) for taxa included in phylogenetic analyses. Brooding morphology and larval measurements were compiled from the literature (Barnhart et al., 2008; Brim Box & Williams, 2000; Dudding et al., 2020; Fritts et al., 2012; Hove et al., 2011; Lea, 1863;

Arnold E. Ortmann, 1912; Arnold Edward Ortmann, 1923; Utterback, 1915; Watters et al., 2009; Williams et al., 2008, 2014) or from museum specimens (*P. necki* – JBFMC8168-1 and JBFMC8168-12; *P. petrina* – JBFMC8690). We estimated the evolutionary history of larval brooding morphology using Bayesian stochastic character mapping (Bollback, 2006; Huelsenbeck et al., 2003) with the “make.simmap” function in the R package phytools v 1.0-1 (Revell, 2011). Bayesian stochastic character mapping used 1000 simulations. To estimate the evolutionary history of larval height, we used the ML based “contMap” function in phytools. The time calibrated phylogeny was pruned to only include taxa with brooding or larval data for each analysis. To further explore congruence between larval morphology and phylogeny, we created bar plots and ran a phylogenetic principal component analysis (pPCA) using larval height, larval length, and our time-calibrated phylogeny in the R packages phylosignal (Keck et al., 2016) and adephylo (Jombart et al., 2010), respectively.

We compiled available information on host infection strategies for taxa included in phylogenetic analyses following the literature (Barnhart et al., 2008; Haag, 2012; Sietman et al., 2012). Host infection strategies were divided into six categories: 1) broadcasting; 2) conglutinate; 3) large mantle lure; 4) large, pale mantle magazine; 5) small, colorful mantle magazine; and 6) small, pale mantle magazine. We pruned the time calibrated phylogeny of taxa with missing data and traced the evolutionary history of host infection strategies using Bayesian stochastic character mapping in phytools. Bayesian stochastic character mapping used 1000 simulations to estimate ancestral character states.

We used the “phyloSignal” function in phylosignal to test the null hypothesis of the absence of phylogenetic signal for each trait used in ancestral character reconstruction. Abouheif’s C_{mean} index (Abouheif, 1999) was used to compute phylogenetic signal, and 1000 replicates were used to assess statistical significance. All analyses were performed in R v 4.1.1.

Hierarchical clustering

We used a hierarchical clustering approach to integrate signal from biogeographic, ecological, molecular, and morphological datasets. First, we pruned all datasets to only include taxa in Quadrulini without missing data across all datasets. We then created distance matrices using the “cophenetic.phylo” function in the R package ape v 5.6-1 (Paradis & Schliep, 2018) from the time-calibrated phylogeny; the “dist.binary” function in the R package ade4 v 1.7-18 (Dray & Dufour, 2007) from the biogeography, larval brooding morphology, and host infection strategy datasets; and the “vegdist” function in the R package vegan v 2.5-7 (Oksanen et al., 2016) from larval measurements. To also incorporate host use information, we first used the “cophenetic.phylo” function to generate a distance matrix from the host phylogeny used in the RevBayes analysis (Table S2). We then used the phylogenetic distance and interaction matrices to create a distance matrix among freshwater mussel taxa using the “comdist” function in the R package

picante v 1.8.2 (Kembel et al., 2010). All resulting distance matrices were combined using the “fuse” function in the R package analogue v 0.17-6 (Simpson, 2007) with equal contribution from each matrix. Hierarchical clustering was performed on the fused matrix using the “hclust” function in the R package stats v 4.1.1. A threshold of 0.3 was used to delimit clusters.

Results

Phylogenetic analyses

All novel AHE reads used in this study are available in the GenBank SRA database (BioProject PRJNA800785). Information regarding material and accession numbers used in this study can be found in Table 1. The final molecular supermatrix consisted of 550 loci represented by 366,360 nucleotides (File S1). Before concatenated phylogenetic analyses, the dataset was grouped into 21 partitions. Concatenated and coalescent based analyses under ML and MP were completely concordant other than a minor topological difference by SVDquartets regarding the placement of *P. succissa* (Fig. S1). Nearly all nodes were fully supported in all ML and MP analyses (BS = 100; Fig. S1), and topologies were generally concordant with Johnson et al. (2018), except for the placement of *C. tuberculata*. *Theliderma* was resolved as sister to *Cyclonaias*, *Quadrula*, and *Pustulosa* with full support (Fig. S1). *Cyclonaias* was resolved sister to *Quadrula* and *Pustulosa*; however, ML and MP analyses did not fully support the placement of *C. tuberculata* (Fig. S1). Topological constraints enforcing the monophyly of previous taxonomic hypotheses for *Cyclonaias* (ML – $p = 0.00115$; MP – $p = 0.0319$) and *Tritogonia* (ML – $p < 0.0001$; MP – $p < 0.0001$) were significantly worse than the estimated topology.

The time-calibrated phylogenetic reconstruction under Bayesian inference was completely concordant with ML and MP analyses (Fig. 2). All nodes were fully supported (PP = 1.0). The crown of Quadrulini was estimated at the early-mid Paleogene (45.29 Ma; 95% highest posterior density (HPD) = 35.48–55.91 Ma) (Fig. 2). *Cyclonaias*, *Quadrula*, *Pustulosa*, and *Theliderma* were estimated to have originated in the late Paleogene to early Miocene (Fig. 2). Partitioning of genera was estimated to have occurred throughout the early-middle Miocene (Fig. 2), with subsequent speciation events estimated to have occurred until the Pliocene. All tree files generated from phylogenetic analyses can be found in supplemental information (Files S2–S6).

Host-parasite coevolution and host repertoire evolution

PACo indicated significant evidence of codiversification between quadrulines and their host fishes ($m^2_{XY} = 0.66$; $p < 0.001$; $n = 1000$), supporting the hypothesis that codiversification with hosts has contributed to the evolution of Quadrulini. Our reconstruction of host repertoire evolution supported generalized host use as the ancestral state of Quadrulini, which included Centrarchidae (PP = 0.63), Per-

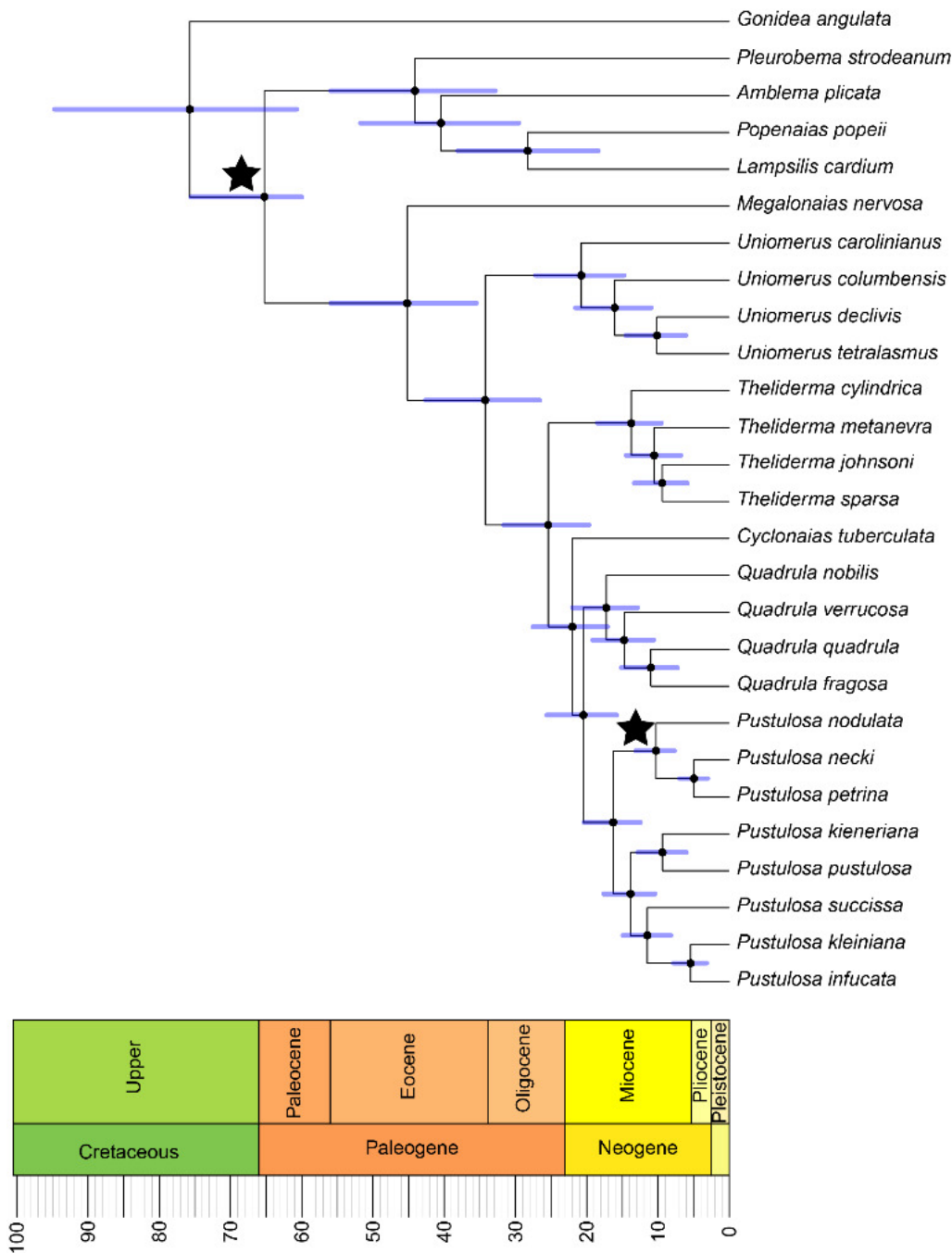


Figure 2. Maximum clade credible tree generated from the calibrated phylogenetic analysis in BEAST.

Divergence time is scaled to million years before present, and node bars represent the 95% highest posterior density. All nodes were fully supported (PP = 1.0). Stars represent crown calibration points.

cidae (PP = 0.66), *Ameiurus* (PP = 0.86), *Ictalurus* (PP = 0.87), and *Pylodictis* (PP = 0.88) (Figs. 3 & S2). Ictalurid specialization, which included *Ameiurus* (PP = 0.90), *Ictalurus* (PP = 0.92), and *Pylodictis* (PP = 0.93) host use, was supported to have occurred in the MRCA of *Cyclonaias*, *Quadrula*, *Pustulosa*, and *Theliderma*. The MRCA of *Theliderma* was strongly supported to have experienced a host switch from ictalurids to leuciscids (PP = 0.999) (Fig. 3 & S2). Most other species in *Cyclonaias*, *Pustulosa*, and *Quadrula* maintained the ancestral state of *Ameiurus*, *Ictalurus*, and *Pylodictis* host use, but the MRCA of *Pustulosa* was strongly supported to have gained *Noturus* as a host (PP = 0.98) (Fig. 3 & S2). The

MRCA of *Quadrula fragosa* and *Quadrula quadrula* was supported as a specialist on *Ictalurus* (PP = 1.0), with no other hosts having PP > 0.5 (Fig. 3 & S2).

Historical biogeography

Comparative historical biogeography failed to reject synchronous origins of Quadrulini and ictalurids in the Mississippi region, with the origin of MRCA of *Ameiurus*, *Ictalurus*, *Noturus*, and *Pylodictis* (42.86 Ma) falling within the 95% HPD (35.48–55.91 Ma) of the crown of Quadrulini (Figs. 4 & S3). *Cyclonaias*, *Quadrula*, *Pustulosa*, and *Theliderma* originated in the Mississippi region (Fig. 4A) where

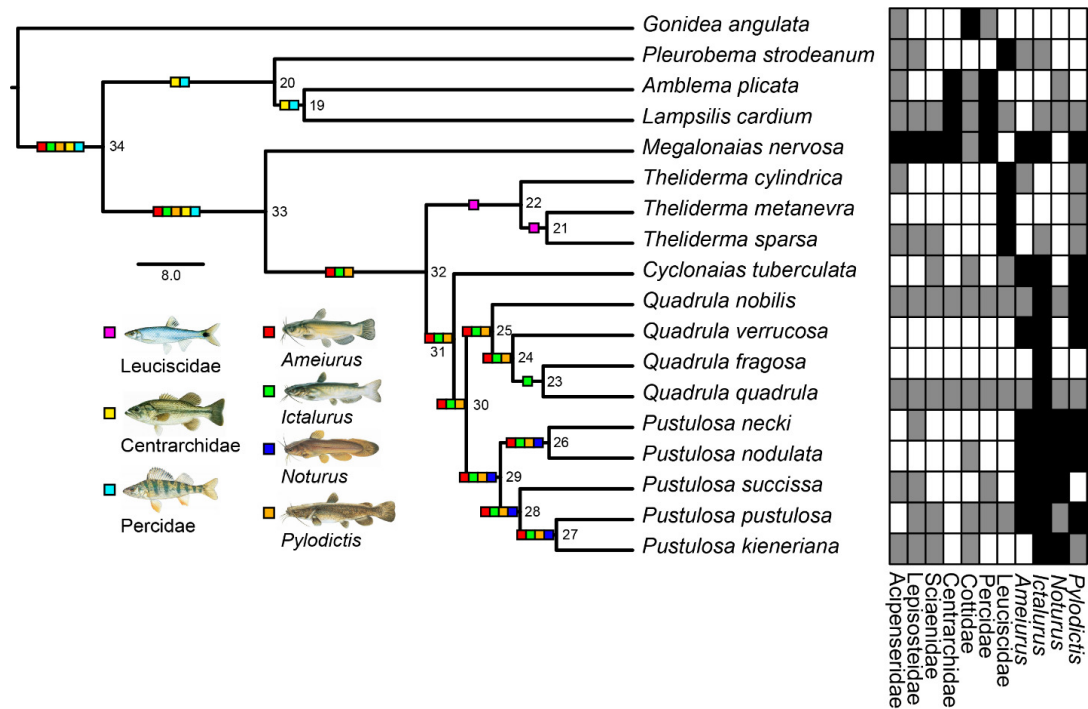


Figure 3. RevBayes reconstruction of host repertoire evolution along the time calibrated phylogeny of Quadrulini.

All interactions displayed are represented by posterior probability ≥ 0.5 . Node numbers are used to match posterior probabilities reported in supplemental information (Figure S2). Host use information for taxa in Quadrulini is provided in cells to the right of terminals. Cell coloration corresponds to the following: black – confirmed host, gray – unknown, and white – confirmed non-host. From top to bottom and left to right, fish photos are as follows: *Cyprinella venusta*, *Micropterus salmoides*, *Perca flavescens*, *Ameiurus melas*, *Ictalurus punctatus*, *Noturus gyrinus*, and *Pylodictis olivaris*. Illustrations courtesy of Joe Tomelleri and the Fishes of Texas Database (<http://www.fishesoftexas.org/>).

they have diversified—with the MRCA of *P. infucata*, *P. kleiniana*, and *P. succissa* invading the Eastern Gulf region in the late Miocene (Fig. 4A). *Pustulosa* and *Noturus* were estimated to have originated synchronously in the Mississippian region during the Miocene (Fig. 4), with the MRCA of *Noturus* (18.52 Ma) falling within the 95% HPD (15.78–25.60 Ma) of the MRCA of *Pustulosa*. These results are coincident with host gain of *Noturus* in *Pustulosa* (Fig. 3) and codiversification between the two groups.

Ancestral character reconstruction

Ancestral character reconstructions of larval height supported a shared evolutionary history of larval miniaturization in *Quadrula* (Fig. 5), which is concordant with their characteristic of parasitic growth. Bar plots and pPCA (Fig. 5) distinguished genera in Quadrulini based on larval characters, which were supported to be phylogenetically conserved ($p = 0.01$). Ancestral character reconstruction of larval brooding morphology supported an independent origination of ectobranchy in *C. tuberculata* (Fig. S4). The lack of phylogenetic signal in larval brooding morphology was rejected ($p = 0.002$). Bayesian stochastic character mapping resolved broadcasting as the most likely ancestral state of Quadrulini (PP = 0.5; Fig. 6). Small, pale mantle magazines were supported to have originated in the MRCA of *Cyclonaias*, *Quadrula*, *Pustulosa*, and *Theliderma* (PP = 0.64; Fig. 6), aligning with the estimated timing of ictalurid specialization. Small, colorful mantle magazines were strongly supported to have evolved in the MRCA of *Theliderma* (PP = 0.99; Fig. 6), which was concordant to the re-

solved host switch to leuciscids. Large, pale mantle magazines were strongly supported to have originated in *Quadrula* (PP = 0.96; Fig. 6), which aligned with the shared evolutionary history of larval miniaturization. Small, pale mantle magazines were strongly supported as the ancestral state of *Pustulosa* (PP = 1.0; Fig. 6). Large, pale mantle magazines ($p < 0.0001$); small, colorful mantle magazines ($p < 0.0001$); and small, pale mantle magazines ($p < 0.0001$) were supported as phylogenetically conserved—while the absence of phylogenetic signal could not be rejected for other infection strategies ($p > 0.05$), likely due to small sample size.

Hierarchical clustering

Twelve taxa in Quadrulini were included in hierarchical clustering analyses, which included all genera currently assigned to the tribe expect *Uniomerus*. Hierarchical clustering of biogeographical, ecological, morphological, and phylogenetic distances delimited 5 clusters within Quadrulini aligning with our taxonomic hypotheses: *Cyclonaias* (*C. tuberculata*), *Megalonaias* (*M. nervosa*), *Quadrula* (*Q. fragosa*, *Q. quadrula*, and *Q. verrucosa*), *Pustulosa* (*P. kieneriana*, *P. necki*, *P. nodulata*, *P. pustulosa*, and *P. succissa*), and *Theliderma* (*T. cylindrica* and *T. metanevra*) (Fig. S5).

Discussion

Our findings suggest the diversification of Quadrulini has been driven, at least in part, by codiversification with their

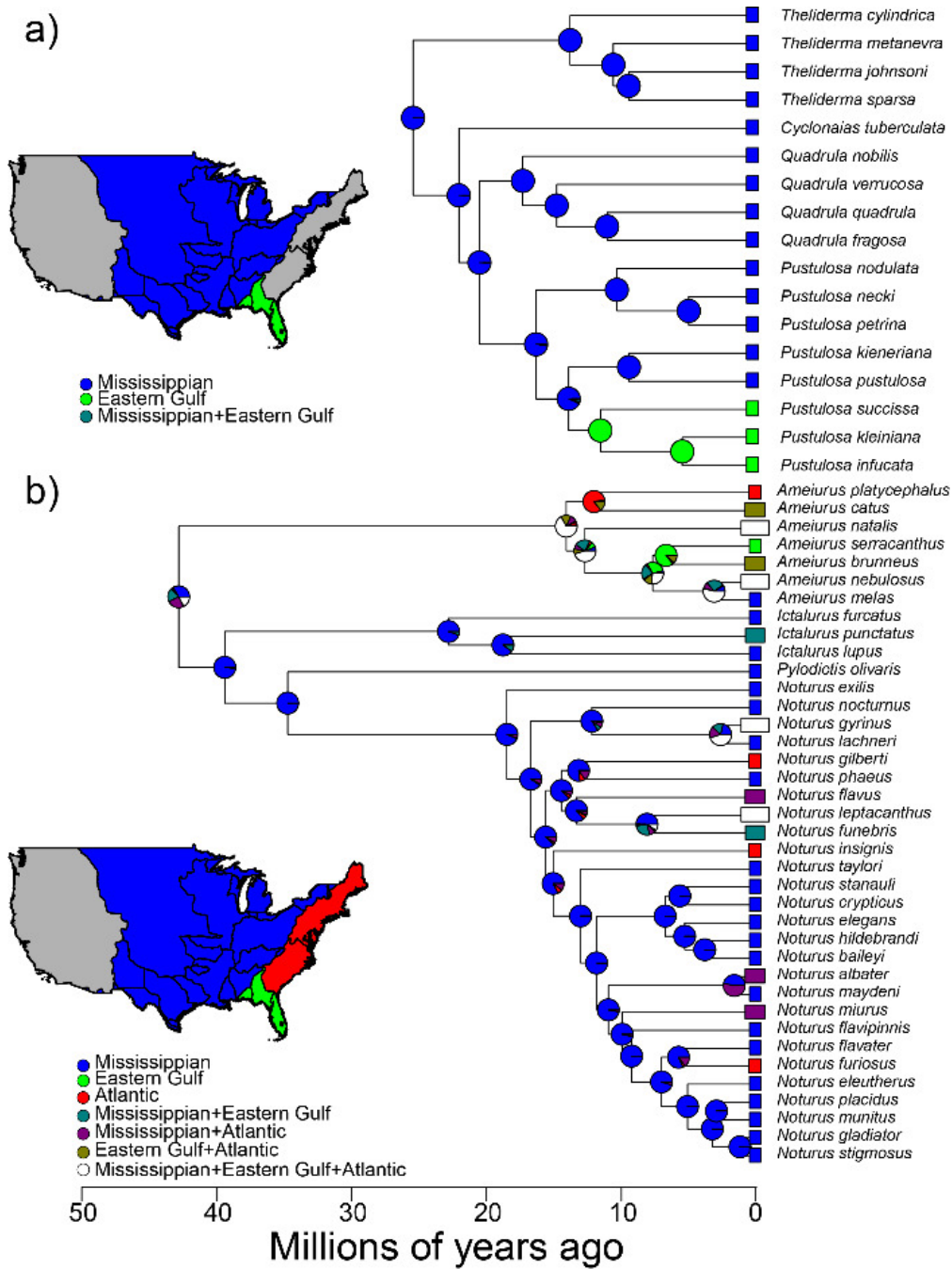


Figure 4. Ancestral-area estimates using the DEC+J model in BioGeoBEARS for a) *Cyclonaias*, *Quadrula*, and *Pustulosa*, and b) *Ameiurus*, *Ictalurus*, *Noturus*, and *Pylodictis*.

Rectangles on terminals represent the current distribution of sampled taxa and size represents the number of areas. Pie charts on nodes represent support for ancestral areas. Maps illustrate the geographic distributions of the Atlantic (red), Mississippi (blue), and Eastern Gulf (green) regions in the United States. Black lines delineate provinces and grey coloration represent regions uninhabited by presented taxa.

host fishes. Life history characteristics associated with a parasitic lifestyle were supported to have coevolved with reconstructed host repertoires, supporting the hypothesis that ecological interactions with host fishes have shaped the evolution of highly specialized traits in this group. Below, we synthesize our results in terms of their ecological and evolutionary significance.

Ecological interactions with host fishes shape life histories in Quadrulini

Our findings suggest the MRCA of Quadrulini used a generalized suite of life history characteristics, including passive host infection (broadcasting) and taxonomically broad host use (Centrarchidae, Percidae, *Ameiurus*, *Ictalurus*, and *Pylodictis*) (Figs. 3 & 6). *Megaloniais*, the sister species to the remainder of Quadrulini, has a more generalized life history strategy in comparison to the rest of the tribe and has retained some plesiomorphic traits, including a taxonomi-

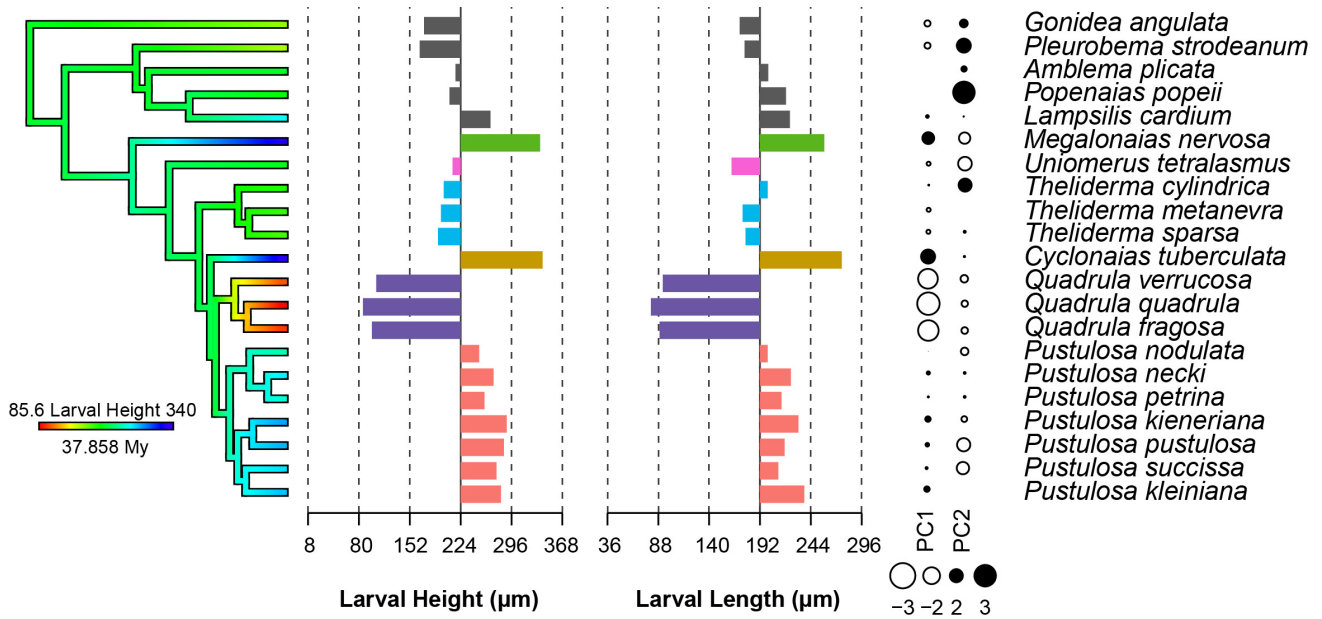


Figure 5. Ancestral character reconstruction of larval height and phylogenetic principal coordinate analysis loadings based on larval height, larval length, and the time calibrated phylogeny on the time calibrated phylogeny generated by BEAST.

Colors in ancestral character reconstruction represent reconstructed trait values. Bar plots are colored by genera in Quadrulini: *Megaloniaias* (green), *Uniomerus* (pink), *Theliderma* (blue), *Cycloniaias* (yellow), *Quadrula* (purple), and *Pustulosa* (red).

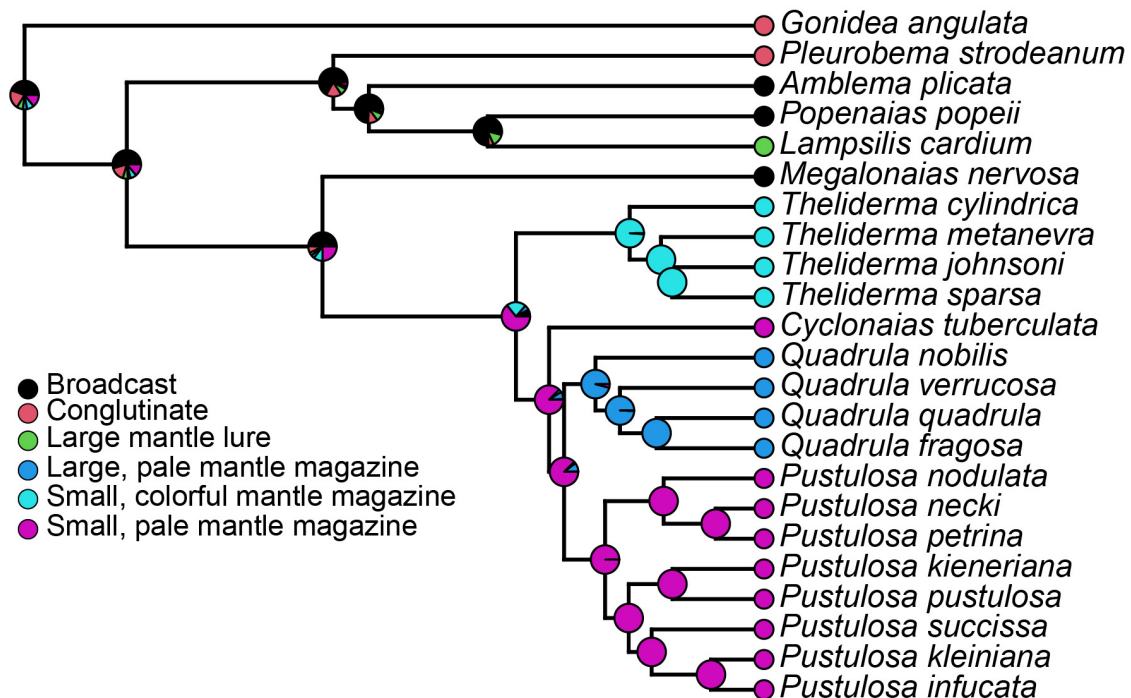


Figure 6. Reconstructed evolutionary history of host infection strategies based on Bayesian stochastic character mapping.

Circles on terminals represent the character state of extant taxa. Coloration in pie charts on nodes represent support for character states.

cally diverse host repertoire and host infection via passive entanglement (Haag, 2012) (Figs. 3 & 6). However, our estimation of quadruline ancestral states would be improved by inclusion of *Uniomerus*, but the life history of the taxon is poorly understood and could not be included in some of

our analyses. We hypothesize that the host repertoire of *Uniomerus* likely at least includes ictalurids or leuciscids, and future life history studies will be useful in testing and resolving the evolutionary history of host use and infection strategies in Quadrulini.

We observed specialized host repertoires and associated life history traits in *Cyclonaias*, *Quadrula*, *Pustulosa*, and *Theliderma*. Ictaluridae specialization and mantle magazines were supported to have evolved in the MRCA of *Cyclonaias*, *Quadrula*, *Pustulosa*, and *Theliderma* (Figs. 3 & 6), suggesting mantle magazines may be a functional trait for successful parasitism of ictalurids. Ictalurid host use was strongly supported as the ancestral state of these genera with a clear instance of host switching from Ictaluridae to Leuciscidae in *Theliderma* during the late Oligocene or early Miocene (Figs. 2 & 3). A previous study has highlighted the use of Leuciscidae in *Theliderma* as a synapomorphy for the genus (Lopes-Lima et al., 2019), but the evolution of the trait had remained untested using phylogenetic comparative methods. The transition to an ecologically and phylogenetically divergent host group coincided with the origin of small, colorful mantle magazines ostensibly adapted to attract sight-feeding minnows (Fig. 6), which supports the hypothesis that ecological interactions with novel host fishes has shaped life history characteristics in *Theliderma* unique to Quadrulini.

Cyclonaias, *Pustulosa*, and *Quadrula* have retained a host repertoire that includes *Ameiurus*, *Pylodictis*, and *Ictalurus* (Fig. 3). However, our analyses supported an independent origination of *Noturus* host gain in *Pustulosa*, which was supplemented by their synchronous origination in the Mississippian region and diversification throughout the Miocene (Fig. 4). This, along with significant evidence of codiversification between quadrulines and their host fishes ($p < 0.001$), suggests that co-speciation with ictalurids may have contributed to the diversification of Quadrulini. *Pustulosa* also has a shared evolutionary history of a small, pale mantle magazine (Fig. 6), which may be a functional trait for successful parasitism of *Noturus*—the smallest members of Ictaluridae. However, it is worth note that a small, pale mantle magazine was resolved as the ancestral state of ictalurid specialists and is also present in *Cyclonaias* (Fig. 4). Despite similar host infection strategies in *Cyclonaias* and *Pustulosa*, the two genera display different levels of species diversity, which we hypothesize to have been driven by co-speciation of *Pustulosa* and *Noturus*, but we cannot reject other drivers of observed codiversification.

Our ancestral character reconstruction of larval size supported a single larval miniaturization event in the evolutionary history of *Quadrula* (Fig. 5). Multiple larval miniaturization events have occurred in Amblemninae, including *Potamilus*, *Quadrula*, and *Truncilla*, each of which is coupled with parasitic growth during encapsulation (Barnhart et al., 2008; Smith et al., 2020). Within Quadrulini, miniaturized larvae and parasitic growth are life history traits unique to *Quadrula* (Hove et al., 2011, 2012), but the functional significance of those traits are uncertain. Larval miniaturization in *Potamilus* and *Truncilla* has been hypothesized to be a functional trait to increase fecundity given putative host infection by maternal sacrifice (Smith et al., 2020). Interestingly, large catfishes (*Ictalurus* and *Pylodictis*) are known to feed on adult mussels (reviewed by Tiemann et al., 2011), but it remains uncertain if they consume mature quadrulines (Sietman et al., 2012). Although there is uncertainty

regarding predation by ictalurids, the brooding period of *Q. verrucosa* coincided with peak ictalurid feeding (May–June) (Hove et al., 2011), which suggests host infection in this species could rely, at least in part, on maternal sacrifice similar to other lineages with miniaturized larvae. However, larval miniaturization may also be a functional trait for dispersal. Given freshwater mussels are largely sessile as adults, parasitism is hypothesized to have evolved to facilitate dispersal (Watters, 2001). Therefore, dispersal capabilities could theoretically be correlated with the time larvae encyst on host fish, and there is some limited evidence favoring this hypothesis over the fecundity hypothesis. First, there appears to be little to no difference in fecundity between *Pustulosa* and *Quadrula* (Haag, 2012), albeit based on very limited sampling. Second, encystment time on host fish has been demonstrated to have extensive variation in *Quadrula*. Life history studies have suggested that the period of encystment typically ranges from approximately one to five weeks in *Cyclonaias*, *Pustulosa*, and *Theliderma* (Dudding et al., 2020; Fritts et al., 2012; Haag & Leann Staton, 2003; Hove, 1997). In *Quadrula*, however, it has been demonstrated that encystment time could be up to nine months under natural conditions (Hove et al., 2012; Steingraeber et al., 2007), but we recognize encystment time shows extensive variability due to water temperature (Steingraeber et al., 2007). We hypothesize larval miniaturization may have evolved for one of these reasons, but future research into early life histories in Quadrulini will be necessary to better understand the functional significance of larval miniaturization in *Quadrula*.

Systematics in Quadrulini

Taxonomy in Quadrulini has been unstable due to systematic hypotheses being primarily based on external shell morphology (Campbell & Lydeard, 2012; Johnson et al., 2018; Lopes-Lima et al., 2019; Serb et al., 2003). Our holistic analysis provides clarity on systematics by using evidence-based explanations for taxonomic recommendations based on biogeographical, ecological, morphological, and phylogenetic datasets (Fig. S5). Our phylogenetic analyses rejected the monophyly of *Cyclonaias* as previously recognized (i.e., *C. tuberculata* + *Pustulosa*; $p < 0.05$), with *C. tuberculata* resolved sister to *Quadrula* and *Pustulosa* (Fig. 2). Our evaluation identified multiple characters that distinguish *Cyclonaias* from *Pustulosa*, including ectobranchy (Fig. S4) and large larval size ($>300 \mu\text{M}$; Fig. 5). In addition, our data supports *Noturus* host use as a synapomorphy for *Pustulosa* (Fig. 3), which is not a known host for *Cyclonaias* (Hove & Kurth, 1997). Given these findings, we designate *Cyclonaias* a monotypic genus consisting of the type species *C. tuberculata*. Designating *Cyclonaias* as monotypic leaves the clade consisting of *P. infucata*, *P. kieneriana*, *P. kleiniana*, *P. necki*, *P. nodulata*, *P. petrina*, *P. pustulosa*, and *P. succissa* without a generic epithet. We resurrect the oldest available generic epithet, *Pustulosa* (type species – *Obliquaria (Quadrula) bullata* Rafinesque, 1820; replacement name in errata for *Bullata* Frierson, 1927 non Jousseume, 1875 [Gastropoda]), to represent this clade. *Obliquaria (Quadrula) bullata* is considered a junior synonym of *P. pus-*

tulosa (Williams et al., 2008), and we therefore designate *P. pustulosa* as the type species of *Pustulosa*. Previous taxonomic treatments have recognized *Pustulosa* as *Amphinaias* (Graf & Cummings, 2007). *Amphinaias* is represented by the type species *Unio couchianus*—an extinct species considered endemic to the Rio Grande drainage (Williams et al., 2017). Multiple attempts were made to include material for this taxon in this study, but we were unable to get usable material for genetic analysis. However, we agree with previous taxonomic treatments that have considered *Unio couchianus* as a member of *Quadrula* based on its quadrate shell shape and sulcus (Williams et al., 2017), which are unlike *Pustulosa*. Therefore, we consider *Amphinaias* a junior synonym of *Quadrula*.

Tritogonia was non-monophyletic, with *T. nobilis* and *T. verrucosa* resolved in a monophyletic group with *Quadrula* (Fig. 2), and our phylogenetic analyses rejected the monophyly of *Tritogonia* ($p < 0.0001$). Previous studies delineated *Quadrula* and *Tritogonia* based on sexual dimorphism of their shells despite their low level of genetic divergence (Lopes-Lima et al., 2019). However, the two genera have many similar life history traits as depicted in our study, including brooding morphology (Fig. S4), host use and infection strategy (Figs. 3 & 6), miniaturized larval morphologies (Fig. 5), and parasitic growth (Hove et al., 2011, 2012; Sietman et al., 2012). We synonymize *Tritogonia* into *Quadrula* based on our findings, which aligns with recommendations from previous studies based on molecular or morphological characters (Arnold E. Ortmann, 1912; Serb et al., 2003; Utterback, 1915).

Conclusions

In this study, we integrated biogeographical, ecological, molecular, and morphological data to provide a holistic view of the evolutionary history of Quadrulini. Our approach emphasized the importance of codiversification with ictalurids in shaping the evolutionary history of Quadrulini and resolved systematics in a taxonomically unstable group of freshwater mussels. Although our approach is robust with available data, there are still many unknowns regarding the host fish species and infection strategies of Quadrulini, especially within common species (Fig. 3). A more thorough understanding of the host repertoires of common species has promise in understanding their apparent resiliency to anthropogenically driven ecosystem state

change. Our findings provide novel information regarding the evolution of host use in Quadrulini (Fig. 3), which can be used to guide future life history studies in poorly explored taxa.

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Data Availability Statement

All molecular data used in this study are available in the GenBank SRA database under BioProject PRJNA800785. Accession numbers are provided in Table 1. The DNA alignment file used in this study (File S1), all tree files (Files S2–S6), and data sources for ancestral character reconstructions (Files S7–S8) are available on [Sciencebase.gov](https://www.ncbi.nlm.nih.gov/genbank/sra/) (Johnson & Smith, 2023).

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**Supplemental Material (See Johnson
& Smith, 2023)**

Table S1. Host taxa confirmed by lab trials included in the codiversification analysis.

Family	Taxa
Acipenseridae	<i>Scaphirhynchus platyrhynchus</i>
Centrarchidae	<i>Ambloplites rupestris</i>
Centrarchidae	<i>Lepomis cyanellus</i>
Centrarchidae	<i>Lepomis gibbosus</i>
Centrarchidae	<i>Lepomis macrochirus</i>
Centrarchidae	<i>Lepomis megalotis</i>
Centrarchidae	<i>Micropterus dolomieu</i>
Centrarchidae	<i>Micropterus salmoides</i>
Centrarchidae	<i>Pomoxis annularis</i>
Centrarchidae	<i>Pomoxis nigromaculatus</i>
Cottidae	<i>Cottus confusus</i>
Cottidae	<i>Cottus marginatus</i>
Ictaluridae	<i>Ameiurus melas</i>
Ictaluridae	<i>Ameiurus natalis</i>
Ictaluridae	<i>Ameiurus nebulosus</i>
Ictaluridae	<i>Ictalurus furcatus</i>
Ictaluridae	<i>Ictalurus punctatus</i>
Ictaluridae	<i>Noturus exilis</i>
Ictaluridae	<i>Noturus funebris</i>
Ictaluridae	<i>Noturus gyrinus</i>
Ictaluridae	<i>Noturus leptacanthus</i>
Ictaluridae	<i>Pylodictis olivaris</i>
Lepisosteidae	<i>Lepisosteus osseus</i>
Lepisosteidae	<i>Lepisosteus platostomus</i>
Leuciscidae	<i>Campostoma anomalum</i>
Leuciscidae	<i>Campostoma oligolepis</i>
Leuciscidae	<i>Clinostomus elongatus</i>
Leuciscidae	<i>Cyprinella camura</i>
Leuciscidae	<i>Cyprinella galactura</i>
Leuciscidae	<i>Cyprinella lutrensis</i>
Leuciscidae	<i>Cyprinella spiloptera</i>
Leuciscidae	<i>Cyprinella venusta</i>
Leuciscidae	<i>Cyprinella whipplei</i>
Leuciscidae	<i>Erimystax dissimilis</i>
Leuciscidae	<i>Erimystax insignis</i>
Leuciscidae	<i>Hybognathus hankinsoni</i>
Leuciscidae	<i>Hybognathus nuchalis</i>
Leuciscidae	<i>Hybopsis amblops</i>
Leuciscidae	<i>Luxilus cardinalis</i>
Leuciscidae	<i>Luxilus chrysocephalus</i>
Leuciscidae	<i>Luxilus cornutus</i>
Leuciscidae	<i>Luxilus zonatus</i>
Leuciscidae	<i>Macrhybopsis storeriana</i>
Leuciscidae	<i>Margariscus margarita</i>
Leuciscidae	<i>Nocomis biguttatus</i>

Family	Taxa
Leuciscidae	<i>Nocomis micropogon</i>
Leuciscidae	<i>Notropis atherinoides</i>
Leuciscidae	<i>Notropis percobromus</i>
Leuciscidae	<i>Pimephales notatus</i>
Leuciscidae	<i>Pimephales promelas</i>
Leuciscidae	<i>Pimephales vigilax</i>
Leuciscidae	<i>Rhinichthys atratulus</i>
Leuciscidae	<i>Rhinichthys cataractae</i>
Leuciscidae	<i>Rhinichthys obtusus</i>
Leuciscidae	<i>Semotilus atromaculatus</i>
Percidae	<i>Etheostoma gracile</i>
Percidae	<i>Perca flavescens</i>
Percidae	<i>Sander vitreus</i>
Sciaenidae	<i>Aplodinotus grunniens</i>

Table S2. Taxon sampling for host tree in host repertoire analyses.

Family	Taxa
Acipenseridae	<i>Scaphirhynchus platyrhynchus</i>
Centrarchidae	<i>Micropterus salmoides</i>
Cottidae	<i>Cottus marginatus</i>
Ictaluridae	<i>Ameiurus melas</i>
Ictaluridae	<i>Ictalurus punctatus</i>
Ictaluridae	<i>Noturus exilis</i>
Ictaluridae	<i>Pylodictis olivaris</i>
Lepisosteidae	<i>Lepisosteus osseus</i>
Leuciscidae	<i>Cyprinella venusta</i>
Percidae	<i>Perca flavescens</i>
Sciaenidae	<i>Aplodinotus grunniens</i>

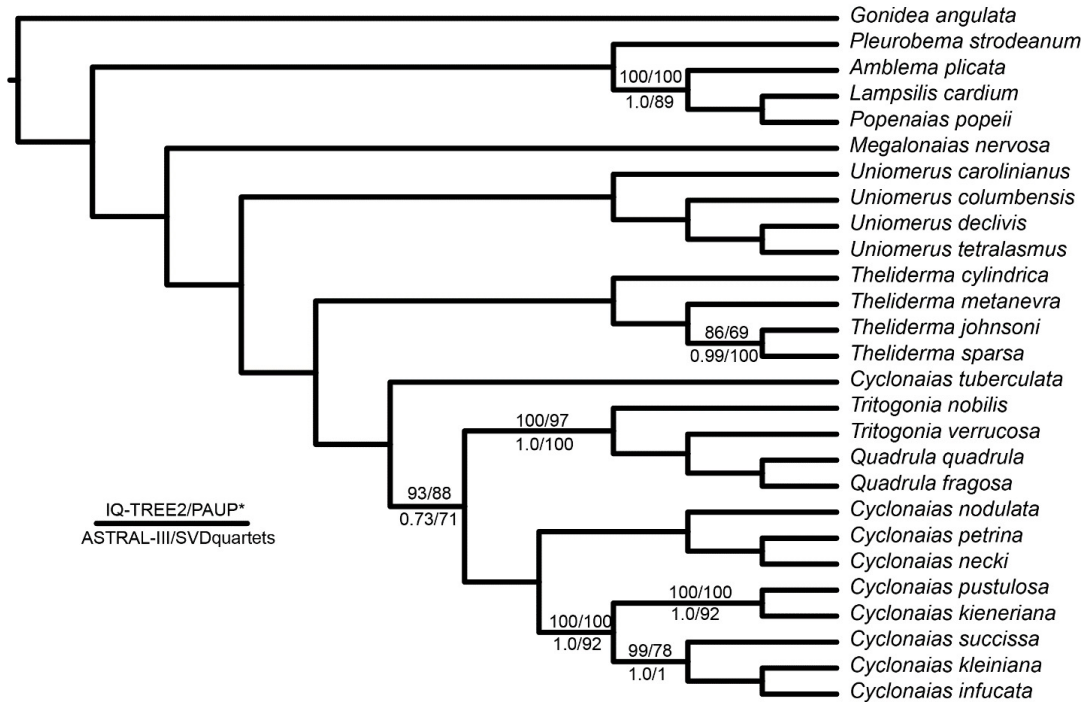


Figure S1. Cladogram generated by maximum likelihood and maximum parsimony analyses.

Tips are labeled with taxonomic hypotheses for *Cyclonaias* sensu Johnson et al. (2018) and *Tritogonia* sensu Lopes-Lima et al. (2019). All trees were concordant except for a minor topological difference due to the placement of "*Cyclonaias succissa*" in SVDquartets (BS = 1). For nodes without full support (i.e., PP < 1.0 or BS/ufBS < 100), support values from concatenated analyses (IQ-TREE2/PAUP*) are denoted above and coalescent-based (ASTRAL-III/SVDquartets) below the branch.

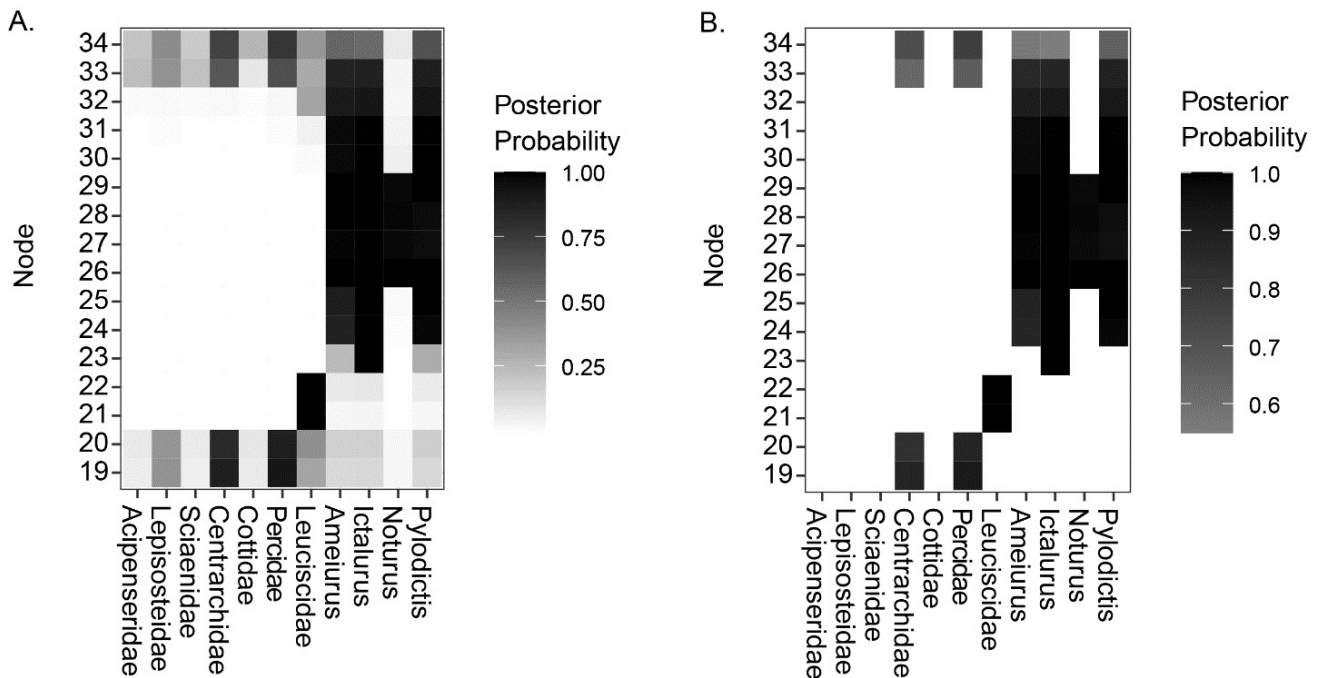


Figure S2. Interactions from the host repertoire evolution analysis supported by A) PP ≥ 0 and B) PP ≥ 0.5.

Node numbers align with node labels presented in Figure 3 in the main text.

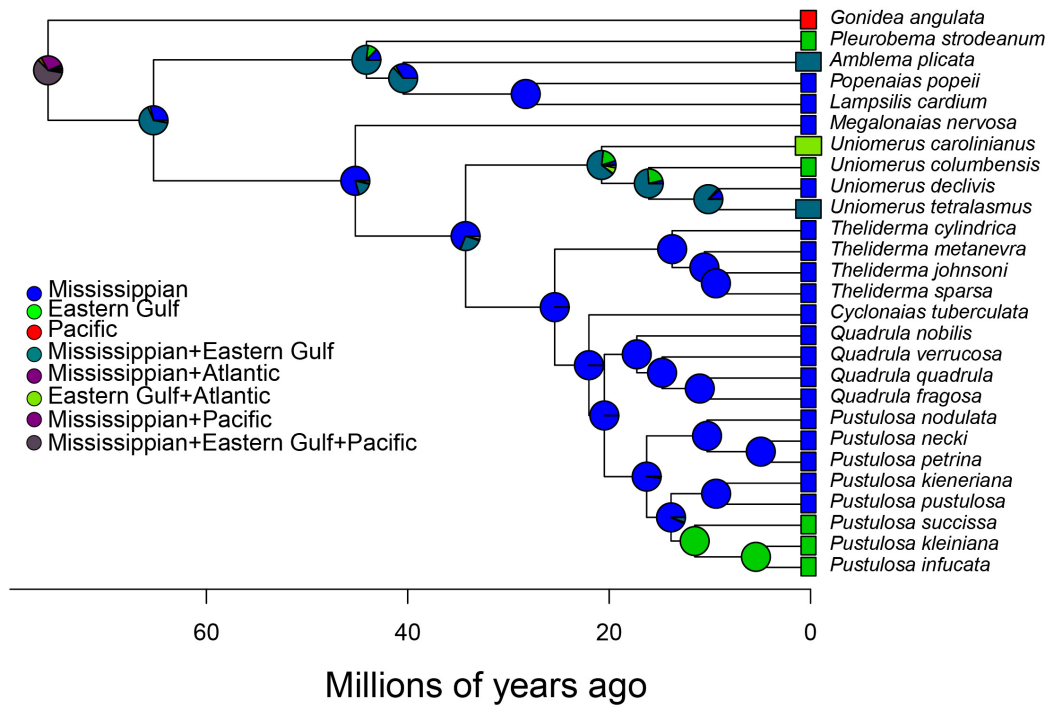


Figure S3. Ancestral-area estimates using the DEC+J model in BioGeoBEARS for sampled freshwater mussel species.

Rectangles on terminals represent the current distribution of extant taxa, and size represents the number of ancestral areas. Pie charts on nodes represent support ancestral areas.

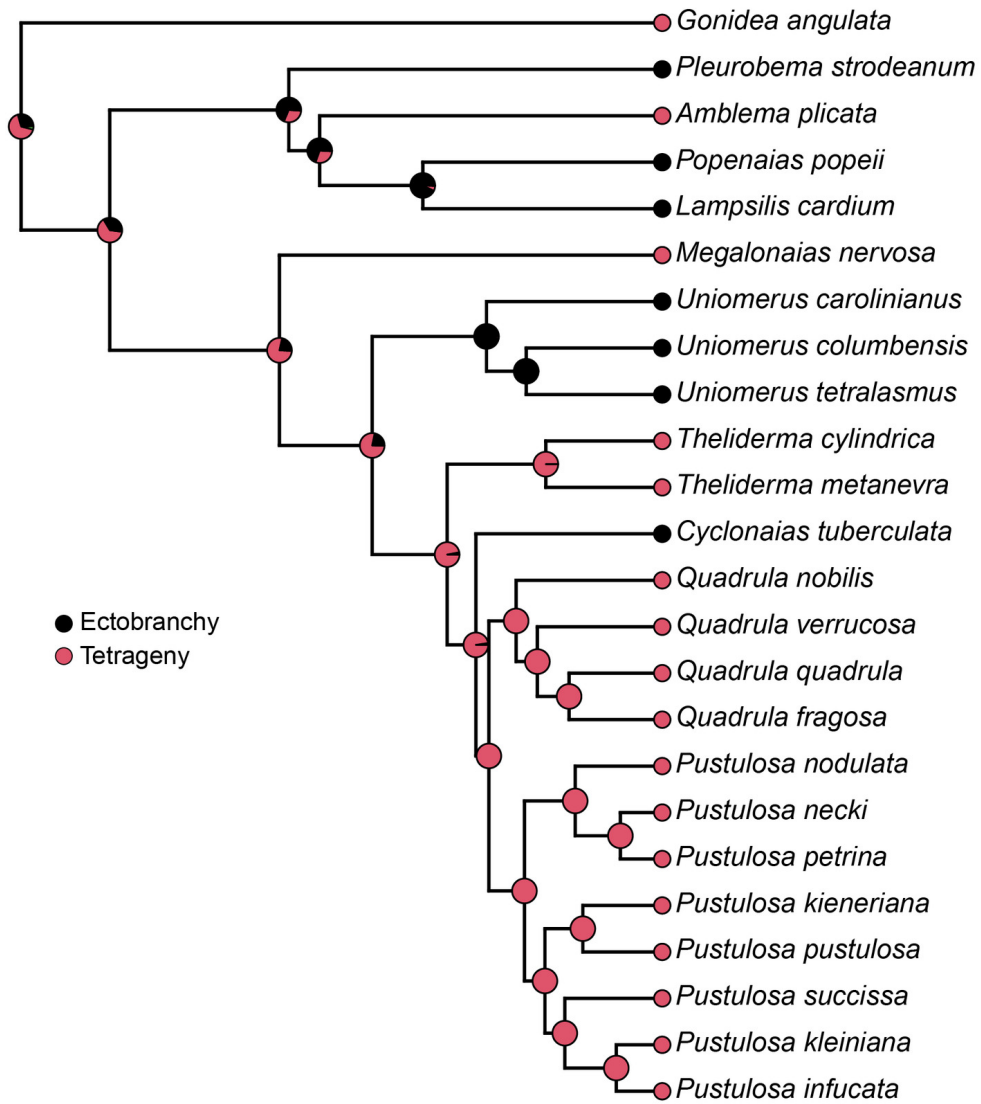


Figure S4. Bayesian stochastic character mapping of brooding morphology on the time calibrated phylogeny of Quadrulini generated by BEAST.

Coloring in pie charts on nodes indicate posterior probability support for character states.

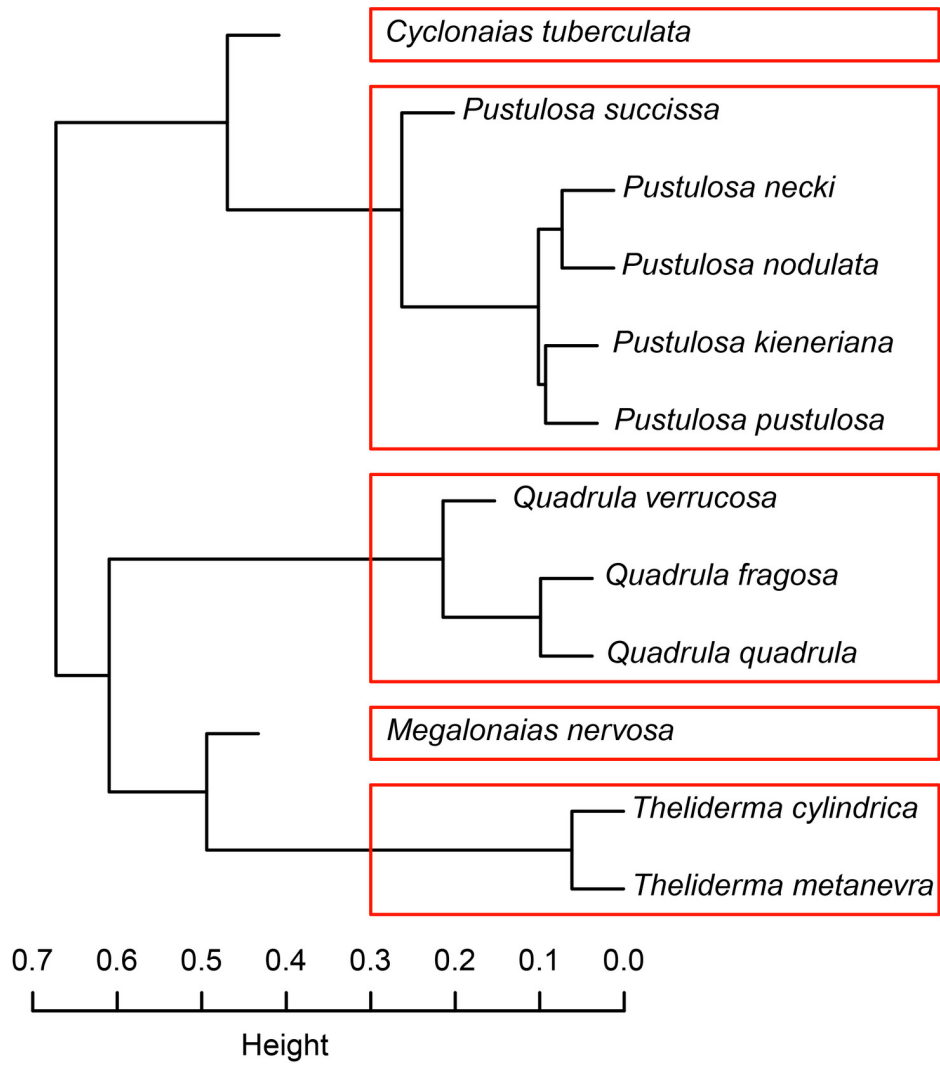


Figure S5. Hierarchical clustering based on biogeographical, ecological, morphological, and phylogenetic distances.

Red boxes indicate cutoffs of clusters based on height of 0.3.