Evolutionary Dynamics of Parasitic Larval Ecology and Lure Mimicry in North American Freshwater Mussels (Unionidae: Bivalvia: Mollusca)

by

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ABSTRACT

Freshwater mussels in the order Unionoida exhibit two outstanding attributes. Although only one of nine freshwater bivalve lineages, they dominate that group representing over 75% of species. They also have an extraordinary life cycle that includes obligatory parasitic larval development on fish hosts, and gravid females have evolved a spectrum of strategies, including mimicry, to increase the probability of host infection. The primary goal of my thesis was to test the hypothesis that the unique larval ontogeny and ecology of unionid freshwater mussels has contributed to the extraordinary diversification of this group. To do this, I focused on North American unionids using three complimentary levels of investigation: at macro-, meso-, and microevolutionary scales. I used available mussel and fish phylogenies, as well as a database of known mussel-host interactions, to address macroevolutionary and ecological aspects of mussel-fish interaction across North American watersheds. Despite the brief duration of their parasitic larval phase, mussels exhibited a similar, right-skewed distribution of host specialization to most other parasite lineages. I also found that genetic distance, range overlap, and number of citations of mussel taxa were most associated with the degree of fish host sharing. Competition for fish hosts is therefore likely to be a major influence on unionid evolution and diversification. I used a phylogenomic approach to reconstruct the evolution of host infection strategies in the diverse and imperiled Lampsiline clade to put host use in an evolutionary context and to estimate speciation rates. Lampsilines have evolved diverse, elaborate mimetic lures to attract fish hosts and the phylogeny indicates an early evolution of mimetic mantle lures, with the subsequent evolution of brood lures. Additionally, I observed strong clade-specific fidelity of host use, as

well as an increase in diversification rates associated with the evolution of a composite host infection strategy involving both mantle and simple brood lures and targeting centrarchid basses. I argue that lampsiline mussels represent a cryptic adaptive radiation, where ecological diversification occurs primarily during the brief parasitic larval stage. That hypothesis is consistent with larval development and ecology being a driver of diversification in the group and may help explain the ecological co-persistence of so many mussel species in North American watersheds without distinct (post-larval) niches.The microevolutionary component was an integrative study of the evolution, behavior, and ecology of diverse mantle lure phenotypes in *Lampsilis fasciola.* I confirmed that this represents a true polymorphism using a phylogenomic approach and by documenting within-brood polymorphism. I then identified putative model species (darters and a leech) for both main phenotypes in the Raisin River study population. Although differing in both coloration and morphology, this polymorphism does not include a significant behavioral component. The discrete nature of its within-brood inheritance suggests that the polymorphism may be regulated by a single locus, and that this system could serve as a model system for identifying underlying genes controlling mantle lure evolution in lampsilines. Each chapter of my dissertation analyzes the role of larval ontogeny on freshwater mussel biodiversity from a different perspective and this research has obtained considerable supporting evidence for the importance of the larval ontogeny in the diversification of freshwater mussels, although it has also highlighted the need for more data at multiple scales. Accurate, high-resolution data of mussel-host interactions in natural settings is necessary to build useful models of this complex evolutionary system.

INTRODUCTION

Understanding global patterns of biodiversity is an unresolved and heavily researched question in the fields of ecology and evolutionary biology (Gaston, 2000; Beaugrand, Kirby & Goberville, 2020). Diversity gradients are influenced by several different factors, from geography and other abiotic factors to ecology and evolutionary history (Ricklefs, 2004). My dissertation addresses a basic question about a distinctive clade of bivalve mollusks: Why is there so many species in the family Unionidae relative to other lineages of freshwater bivalves?

Mollusca is the second most diverse animal phylum, and bivalves comprise a large percentage of all molluscan fauna (Ruppert, Fox & Barnes, 2004; Smith et al., 2011). The vast majority of bivalves are marine organisms (McMahon & Bogan, 2001; Bogan, 2007), yet there have been 9 independent colonizations of freshwater habitats by bivalve lineages (Bogan, 1993, 2007; Park & Foighil, 2000). Among these freshwater bivalve lineages, the order Unionoida comprises about ~80% of all known species of freshwater bivalve globally (Graf & Cummings, 2007), and within that, about 75% of Unionoida (~700 species) are in the family Unionidae (Graf & Cummings, 2006). The pronounced dominance of freshwater bivalve diversity by unionids (commonly known as freshwater mussels) is enigmatic and my dissertation tests the hypothesis that it stems, in significant part, from their extraordinary larval ontogeny and ecology.

The larval ecology of unionid mussels is unique among bivalves. They are obligate parasites of fishes (Barnhart, Haag & Roston, 2008) and lack the planktonic larval development, or benthic direct development, typical of most bivalve species (McMahon & Bogan, 2001; Bogan, 2007). This brief host-parasite interaction is critical for overall freshwater mussel fitness,

as recruitment will not occur if the larvae are unable to parasitize a suitable host (Haag, 2012), and it is believed to have evolved as a mechanism for promoting upstream dispersal in lotic habitats (Barnhart, Haag & Roston, 2008). With ~300 species, North America harbors a disproportionally large percentage of global unionid biodiversity (Graf & Cummings, 2007; Bogan & Roe, 2008; Haag, 2012). They have collectively evolved a spectrum of strategies for increasing the probability of successful infecting hosts with their larvae, including many remarkable examples of aggressive mimicry (Barnhart, Haag & Roston, 2008; Jamie, 2017).

The parasitic interaction between unionid mussels and their fish hosts provides additional ecological complexity to the freshwater bivalve niche (Hutchinson, 1957), and my hypothesis is that this new niche axis has been influential in the diversification of the Unionidae. My dissertation investigates the ecological and evolutionary consequences of the short but critical parasitic larval ontogeny of unionid mussels. This research will focus on three different scales: a macroevolutionary scale which investigates evolutionary relationships between North American mussels and their host fishes, as well as building ecological models of patterns of host use and host specialization; a mesoevolutionary scale which investigates the evolutionary history of mimetic lures in the highly diverse lampsiline clade; and a microevolutionary scale which investigates polymorphic lure mimicry in *Lampsilis fasciola*, and defines this as a model system for discovering the genetic basis of lure development and evolution.

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CHAPTER 1

Ecological Correlates and Phylogenetic Signal of Host Use in North American Unionid Mussels

ABSTRACT

Mussels in the order Unionoida comprise ~75% of the world's freshwater bivalve species and are free-living apart from a brief larval stage that parasitizes fish. We investigated the relationships among species of North American unionid mussels and their known host fishes from a macroevolutionary perspective to test whether and how ecological and evolutionary factors correlate with patterns of host use. A subset of 69 mussel species were chosen based on data availability regarding their fish host repertoires, phylogenetic relationships, and ecology. Despite the brevity of their parasitic life stages, the mussels conformed to the right-skewed distribution of host ranges typical of parasitic taxa, in which most species are specialists and a few are generalists. Phylogenetic least squares regression models identified affinity for low-gradient and riffle habitats and colonization of post-glacial watersheds as the best predictors for number of fish host species per mussel. However, the second-best model identified citation number as a predictor of the number of hosts, implying that many mussel–host interactions still remain to be identified. A Multiple Regression Mantel test was performed to identify factors associated with the proportion of hosts shared between pairs of mussel species. Range overlap, citations, genetic distance, and similarity in host infection strategy were significantly correlated with proportion of hosts shared, yet total variation explained by the best model was low (R^2 = 0.14). There was evidence of topological association between mussels and their host (P = 0.001)

and a significant phylogenetic signal of host specificity ($\lambda = 0.81$, $P = 0.003$), indicating closely related mussels that overlap in range are more likely to be competing for hosts. Our results provide an initial macroevolutionary framework for studying the evolution of host infection strategies in these mussels but also highlights gaps still remaining in our fundamental ecological knowledge of this endangered clade.

INTRODUCTION

It was once assumed that organisms generally become more specialized over evolutionary time, eventually leading to "evolutionary dead ends", where species are incapable of reversing this process (Moran, 1988). More recently, evolutionary transitions from specialist to generalist have been documented in a variety of lineages, implying that specialization is favored in some circumstances, while generalization is favored in others (Armbruster and Baldwin, 1998; Barrett, 2013). Studies examining the phylogenetic signal of specialization often come to conflicting conclusions; some show specialization to be highly conserved (Brandle et al., 2002) and others find no such phylogenetic signal (Nosil, 2002; Sargent and Vamosi, 2008). In a large study comparing 116 genera across the tree of life, (Gomez et al., 2010) found significant phylogenetic conservation in 69% of antagonistic (parasitic or predatory) biotic interactions. Parasite taxa vary substantially in the number and phylogenetic breadth of hosts they are able to infect (Combes, 2001). This trait (host specificity) is straightforward to quantify, making host–parasite interactions a convenient system for studying the evolution of specialization.

The number of parasite taxa has been estimated to exceed that of "free-living" (i.e., non-parasitic) taxa (Dobson et al., 2008) and, in some systems, the total biomass of parasites rivals

that of large, conspicuous faunal groups such as fish or birds (Price, 1980; Kuris et al., 2008; Preston et al., 2013). Parasites have strong effects on community dynamics by altering host vital rates and behavior (Price et al., 1986; Moore and Gotelli, 1990; Poulin, 1999; Wood et al., 2007). Parasitism is an important evolutionary driver, and the host range (number of hosts used) of parasites is suspected to have strong effects on the evolutionary rates and epidemiology (García-Arenal and Fraile, 2013; Frenken et al., 2017). Host specialists tend to have higher speciation rates, due either to co-speciation with their host or to host switching (Poulin and Keeney, 2008; de Vienne et al., 2013). Specialists also tend to have higher extinction rates, presumably due to their dependence on only a few host taxa and small geographic ranges (Jablonski, 1987; Krasnov et al., 2005). Therefore, investigating the ecological and evolutionary correlates of host specialization is important for understanding macro-evolutionary patterns of biodiversity.

Most bivalve mollusks either produce planktonic, free-living larvae or have direct development (Bogan, 2008; McMahon and Bogan, 2009) but one lineage of freshwater mussels has uniquely evolved an obligate parasitic relationship with fish hosts (Barnhart et al., 2008). The Unionoida is an ancient (~200 mya) clade and is by far the most diverse freshwater bivalve order, representing 796 out of 1026 of the described species of freshwater bivalves (Graf and Cummings, 2007). These mussels are typically large, easy to locate, and vary substantially in their degree of host specialization (Cummings and Watters, 2016). Also, due to their historic importance for the button industry (before the use of plastics) and present threatened status, a large amount of data has been collected detailing North American mussel–fish parasitic interactions (Haag, 2012; Cummings and Watters, 2016). These characteristics make unionid mussels an attractive but under-utilized system for studying host–parasite interactions.

Freshwater mussels in the order Unionoida have a unique life cycle in which specialized larvae, called glochidia, are obligate short-term parasites on fish hosts (Barnhart et al., 2008). Typically, glochidia attach to and encyst in the fish's gill tissues and although some nutrition is derived from the host, enhanced upstream dispersal capability is hypothesized to be the primary adaptive driver of this parasitic life history, with the fish hosts acting as dispersal vectors (Watters, 2001; Fisher and Dimock, 2005; Barnhart et al., 2008). After the short 2–4 weeks transformational period that occurs during attachment to the host, the newly formed juvenile resembles a small adult mussel, exits the fish host and is capable of surviving in the sediment (Araujo et al., 2002). Following infection with glochidia, fish become temporarily immune to further infection (Reuling, 1919; O'Connell and Neves, 1999; Dodd et al., 2006).

The family Unionidae comprise the majority of species (620 of 796) within Unionoida (Bogan and Roe, 2008) and almost half (300) of these are endemic to North America (Bogan and Roe, 2008; Graf and Cummings, 2007; Haag, 2012). Some unionid mussels use a "broadcast" host infection strategy, releasing glochidia into the water column, either singly or within a mucous net, but many other species have evolved novel lure mechanisms for attracting particular host species leading many researchers to suspect strong specialization in these taxa (Figure 1; Haag and Warren, 1999; Barnhart et al., 2008).

North America harbors a disproportionate share of global unionid biodiversity (Graf and Cummings, 2007; Bogan and Roe, 2008; Haag, 2012). However, this faunal group is imperiled due to habitat destruction, historical overharvesting, the introduction of invasive species, pollution, and changes in land use (Strayer et al., 2004; Downing et al., 2010; Haag, 2012). Freshwater mussels are an important component of freshwater ecosystems, providing unique microhabitats for benthic invertebrates and increasing available nutrients for the benthic

community (Spooner and Vaughn, 2006). Unionids are found in extremely diverse and dense aggregations, called beds, with up to 68 different species occurring in a 80-km reach of a single river (Garner and McGregor, 2001; Haag, 2012). Despite the ecological and conservation value of this clade, the evolutionary processes that led to the diversification of the Unionidae are poorly understood (Graf and Cummings, 2006).

Given the historical existence of diverse assemblages of unionids and the immune-mediated limitation of host infection, hosts could be a limiting resource for unionid mussels. If this were the case, we would expect fewer shared hosts between competing species (i.e., species that are closely related and have overlapping ranges) than between species that are not competing. (Rashleigh and DeAngelis, 2007) developed a model that predicts coexistence between mussel species if they differ in host encounter rate among different fish species, suggesting that resource partitioning of hosts may be important for mussel coexistence. A field experiment by (Haag and Stoeckel, 2015), however, did not find evidence for reduced recruitment for two mussel species competing for the same host. If adaptation to new host species is a relatively slow evolutionary process, we would expect closely related mussels to have more shared hosts due to inherited immunological compatibility. It is currently unknown whether host range is a conserved or labile trait in the Unionidae. We also do not understand whether the frequency distribution of unionid host range conforms to the patterns observed in other parasite taxa or what traits are associated with specialist or generalist species. These gaps in knowledge are important, given the consequences of host range for diversification rates (Krasnov et al., 2005). Parasite taxa generally have a right-skewed distribution of host ranges, where most species are specialists and a few are generalists (Gregory et al., 1991; Poulin, 1992; Vazquez et al., 2005).

Here, we investigate the relationship between unionid mussels and their host fish from a macroevolutionary perspective, to elucidate ecological and evolutionary predictors of host use. Specifically, a series of five questions are addressed: (1) How does host range (number of host species infected) vary among species of unionid mussel? (2) What factors are associated with similarity of host assemblages between species of unionid mussel? (3) Is there evidence for topological congruence between North American unionid mussels and their hosts? (4) Is there phylogenetic signal of host specificity in Unionid mussels? (5) What ecological and evolutionary factors are associated with host specificity in unionid mussels?

MATERIALS AND METHODS

Database Assembly

We compiled a dataset for 69 North American unionid species and their fish hosts. These taxa were chosen because their phylogenetic relationships are known, (Campbell et al., 2005) and as are their fish hosts (Cummings and Watters, 2016). Campbell et al.'s (2005) phylogeny represented all 37 recognized genera of Amblemine (a subfamily that comprises about 250 of the ~300 North American unionids) and we chose a subset of 69 eastern North American freshwater mussel taxa encompassing all North American species included in that study. Cummings and Watters (2016) compiled a publicly available database (INHS) detailing all known North American mussel–host interactions, and this database was queried for the 69 species of interest to obtain data on the number of fish host species, host genera, and host families (Cummings and Watters, 2016). The mussel–host database is incomplete, and many of its records were determined using different types of evidence. Nevertheless, we included all INHS database mussel–host interactions in our analyses, because they represent the best available data on North American mussel–host interactions. To account for differences among mussel species in

sampling effort (which could affect the number of hosts species reported for each mussel species), each mussel's binomial name was queried in Web of Science (Reuters, 2012) and the total number of citations was recorded. Genetic sequence data for freshwater mussels were obtained from Campbell et al. (2005), consisting of concatenated fragments of COI, 16S, and ND1 (see Campbell et al. [2005] for further information regarding gene sequencing and amplification). Habitat preference data were collected from NatureServe (NatureServe, 2007), by recording habitat types listed for each mussel species. Possible categories for these data include: river, creek, high-gradient stream, low-gradient stream, pool, riffle, and lentic, and it is common for individual mussel species to span multiple habitat categories. Range maps were estimated for all 69 species of North American mussels using GBIF occurrence point data, extracted using the 'dismo' package in R (R Core Team, 2014; Hijmans et al., 2015; GBIF Secretariat, 2017) . To remove outliers, GBIF data were screened for points that occur outside of North America and those points were removed from the dataset. Species distributions were estimated by creating a minimum convex hull polygon using the 'rgdal' package in R and range area was converted to square kilometers using the areaPolygon function in the 'geosphere' package in R (Bivand et al., n.d.; Hijmans et al., 2012). A phylogenetic tree for 7,822 species of fish created using a mega-phylogeny approach (Smith et al., 2009) was obtained from Rabosky et al. (2013).

Frequency Distribution of Host Range

The number of fish species identified as hosts in the INHS mussel host database was tallied per mussel species. A histogram was created to show the distribution of host range (number of hosts per mussel) across this subset of North American unionids (Figure 2). This frequency distribution was then compared to several ideal distributions (Poisson, negative binomial, log-normal, normal, exponential, geometric, and Weibull). Using the MASS package,

implemented in R, the unionid host range data were fitted to these distributions and all distributions were compared using log-likelihood values to determine which distribution best fit the data (Venables and Ripley, 2013).

Multiple Regression on Distance Matrices

We performed a Multiple Regression on Distance Matrices (MRM) to determine whether genetic distance, range overlap, number of citations, or similarity in host infection strategy were significantly correlated with the proportion of shared host species among unionid mussel species (Lichstein, 2007). We used the proportion of shared hosts rather than the total number of shared hosts to account for the confounding influence of total number of host on the number of shared hosts. Pairwise data lack independence, and a General Linear Model cannot therefore be used to assess significance for these data. The MRM was performed in R using the 'ecodist' package (Goslee and Urban, 2007) with the response variable [number of shared hosts between mussel species pairs], calculated using the data from the INHS database. Genetic data for three mitochondrial loci obtained from Campbell et al. (2005) were used to calculate a pairwise matrix of genetic distance (K80; (Kimura, 1980)) between all pairs of mussel species used in this analysis using the 'ape' package in R (Paradis et al., 2004). To estimate proportion of range overlap, a separate spatial polygon representing the overlap between ranges was created for each pair of species using the gIntersection function in the 'rgeos' package in R. The area of the intersection (in km^2) was calculated using the 'geosphere' package, and the proportion of range overlap was obtained by dividing the area of overlap by the union of the two ranges. To estimate the number of citations for each pair of mussel species, we calculated the product of the log number of citations from Web of Science.

A binary pairwise matrix was created to describe similarity between mussel host infection strategies. Mussels were categorized into two basic host infection strategies – lure and non-lure – based on whether the infection strategy elicits a change in behavior in the host. Host infection strategies were first categorized using a variety of sources (Zanatta and Murphy, 2006a; Barnhart et al., 2008; Haag, 2012). Specific categorization of mussel host infection strategies can be found in Supplementary Table 1. Mussels that create conglutinates, superconglutinates, mantle lures, mantle magazines, or used a female sacrifice strategy were classified as 'lure' because their infection strategy relies on inducing predatory behavior in the fish hosts. Mussels that broadcast free glochidia or mucous webs containing glochidia were classified as 'non-lure'. All pairs of mussels were compared; pairs that shared the same basic strategy (lure or non-lure) received a value of 1 in the pairwise matrix, and all other pairs received a value of 0.

ParaFit Test of Topological Congruence

To assess whether there was topological congruence between unionid mussels and their hosts, we performed a ParaFit analysis, implemented in R using the 'ape' package (Legendre et al., 2002). It is important to note that ParaFit only tests for topological congruence between the associated hosts and parasites (i.e., nonrandom associations). Topological congruence is expected from two clades that have coevolved, but it is impossible to falsify the hypothesis that host and parasite clades are congruent through allopatric processes alone using this approach (Brooks, 1979). The data used to create the association matrix for the ParaFit analysis were collected from the INHS database and the host fish phylogenetic distance was calculated from the phylogenetic tree provided in Rabosky et al. (2013). Mussel genetic distance (K80) was calculated using the DNA sequence data provided by Campbell et al. (2005). ParaFit was performed with 999 permutations, using the Lingoes correction (Lingoes, 1971) to adjust for

negative eigenvalues. A Bayesian phylogenetic tree was created in Mr Bayes vs 3.2.6 using the mussel mitochondrial data (Huelsenbeck and Ronquist, 2001; Campbell et al., 2005). Bayesian analyses was performed for these mussels as described in Campbell et al. 2005. However, highly variable regions of 16S were included in the final analysis and the number of chains used was left at the default value of 4. For visualization purposes, the mussel and fish phylogenetic trees were plotted with interaction lines connecting associated tips from host to parasite using the 'cophyloplot' function from the 'ape' package in R.

Phylogenetic Signal of Host Range

We used the Bayesian phylogenetic tree created for unionid mussels (see above) and attached the number of hosts to each tip using the 'phylobase' package in R (Hackathon et al., 2013). Multiple indices were calculated to assess the phylogenetic signal of the number of hosts including: Moran's I index (Moran, 1948), Abouheif's C_{mean} index (Abouheif, 1999), Blomberg's K and K^* (Blomberg et al., 2003), and Pagel's λ (Pagel, 1999). All indices and tests were calculated and performed using the 'phyloSignal' and 'lambdaTest' functions in the 'phylosignal' package, implemented in R, with 999 reps (Keck et al., 2016). The number of hosts for each mussel species was plotted next to each tip on the phylogeny using the 'barplot.phylo4d' function from the 'phylosignal' package in R.

Phylogenetic Least Squares Regression Analysis

A series of generalized least-squares regression analyses were used to determine which variables were correlated with the number of hosts per mussel. Variables included in the analysis were geographic range, log number of citations for each mussel species, host infection strategy (reduced to either active or passive), a binary variable expressing whether mussel geographic range overlaps with previously glaciated areas during the Pleistocene (performed by manually

comparing range maps of each species to known glacial maxima), and multiple binary variables describing habitat preferences as indicated by NatureServe.org (NatureServe, 2007) including: river, creek, high-gradient stream, low-gradient stream, pool, riffle, and lentic. All combinations of main effects of these variables were considered in the models, and models were ranked using AIC (Akaike, 1987). Residuals of the best models were checked for normality using a Shapiro-Wilks test and, if necessary, the response variable was modified to conform to the assumption of normally distributed residuals (Mundry, 2014).

Phylogenetic least squares regression is typically used to account for non-independence among species and is one of the most commonly used methods for phylogenetic comparative analysis (Symonds and Blomberg, 2014). This approach takes the varying degrees of relatedness between species into account, by incorporating hypotheses of shared branch lengths between species as a correlate in the analysis. This is accomplished by setting the error term of the model to a phylogenetic correlation matrix. The phylogenetic correlation matrix was created from the Bayesian phylogenetic tree (described above) using the unionid mitochondrial sequence data from Campbell et al. (2005) and then using Brownian motion to model the expected variances based on shared branch lengths. If, however, a phylogenetic correction is applied to a model when there is no phylogenetic signal in the residuals, the Type 1 error will be inflated (Revell, 2010). To correct for this potential problem, a second parameter, Pagel's λ, was added to the model (Pagel, 1999). This parameter estimates phylogenetic signal and was optimized using maximum likelihood to estimate the phylogenetic signal in the residuals of each model. The phylogenetic covariance matrix is then multiplied by Pagel's λ to scale the extent of phylogenetic dependence included in the model (Luis et al., 2015). If $\lambda = 1$, the error structure of

the model is perfectly correlated to the phylogenetic covariance matrix. If $\lambda = 0$, there is no phylogenetic signal in the error structure.

RESULTS

Database Assembly

We searched the INHS mussel host database for the 69 species of unionid mussel for which we had data on phylogenetic relationships and found 171 species of host fish described for this subset of mussels. Of the 171 identified hosts, 150 were included in the phylogeny provided by Rabosky et al. (2013).

Frequency Distribution of Host Range

The frequency distribution of the number of hosts per mussel was right skewed (Figure 2), consistent with the frequency distribution of host range for many other parasitic taxa (Gregory et al., 1991; Poulin and Mouillot, 2003). Log-likelihood values indicated that the best-fit distribution was log-normal (Table 1).

Multiple Regression on Distance Matrices

The MRM model that explained the most variation ($R^2 = 0.14$; $P < 0.001$) incorporated all of the variables (Table 2). This was expected, because these models are not penalized for additional parameters, so the model with the greatest number of parameters is likely to perform best. However, all models including only a single variable (range overlap, genetic distance, citations, and strategy similarity) were significant. Range overlap, genetic distance, and citations were highly significant ($P < 0.001$), but the variation in the proportion of hosts shared explained by any of the MRM models was very low. Proportion of hosts shared over genetic distance was plotted to visualize the distribution of hosts shared across a gradient of relatedness (Figure 3).

ParaFit Test of Topological Congruence

The ParaFit test of topological congruence is a statistical test designed to assess whether two associated groups of organisms (e.g., hosts and parasites) are associated randomly or not. There was topological congruence between unionid mussels and their host fish ($P = 0.001$). Figure 4 displays the mussel and fish phylogenies used in this analysis, including all known parasitic interactions between the two groups.

Phylogenetic Signal of Host Range

Significant phylogenetic signal was detected for mussel host range using Pagel's Lambda ($\lambda = 1.00$, $P = 0.001$), Bloomberg's K ($K = 0.39$, $P = 0.015$), and Bloomberg's K* ($K^* = 0.52$, $P = 0.002$). Phylogenetic signal was not significant when using the spatial autocorrelation metrics, Moran's I index ($I = 0.008$, $P = 0.126$; Moran, 1948) or Abouheif's C_{mean} index ($C = -0.018$, $P = 0.51$; Abouheif, 1999). Trait data (host range) is displayed next to each tip on the unionid phylogeny and centered visualization (Figure 5).

Phylogenetic Least Squares Regression Analysis

The results for the top five models of this analysis are displayed in Table 3. Each of the top five models performed similarly, as indicated by the AIC values, and the residuals of each model were normally distributed after log-transforming the response variable ($P < 0.05$). The best model for predicting the number of hosts of unionid mussels included the variables glacial (corresponding to mussels with geographic ranges extending into areas that were glaciated during the previous glacial maximum; $P = 0.036$), low-gradient habitat ($P = 0.001$), and riffle habitat ($P = 0.02$). The variable glacial was positively associated with number of hosts (estimated $\beta = 0.54$) and the low-gradient and riffle habitat types were negatively associated with the number of hosts (estimated β's = -0.91 and -0.71 respectively). This model also had a high estimated phylogenetic signal in the model residuals (Pagel's $\lambda = 0.88$).

The second-best model for predicting the number of hosts included four variables; the log number of citations ($P = 0.002$; $\beta = 0.42$), host infection strategy ($P = 0.010$; $\beta = -0.57$), low-gradient habitat ($P = 0.013$; $\beta = -0.75$), and riffle habitat ($P = 0.013$; $\beta = -0.45$). The second-best model had very low estimated phylogenetic signal in the model residuals (Pagel's $\lambda = 0.00$). To assess why this model does not show phylogenetic signal in the residuals, we tested whether the number of citations associated with each mussel had a phylogenetic signal using the 'phyloSignal' function as described above. The citations variable had significant phylogenetic signal ($\lambda = 0.91$, $P = 0.005$), which explains why the model that includes the citations variable had a low estimated phylogenetic signal in the residuals.

DISCUSSION

This study identifies both habitat and phylogeny as important predictors of host range (number of hosts per mussel) in a subset of North American unionid mussels. There is significant phylogenetic signal of host range in unionid mussels and, after controlling for phylogeny, certain habitat characteristics (low-gradient and riffle habitat) were associated with host range. Mussels and their host fish show evidence of topological congruence, which may be due to cospeciation or speciation via host-shifts (de Vienne et al., 2013). Genetic distance, proportion of range overlap, number of citations, and strategy similarity were all significantly associated with the proportion of hosts shared between mussels. However, even the best-performing model, which included all variables, was only able to explain 14% of the variation in the proportion of hosts shared. The response variable in the MRM was overwhelmingly biased towards zero, with 76% of all mussel pairs lacking any hosts in common (1774 out of 2346 comparisons). The phylogenetic signal of host range, coupled with the greater proportion of shared hosts between closely related mussels, suggests that host assemblage is relatively conserved through

evolutionary time (Revell et al., 2008). Fish, once infected by glochidia, mount a temporary immune response that reduces the probability of future glochidial infection (Reuling, 1919; O'Connell and Neves, 1999; Dodd et al., 2006). The strength of the immune response of a fish host to a secondary glochidial infection is negatively correlated with genetic distance from the initial mussel species, which would make competition for hosts stronger among closely related mussel species (Dodd et al., 2005). This suggests that closely related mussels are competing for hosts. In this scenario, mussels that have an advantage in infecting hosts first would be favored. Over time, this selective pressure could lead to the complex structures used to attract hosts that we observe in many of the Lampsilini mussels (Zanatta and Murphy, 2006b; Barnhart et al., 2008). However, this study also found the number of citations to be a significant predictor for the number of hosts in the second-best PGLS model, as indicated by AIC. This is an important caveat that implies there are probably many undocumented hosts. Also, all host–parasite interaction data from the INHS mussel host database were included in the analysis, including multiple different types of evidence for each interaction. This means that for well-studied taxa, some of the documented host–parasite interactions include laboratory infections that may not be ecologically meaningful.

Many parasites have complex lifecycles (Combes, 2001). Our understanding of parasite ecological and evolutionary dynamics is primarily derived from empirical studies on organisms that are parasitic for all of or most of their life (Minchella and Scott, 1991; Poulin, 1992), but many parasites switch hosts at different stages of their lifecycle and some organisms are only parasitic for a brief period of during their lifecycle. The results from these analyses suggest that unionid mussels show a similar frequency distribution in the number of hosts per mussel as do other parasite taxa (Gregory et al., 1991; Poulin and Mouillot, 2003). This is an important insight

because freshwater mussels are parasitic for only a very small proportion of their life cycle. Some species of freshwater mussel can live for over 100 years, and the larval life stage usually lasts for only three to four weeks. Despite the brevity of the parasitic stage, the distribution of the number of hosts for unionid mussels conforms to the general patterns observed among other parasites.

Our data showed that host range (number of hosts per mussel) had a strong phylogenetic signal across a variety of different metrics. All estimates of phylogenetic signal based on explicit evolutionary models under Brownian motion (Blomberg's K and K*, and Pagel's λ) were significant (P = 0.015, 0.002, and 0.001 respectively), but the two estimates of phylogenetic signal derived from spatial statistics were not significant. Systems with strong phylogenetic signal in their ecological interactions are associated with higher modularity, and species belonging to the same module are likely to share few hosts (Gomez et al., 2010). The host range of unionid mussels is inherited, creating clades of predominately specialist and predominately generalist taxa. Given that host range in unionid mussels is inherited, and host range influences evolutionary rates, different unionid clades may have different speciation rates (Poulin and Keeney, 2008; de Vienne et al., 2013).

The phylogenetic least-squares regression analysis reported that all of the top five models performed similarly (ΔAIC < 2). Among models predicting the number of hosts for a species of unionid, the best model included a variable representing whether the species currently inhabits area that was covered by the Laurentide ice sheet during the last glacial maximum, a variable indicating low-gradient habitat type, and a variable indicating riffle type habitat. This model also had a very high estimated phylogenetic signal in the model residuals (Pagel's λ = 0.88), meaning more closely related mussels were more likely to have a similar number of hosts. Throughout the

Pleistocene (~2.5 mya–10,000 years ago), massive ice sheets advanced and retreated across much of northern North America, with the Laurentide ice sheet covering much of eastern and central North America (Pielou, 2008). After the glaciers retreated, the newly exposed landscape became available for colonization by unionids, using their host fish as dispersal vectors. This model suggests that, after accounting for phylogeny, mussel species that colonized the recently glaciated landscape tend to have more hosts than other mussels. This scenario is to be expected, given that generalists should have greater dispersal capabilities (Nurmi and Parvinen, 2011). Our model also suggests a possible difference in habitat type between generalists and specialists. Species that occur in low-gradient habitat were likely to have fewer hosts than other mussel species, after controlling for phylogeny. This may be due to habitat variability, as high-gradient streams are likely to be more temporally variable (Schlosser, 1990). This variability in the physical characteristics of high-gradient habitats probably leads to variability in the composition of fish assemblages. In habitats where resource levels are highly variable, ecological niche theory predicts that specialists will be less successful (Levins, 1968).

The second-best model included the log number of citations for a mussel species on Web of Science, as well as the low-gradient habitat variable. The citation variable was included as a proxy for the amount of sampling effort invested into each species, and this result suggests that – despite the volume of data collected on mussel–host relationships for over a century – many mussel–host relationships probably remain undocumented. The estimated phylogenetic signal in the residuals of this model was very low (Pagel's $\lambda \sim 0.00$). This suggests that the effect of phylogeny on host range was already incorporated in the model by variables that have phylogenetic dependence (Revell, 2010; Luis et al., 2015). The citations variable was found to

have strong phylogenetic signal ($\lambda = 0.91$, $P = 0.005$), explaining the lack of phylogenetic signal in the model's residuals (Revell, 2010).

Parasitism provides a new axis in the bivalve niche that enables closely related species to diverge (Hutchinson, 1957). Unionid mussels are by far the most diverse group of freshwater bivalves and parasitism may have contributed to the success of this clade (Bogan and Roe, 2008). Due to the historically high abundance of freshwater mussels (Garner and McGregor, 2001; Haag, 2012) and the acquired immunity of the hosts (O'Connell and Neves, 1999), it seems likely that competition for hosts may be an important factor driving the evolution of the Unionidae. If freshwater mussels were competing for hosts, we would expect competition to be stronger between two coexisting mussel species that are closely related, and these mussels would be more likely to differentiate in their host usage (Webb et al., 2002). Our results suggest closely related mussel taxa are likely to have a similar number of hosts and are more likely to share the same host species than are more distantly related mussel taxa. However, there is a large amount of variation in the proportion of hosts shared, even among closely related taxa (Figure 2). Furthermore, it is unclear which particular host species are the most ecologically important to the reproductive success of different mussel species. Closely related mussels could share numerous hosts through inherited immunological compatibility yet use different subsets of host species. This analysis compares all documented host–parasite interactions equally, although there can be tremendous variation in transformation success rate between different host species and even different populations of the same host species (Riusech and Barnhart, 2000; Rogers et al., 2001; Barnhart et al., 2008).

Understanding the subtle differences between mussel host infection strategies and the phenotypic diversity of structures used to entice hosts, as well as gathering more data on mussel–

fish interactions will help to elucidate the role of parasitism on the success of this diverse and imperiled clade. Understanding host infection strategies in greater detail will enable us to better test how variation in host infection strategy relates to reproductive success of unionid mussels. Mussel species that produce mimetic mantle flaps, for example, often vary considerably in the size, shape, and pigmentation of their mantle flaps (Zanatta et al., 2007; Barnhart et al., 2008). Studying these traits in detail, and how variation in phenotype relates to variation in fitness may help us understand the evolution of these active host infection strategies. Host range (number of hosts per mussel) was shown to have strong phylogenetic signal in this system. Given the theoretical importance of host range on speciation and extinction rates, diversification rates should be estimated for the Unionidae to determine if these rates correlate with host range. The development of a large and comprehensive unionid phylogeny using high-throughput sequencing will increase the accuracy of these estimates.

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Table 1.1: The log-likelihood values for fitting the frequency distribution of the number of hosts per mussel to 7 different frequency distributions.

Distribution Name	Log-likelihood Value
Log-normal	-198.869
Weibull	-204.5227
Exponential	-204.9611
Negative Binomial	-207.2542
Geometric	-209.5612
Normal	-237.5575
Poisson	-325.0759

Table 1.2: Results from the MRM model. Response variable is the number of shared hosts between mussel species normalized by the total number of unique hosts between mussel species.

Model	R^2	P
Genetic Distance + Strategy Similarity + Range Overlap + Citations	0.14	<0.001
Range Overlap	0.06	<0.001
Genetic Distance	0.05	<0.001
Strategy Similarity	0.04	<0.001
Citations	0.02	<0.001

Table 1.3: Results from the PGLS models displaying AIC, ΔAIC, model Weight, and Pagel's λ estimated for the residual error structure in the given model. Response variable is the number of hosts per mussel species. Lowgrade, Riffle, Highgrade, and Creek all refer to different habitat preferences recorded from NatureServe.org. Glacial refers to a binary variable indicating species that range currently extends beyond the glacial maxima from the Pleistocene glaciations. Citations refers to the number of citations for each species on Web of Science.

Model	AIC	ΔAIC	Weight	λ
Glacial + Lowgrade + Riffle	224.5	0	0.015	0.88
Citations + Infection Strategy + Lowgrade + Riffle	224.6	0.1	0.014	0
Citations + Strat + Lowgrade	224.7	0.2	0.014	0
Citations + Lowgrade	224.8	0.3	0.013	0
Glacial + Creek + Lowgrade + Riffle	224.8	0.3	0.013	0.92

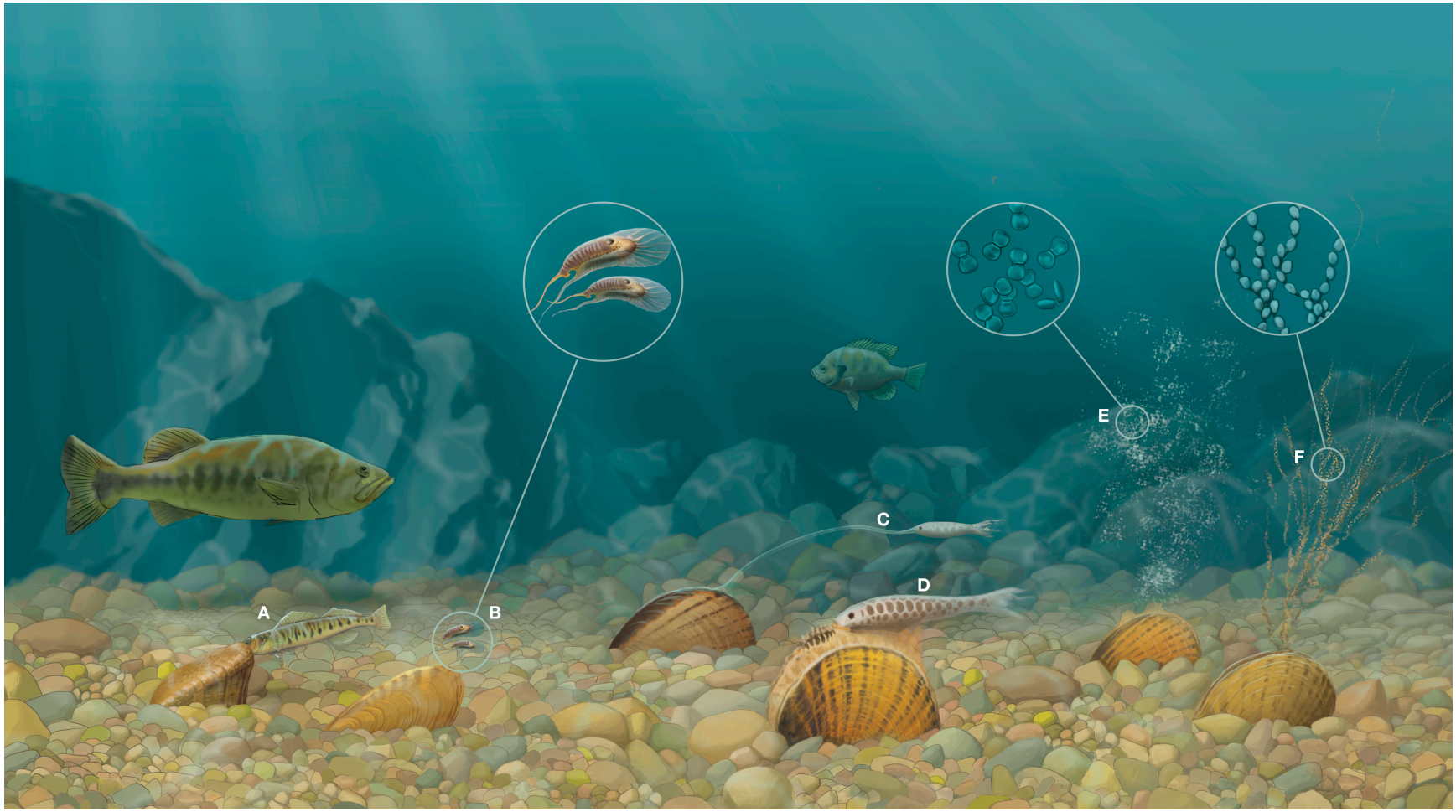

Figure 1.1: A hypothetical benthic assemblage showing exemplars of North American freshwater mussel host infection strategies (Barnhart et al., 2008; Haag, 2012). A) *Epioblasma triquetra*: physical entrapment of the host. B) *Ptychobranchus subtentum*: releasing conglutinates containing glochidia that mimic invertebrate prey items. C) *Hamiota perovalis*: "fishing behavior" using a tethered superconglutinate containing glochidia. D) *Lampsilis cardium*: displaying mantle lure. E) *Anodonta implicata*: broadcasting individual glochidia. F) *Pyganodon grandis*: releasing a mucous web of glochidia. A-D represent "lure" strategies that induce a predatory response in the fish host and E&F represent "non-lure" broadcast strategies.

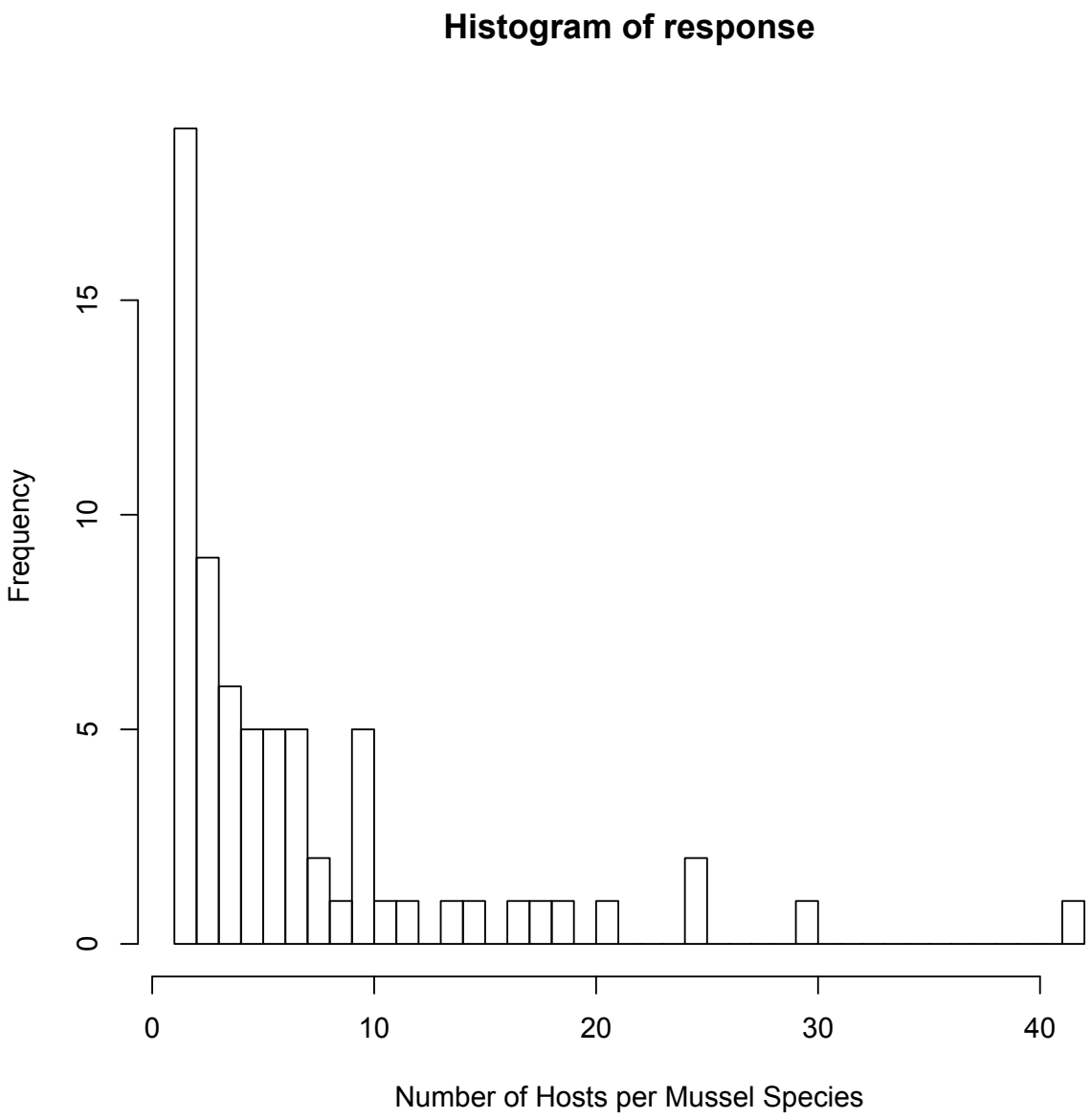

Figure 1.2: A histogram displaying the frequency distribution of the number of hosts per mussel species

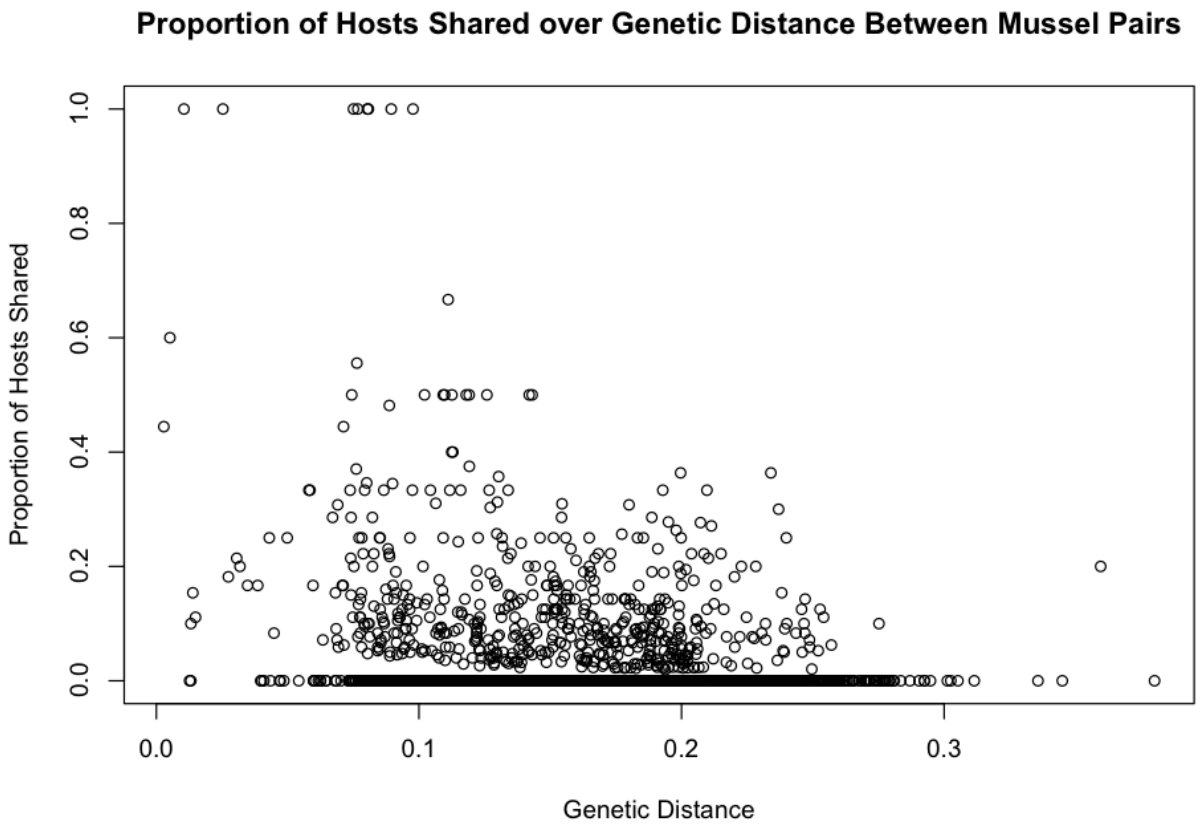

Figure 1.3: A scatterplot displaying the proportion of hosts shared over the genetic distance (K80; Kimura (1980)) for all pairs of unionid mussels in this analysis. Using a Multiple Regression Mantel test, genetic distance is significantly associated with proportion of hosts shared ($P = 0.0001$)

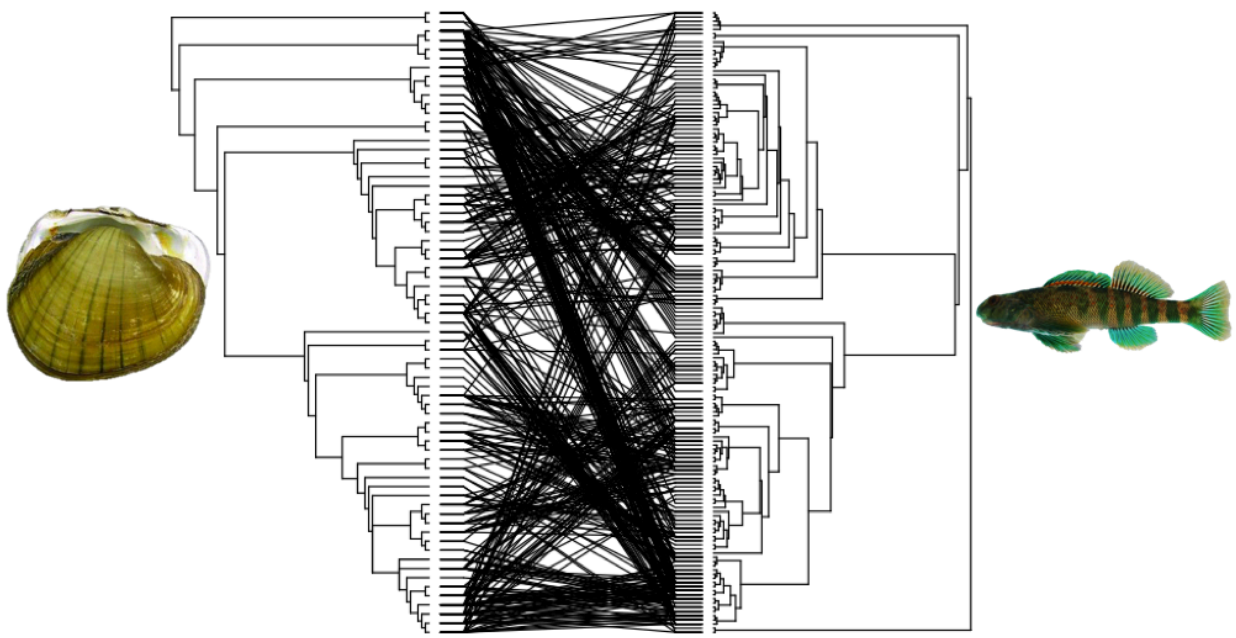

Figure 1.4: A phylogeny of a subset of freshwater mussels (left) and a phylogeny of their host fish (right). Connecting lines represent all known parasitic interactions. The Parafit test of topological congruence suggests the interactions between mussels and fish are non-randomly distributed ($P = 0.001$)

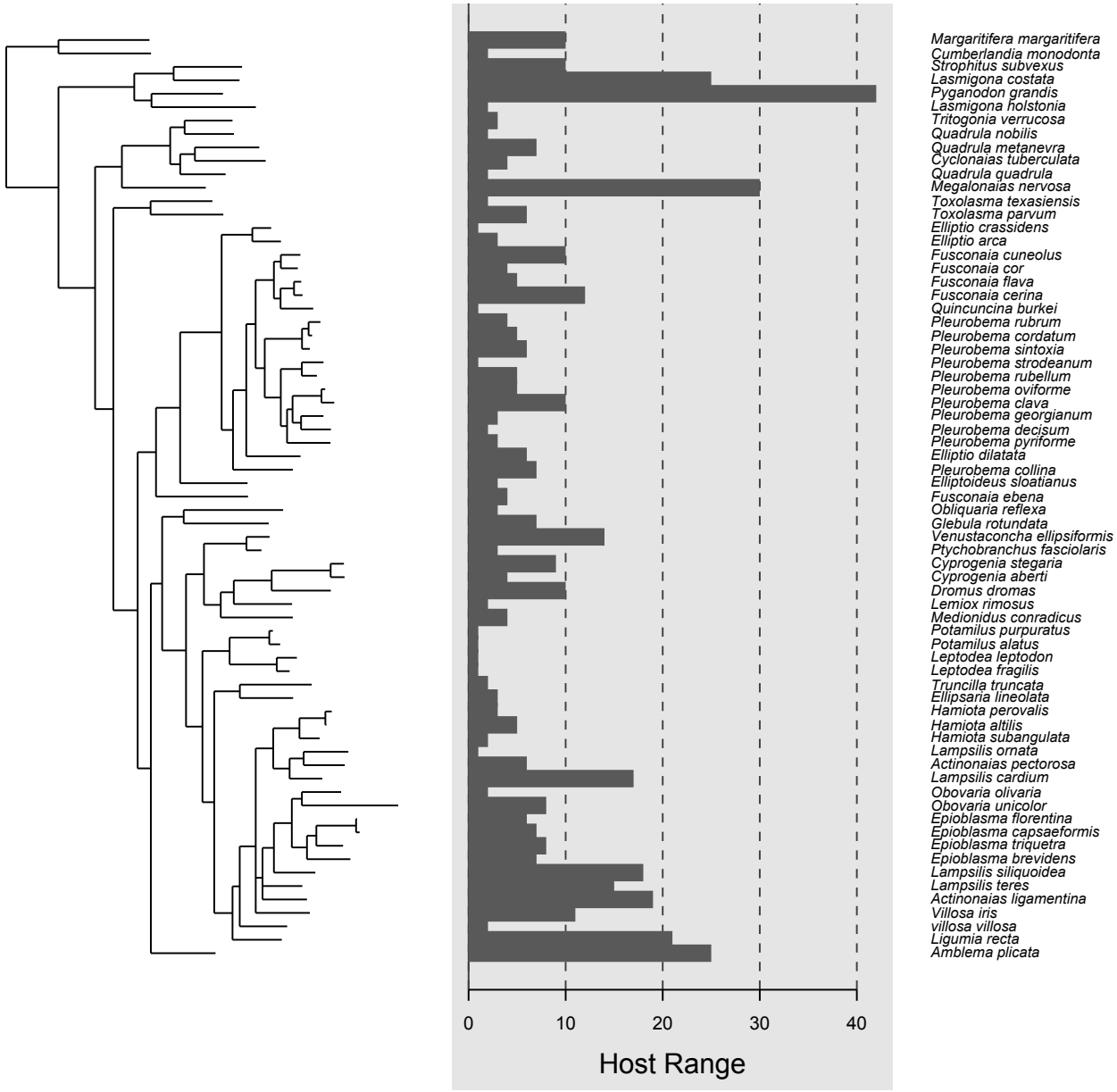

Figure 1.5: Displays the mussel phylogenetic tree alongside the trait value host range for each species of mussel. Phylogenetic signal was detected for host range in unionid mussels ($\lambda = 0.81$, $P = 0.003$).

CHAPTER 2

Evolution of Diverse Fish Host Infection Mechanisms Delineates an Adaptive Radiation of Lampsiline Freshwater Mussels Centered on their Larval Ecology

ABSTRACT

North American watersheds contain a high diversity of freshwater mussels (Unionoida). During the long-lived, benthic phase of their life cycle, up to 40 species can co-occur in a single riffle and there is typically little evidence for major differences in their feeding ecology or microhabitat partitioning. In contrast, their brief parasitic larval phase involves the infection of a wide diversity of fish hosts and female mussels have evolved a spectrum of adaptations for infecting host fish with their offspring. Many species use a passive broadcast strategy: placing high numbers of larvae in the water column and relying on chance encounters with potential hosts. Many other species, including most members of the Lampsilini, have a proactive strategy that entails the use of prey-mimetic lures to change the behavior of the hosts, *i.e.*, eliciting a feeding response through which they become infected. Two main lure types are collectively produced: mantle tissue lures (on the female's body) and brood lures, containing infective larvae, that are released into the external environment. In this study, we used a phylogenomic approach (ddRAD-seq) to place the diversity of infection strategies used by 54 North American lampsiline mussels into an evolutionary context. Ancestral state reconstruction recovered evidence for the early evolution of mantle lures in this clade, with brood lures and broadcast infection strategies

both being independently derived twice. The most common infection strategy, occurring in our largest ingroup clade, is a mixed one in which mimetic mantle lures are apparently the predominant infection mechanism, but gravid females also release simple, non-mimetic brood lures at the end of the season. This mixed infection strategy clade shows some evidence of an increase in diversification rate and most members use Centerarchids (*Micropterus* & *Lepomis* spp.) as their predominant fish hosts. Broad linkage between infection strategies and predominant fish host genera is also seen in other lampsiline clades: worm-like mantle lures of *Toxolasma* spp. with sunfish (*Lepomis* spp.); insect larvae-like brood lures (*Ptychobranchus* spp.), or mantle lures (*Medionidus* spp., *Obovaria* spp.), or mantle lures combined with host capture (*Epioblasma* spp.) with a spectrum of darter (*Etheostoma* & *Percina* spp.) and sculpin (*Cottus* spp.) hosts, and tethered brood lures (*Hamiota* spp.) with bass (*Micropterus* spp.). Our phylogenetic results confirm that discrete lampsiline mussel clades exhibit considerable specialization in the primary fish host clades their larvae parasitize, and in the host infection strategies they employ to do so. They are also consistent with the hypothesis that larval resource partitioning of fish hosts is an important factor in maintaining species diversity in mussel assemblages. We conclude that, taking their larval ecology and host-infection mechanisms into account, lampsiline mussels may be legitimately viewed as an adaptive radiation.

INTRODUCTION

Adaptive radiation is a form of speciation, enabled by ecological opportunity, in which lineages evolve divergent ecologies and phenotypes to exploit distinct ecological niches (Schluter, 2000; Gavrilets & Losos, 2009). This process is widespread in nature and there are many famous examples of adaptive radiations including Darwin's finches, cichlid fishes in the East African Great Lakes, and Caribbean anoles (Grant, 1999; Schluter, 2000; Seehausen, 2006).

The classic concept of adaptive radiation involves relatively rapid speciation with highly conspicuous phenotypic and ecological differentiation (Schluter, 2000). However, in recent years, these criteria have been expanded to include radiations that have developed over longer temporal scales (Losos, 2010; Arbour & López-Fernández, 2016) as well as radiations characterized by cryptic ecological (Pillon et al., 2014) and phenotypic divergence (Gittenberger & Gittenberger, 2011).

At first glance, most members of the 298 species of unionid mussels found throughout the US and Canada (Williams et al., 2017) would not appear to meet adaptive radiation expectations with regard to ecological distinctiveness. Up to 40 species can co-occur in a single riffle (Haag & Warren, 1998), but there is little evidence for obvious microhabitat partitioning in multispecies aggregations (Strayer, 1981; Strayer & Ralley, 1993), and their nutrition is derived from a combination of ingested sediments (Nichols et al., 2005) and suspended particles (Nichols & Garling, 2000; Vaughn, Nichols & Spooner, 2008). Previous studies have found little evidence of significant resource partitioning in diet among co-occurring species (Coker et al., 1921; Bronmark & Malmqvist, 1982; Raikow & Hamilton, 2001), although a recent study by Tran & Ackerman (2019) found some evidence of differential clearance rates of some planktonic microalgal species in flowing conditions and Atkinson et. al. (2020) found variation in tissue stoichiometry among unionid mussels that correlate with phylogeny. The consensus view (Coker et al., 1921; Bronmark & Malmqvist, 1982; Rashleigh & DeAngelis, 2007; Vaughn, Nichols & Spooner, 2008; Haag, 2012) is that post-larval resource partitioning alone is an insufficient mechanism to explain the persistence of diverse mussel assemblages in intact US and Canadian rivers.

The above studies concern the habitat preferences and feeding ecology of long-lived,

macroscopic, post-larval stage of the unionid life cycle (Fig. 1). However, once details of their larval life history and reproductive ecology are taken into account, a large amount of ecological and phenotypic divergence is apparent in this group (Barnhart, Haag & Roston, 2008; Haag, 2012). Uniquely among bivalves, freshwater mussel (Unionoida) larvae are obligate, short-term parasites of fishes (Bogan, 2007; Barnhart, Haag & Roston, 2008; Haag, 2012). This early ontogeny is thought to have evolved as an upstream dispersal mechanism (Watters, 2001; Araujo, Cámara & Ramos, 2002; Barnhart, Haag & Roston, 2008). Co-occurring freshwater mussel species may differ substantially in the fishes used as hosts, the degree of host specialization, the host infection mechanisms used by gravid females, and the seasonality of host infection (Barnhart, Haag & Roston, 2008; Haag, 2012; Cummings & Watters, 2017; Hewitt, Wood & Ó Foighil, 2019). Rashleigh & DeAngelis (2007) used ecological modeling to examine partitioning of host use as a mechanism for coexistence in freshwater mussels and found that coexistence via competition for host fish was possible given 1) a high diversity of fish species in the environment; and 2) the ability to target specific fish hosts in the environment. The latter criterion rules out clades largely composed of known fish host generalists such as the subfamilies Unioninae (Barnhart, Haag & Roston, 2008). For fish host specialists, however, we predict that this hypothesized ecological process (Rashleigh & DeAngelis, 2007), if valid over longer timescales, would lead to the evolution of adaptive radiations centered on the brief larval life history stage, and characterized by the evolution of host specialization and of specialized host-infection behaviors.

The goal of our study is to test that prediction by analyzing the evolutionary history of host preference and host infection mechanisms in 54 species of lampsiline mussels using the first genomic (ddRAD-seq) phylogeny of the group. We chose this clade because of its high diversity,

the availability of extensive background information about host fish specificity (Barnhart, Haag & Roston, 2008; Cummings & Watters, 2017), and, most importantly, because they are predominantly specialist parasites (Haag & Warren, 1998). A given species will typically specialize on a few closely related fish taxa as hosts, *e.g.*, darters, or basses, or drum, or sculpins, or percids. They also have a wide diversity of well-documented host fish infection mechanisms. Some species use broadcast release, which relies on passive distribution of larvae in the water column to contact and infect a host (Fig. 2a), but most species have a proactive strategy that entails the use of lures by gravid females to elicit a host feeding response through which they become infected. There are two main lure types (Lefevre & Curtis, 1912; Barnhart, Haag & Roston, 2008): mantle tissue lures on the female's body (Fig. 2b-d) and brood lures (i.e., conglutinates and superconglutinates) containing larvae, that are released into the environment (Fig. 2e-h).

Brood lures are encapsulated aggregates of larvae that form in the female gill demibranch marsupia (Lefevre & Curtis, 1912; Barnhart, Haag & Roston, 2008) and range in complexity from simple, fragile structures that break up upon release (Fig. 2e), to durable aggregations with striking mimicry of prey items including insect larvae (Fig. 2f) and fish fry, to baited worm-like lures partitioned into non-infective and infective sections (Fig. 2g), to tethered lures that resemble prey fish (Barnhart, Haag & Roston, 2008; Haag, 2012). Many lampsiline species employ a mixed strategy that involves mantle lure displays (Fig. 2d) for most of the infection season (usually late spring/early summer) and release of simple non-mimetic brood lures (Fig. 2e) at its end (Corey, Dowling & Strayer, 2006; Barnhart, Haag & Roston, 2008).

An earlier study by Zanatta & Murphy (2006) used a mitochondrial phylogeny to investigate the evolution of host infection strategies in 49 lampsiline species. They recovered

evidence for an early evolution of mantle lures in this clade together with a number of secondary losses, in some cases involving the evolution of brood lures (conglutinates/superconglutinates), but many higher-level relationships in their mitochondrial gene trees were poorly supported. We built on their pioneering study by constructing the first genomic lampsiline phylogeny in order to place the diversity of host use, and host infection strategies, into a robust evolutionary context. We were also interested in testing for evidence of a cryptic adaptive radiation, centered on the brief, microscopic, and ecologically diverse, parasitic larval life history stage of this clade, but also incorporating maternal host infection mechanisms.

MATERIALS AND METHODS

Sample Collection

Our sampling strategy, for both ingroup and outgroup taxa, was primarily guided by the Zanatta & Murphy (2006) study, although we were not successful in obtaining, and/or genotyping, all of the species they included. Tissues samples from a total of 84 species were collected from the field (N=13) as well as obtained from various research collections (N=71) including the Illinois Natural History Survey, The University of Florida, North Carolina Museum of Natural Sciences, and from the Alabama Aquatic Biodiversity Center. Our final dataset consisted of 109 sequenced individuals representing 54 species across 22 different genera (Table 1).

Among the Zanatta & Murphy (2006) taxa that we were unable to source was the genus *Popenaias*, that positioned within the Amblemini in mitochondrial gene trees (Campbell et al., 2005; Zanatta & Murphy, 2006). However more recent studies, using data from the large nuclear ribosomal gene in addition to mt sequences (Pfeiffer et al., 2019), and from an anchored hybrid

phylogenomic approach (Pfeiffer, Breinholt & Page, 2019) recovered this genus as members of a newly recognized Mesoamerican and Rio Grande clade, Popenaiadini, sister to Lampsilini.

A non-lethal biopsy technique developed by Berg et al. (1995) was used to collect tissue samples from mussels in the field. Mussel species were categorized based on presence or absence of mantle lure and type of brood lure (simple, complex, or tethered). Mantle lures and brood lures were treated as separate variables because they are not mutually exclusive with many species having both mantle lures and brood lures. The wide spectrum of mantle lure phenotypes found across the clade (Barnhart, Haag & Roston, 2008; Haag, 2012) complicated discrete sub-categorization so this variable was scored simply into presence or absence states. Brood lures were broken down into four categories: absence of brood lure, simple/fragile brood lure, complex brood lure, and tethered brood lure. Information regarding primary hosts, and host infection strategies, for each mussel species (Table 1) was compiled from various literature sources. Reference literature used for each species listed and cited in Supplementary Table 1.

ddRADseq Data Collection and Bioinformatics

Genomic DNA was extracted from tissue samples using the E.Z.N.A. Mollusk DNA kit (Omega Bio-Tek, Norcross, GA) according to manufacturer's instructions and then stored at -80°C. The quality and quantity of DNA extractions were assessed using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA) and ddRADseq libraries were prepared following the protocols of Peterson et al. (2012). We then used 200 ng of DNA for each library prep. This involved digestion with Eco-RI-HF and MseI (New England Biolabs, Ipswich, MA) restriction enzymes, followed by isolating 294-394 bp fragments using a Pippen Prep (Sage Science, Beverly, MA) following the manufacturer's instructions. Prepared ddRADseq libraries then were submitted to the University of Michigan's DNA sequencing core and run in three different lanes

using 150 bp paired-end sequencing on an Illumina HiSeq 2500. Two control individuals of *Lampsilis fasciola* were run in each lane and reads for both individuals clustered together in every analysis with 100% bootstrap support, indicating no lane effects on clustering across individuals. Raw demultiplexed data were deposited at genbank under the bioproject ID PRJNA704566 with accession numbers SAMN18093783-SAMN18093865.

The alignment-clustering algorithm in ipyrad v.0.7.17 (Eaton, 2014; Eaton & Overcast, 2020) was used to identify homologous ddRADseq tags. Ipyrad is capable of detecting insertions and deletions among homologous loci which increases the number of loci recovered at deeper evolutionary scales compared to alternative methods of genomic clustering (Eaton, 2014). Demultiplexing was performed by sorting sequences by barcode, allowing for zero barcode mismatches (parameter 15 setting 0) and a maximum of five low-quality bases (parameter 9). Restriction sites, barcodes, and Illumina adapters were trimmed from the raw sequence reads (parameter 16 setting 2) and bases with low-quality scores (Phred-score <20, parameter 10 setting 33) were replaced with an N designation. Sequences were discarded if they contained more than 5 N's (parameter 19). Reads were clustered and aligned within each sample at two different similarity thresholds, 85 and 90% and clusters with a depth < 6 were discarded (parameters 11 and 12). We also varied the number of individuals required to share a locus from ~25% (N = 27) to ~46% (N = 50). Ipyrad output files were used for further downstream analyses and are available on Dryad at the following DOI: https://doi.org/10.5061/dryad.c866t1g62.

Phylogenomic Analyses

We analyzed the four concatenated ddRAD-seq alignment files (85% and 90% clustering similarity and 25% and 46% minimum samples per locus) using maximum likelihood in RAxML v8.2.8 (Stamatakis, 2014). A general time-reversible model (Lanave et al., 1984) was used for

these analyses that included invariable sites and assumed a gamma distribution. Support was determined for each node using 100 fast parametric bootstrap replications. Due to the relatively deep phylogenetic scale comprised by our taxon sampling, we recovered many more loci with a minimum of 25% individuals per locus and 85% clustering threshold (4725 loci) compared to runs that included 46% individuals per locus at the same clustering threshold (664 loci). The 90% clustering threshold produced even fewer loci and was not very useful for our phylogenomic analyses. Relationships were robust for most nodes with the 85% clustering threshold, and downstream analyses were performed using both of these datasets (85%-25% and 85%-46%).

The maximum likelihood phylogeny output from RAxML was trimmed to remove the outgroup taxa (*Quadrula quadrula, Amblema plicata, Fusconaia flava,* and *Eurynia dilata*) as well as all multiples of each species using the 'ape' package in R version 3.5.2 (R Core Team, 2018; Paradis & Schliep, 2019). This tree with a single individual of each species was used to create an ultrametric tree with two comparable methods using penalized maximum likelihood approaches (Sanderson, 2002; Kim & Sanderson, 2008); one implemented in R using the 'ape' package with a correlated rate model (Paradis & Schliep, 2019), and another using treePL (Smith & O'Meara, 2012).

Ancestral State Reconstruction

We analyzed the evolution of mantle lures and brood lures separately because these host infection strategies are neither homologous characters, nor mutually exclusive with many species using both mantle lures and brood lures (Corey, Dowling & Strayer, 2006; Barnhart, Haag & Roston, 2008). For each species of mussel, we independently assessed the mantle lure and brood lure characters and categorized them into binary, present or absent, character states based on the

current available data (Supplementary Table 1). Ancestral State reconstructions for both mantle lures and brood lures were performed using the rerooting method (Yang, Kumar & Nei, 1995), implemented in the 'Phytools' package in R (Revell, 2012; Paradis & Schliep, 2019), and using both a one-rate model (ER; equal transition rates among all character states) and a symmetric model (SYM; rates can vary among different traits but forward and reverse transition are constrained) where rates are allowed to differ between transitions but are constrained between forward and reverse transitions.

Lampsiline Diversification Rates

Two different approaches were used to investigate the potential influence of host infection strategies on diversification rates in the Lampsilini. The first method used State Speciation and Extinction models to explicitly test the association between host infection strategies and diversification rates, the second method used BAMM to estimate diversification rates and evidence of rate shifts in the lampsiline phylogeny

Hidden State Speciation and Extinction models were implemented using the 'hisse' package in R (Beaulieu & O'Meara, 2016). Four models were performed independently for each trait (presence of mantle lure, presence of brood lure, broadcast release); a binary state-dependent model (BiSSE), a hidden state dependent model (HiSSE), a two-state character-independent model, and a four-state character independent model. The two-state and four state character-independent models were included as null models to compare to the BiSSE and HiSSE models. Rabosky & Goldberg, (2015) found that BiSSE models tend to have a high type-1 error rate when compared to a null model that assumes homogenous diversification rates across the tree. The two-state and four-state character-independent models were proposed as an alternative null model which allows for rates to vary, independent of the trait value, and reduces type-1 error

rates (Beaulieu & O'Meara, 2016). All models allowed extinction rates to vary independently for each character state, and transition rates between states were fixed to simplify the models. The revised freshwater mussel taxonomy by Williams et al. (2017) was used to estimate sampling frequency for each trait category. This analysis was performed with both the ultrametric tree derived from the 'ape' package as well as the one derived using TreePL. To further explore state-dependent models, the R package 'Diversitree' was used to estimate and visualize diversification rates using an MCMC approach (FitzJohn, 2012).

For the second method, we used Bayesian Analysis of Macroevolutionary Mixtures (BAMM) software package (v. 2.5) and the R package "BAMMtools" to estimate diversification rates in the Lampsilini phylogeny, (Rabosky, 2014; Rabosky et al., 2014). BAMM uses a reversible-jump Markov Chain Monte Carlo to automatically detect clades that share common evolutionary parameters of diversification (Rabosky et al., 2013). BAMM was performed using 10,000,000 generations, sampling every 5000 generations. Priors for the model were selected using the setBAMMpriors function in R (Rabosky et al., 2014). To account for incomplete taxon sampling, we used previously published mitochondrial phylogenies for this group (Campbell et al., 2005; Zanatta & Murphy, 2006) and the revised list of freshwater mussels of the United States and Canada by Williams et al. (2017) to estimate clade-specific frequencies of sampling biases.

RESULTS

ddRADseq Data Collection and Bioinformatics

Illumina sequencing returned raw reads ranging from 287,978 to 14,377,252 per individual across the 83 unionid samples included in the analyses. Mean coverage depth, for the

85% clustering threshold, ranged from 1.48 (*Toxolasma lividum*) to 5.25 (*Lampsilis virescens*) (Table1, Supplementary Table 2).

We identified between 4,745 and 664 homologous loci across the two best ddrad datasets (85%-25% and 85%-46%) and, in general, much higher numbers of loci were recovered for the core Lampsilini ingroup (> 1,000 loci) relative to the outgroups (<100 loci). Although lowering clustering thresholds produced a much greater amount of missing data in the ddrad supermatrix, they also greatly increased the number of loci which could be used, *e.g*., for the 85% clustering threshold, 664 loci were recovered when a minimum of 46% individuals were included, whereas a 25% minimum yielded 4,745 loci. Simulation studies and empirical analyses both suggest that large amounts of missing data may be relatively unproblematic for phylogenetic reconstructions, especially if the total dataset is large (Rubin, Ree & Moreau, 2012; Huang & Knowles, 2016; Eaton et al., 2016). Datasets recovered from both the 25% and 46% minimum samples per locus clustering thresholds were used in all our phylogenomic analyses.

Phylogenomic Analyses

The ddRADseq gene tree topologies we recovered were highly consistent across all of the parameter settings analyzed, with a few differences in placement of poorly supported nodes (Fig. 3 and Supplementary Figure 1). All our phylogenetic trees recovered the monophyletic genus *Toxolasma* as sister to the other members of the Lampsilini tribe included in the study. The latter formed four well-supported crown clades, each composed of members of >1 genus: a 2-species clade with *Glebula* and *Cytronaias* spp., a 10-species clade with *Medionidus*, *Lemiox*, and *Pytchobranchus* spp., a 5-species clade containing *Leptodea*, *Potamilus* and *Truncilla* spp., and a 33-species clade containing *Ligumia*, *Epioblasma*, *Obovaria*, *Venustaconcha*, *Hamiota*, *Villosa, Sagittunio, Cambarunio, Leaunio* and *Lampsilis* spp. Across our topologies, some genera were

recovered as monophyletic [*Toxoplasma* (4 species), *Obovaria* (2 species), *Venustaconcha* (2 species) *Hamiota* (4 species), *Lampsilis* (14 species), *Pytchobranchus* (3 species), *Sagittunio* (2 species; see Watters, 2018), *Leaunio* (2 species; see (Watters, 2018)], but some others did not [*Medionidus* (6 species), *Leptodea* (2 species)]. The new reclassification of *Villosa* suggested by Watters (2018) is supported in our analyses for the species we have included.

An ultrametic tree (Fig. 4) was created with TreePL from the 85% clustering similarity with 25% minimum samples per locus topology (Fig. 3) and manually pruned to one individual per species according to read count. The mussel species are color-coded according to their host infection strategy and their primary (most frequently used) host taxa are indicated. A striking feature of this topology is the high degree of conservation shown by ingroup mussels in their primary fish host taxa, e.g., the mantle-lure producing *Toxolasma* spp. clade with sunfishes (*Lepomis* spp.), the mixed strategy dominated 33-species clade primarily with bass (*Micropterus* spp.), the mantle lure or brood lure 10-species *Medionidus*/*Lemiox*/ *Ptychobranchus* spp. clade with darters (*Etheostoma* spp.) and sculpins (*Cottus* spp.), and the 5-species *Leptodea*/*Potamilus*/ *Truncilla* spp. clade–some broadcasting larvae, some with mantle lures–with freshwater drum (*Aplodinotus grunniens*).

Ancestral State Reconstruction

Ancestral state reconstructions were performed for both mantle lures and for brood lures using two different models for transition rates (ER and SYM). The likelihood values for the mantle lure models are ER = -24.65 and SYM = -24.65. For brood lure reconstructions the likelihood values are ER = -26.14 and SYM = -23.81. Using the SYM model, estimated probabilities of character states at each node were plotted on the ultrametric tree (Fig. 5; Supplementary Figure 2). These results imply that mantle lures evolved early in the Lampsilini

phylogeny, being present in the ingroup's last common ancestor, with four to six subsequent losses. Brood lures are inferred to have independently evolved twice in this phylogeny.

Gain of a complex brood lure was coupled with loss of a mantle lure in *Pytchobranchus* (Fig. 5), although this transition was not associated with a change in primary host fishes (darters/sculpins; Fig. 4). Gain of a simple brood lure in ancestor of the 33-species, 10-genus, predominantly bass host specialist clade did not result in the loss of a mantle lure. However, within that clade, the subsequent evolution of a complex, tethered brood lure in *Hamiota* was associated with the loss of a mantle lure in 3/4 species (Fig. 5), but no change in primary host fishes (Fig. 4). Eleven of the 33 species in this clade have primary fish hosts other than bass [darters/sculpins (7), sunfishes (3), and walleye (1)] and, while all of them have retained mantle lures, 3 of the 7 species targeting darters/sculpins–*Obovaria subrotunda*, *Obovaria choctawensis* and *Epioblasma triquetra*–have lost simple brood lures, with the latter species physically capturing host fish to enable larval infection (Fig. 2c). The remaining cases of mantle lure loss are associated with the gain of broadcast larval release in two clades: one containing the gar specialist *Cyrtonaias tampicoensis* and the sunfish specialist *Glebula rotundata*, the other involving three members of the 5-species *Leptodea*/*Potamilus*/ *Truncilla* spp. clade: the drum (*Aplodinotus grunniens*) specialists *Truncilla macrodon* and *Potamilus ohiensis* and the white perch (*Morone americana*) specialist *Leptodea ochracea* (Figs 4, 5).

Lampsiline Diversification Rates

Three traits were assessed independently (mantle lure, brood lure, and broadcast release) using four different models (BiSSE, HiSSE, 2 state character independent, and 4 state character independent; Table 2). The best-performing model (AICc) for the mantle lure trait was the two-state independent model, suggesting no relationship between mantle lures and net diversification

rates. The BiSSE model was the best-performing model (AICc) for the brood lure trait by a small margin, suggesting an increase in net diversification rate for species with brood lures (estimated net diversification rate of 11.7 for species with brood lure versus 8.5 for those without) and largely similar estimates for extinction fraction, which is the ratio of extinction rate/speciation rate (0.38 versus 0.41 respectively). This result was consistent across both the 25% minimum samples per locus topology (Table 2) and the 46% topology (Supplementary Table 3), regardless of how the ultrametric tree was derived. To explore these models further, we used an MCMC modeling approach, implemented in the R package 'diversitree' (FitzJohn, 2012) to estimate diversification rates for species with and without brood lures. The distributions for the parameter estimates have some overlap (Fig. 6) but display two distinct peaks and the species with brood lures have a higher estimated diversification rate. When analyzing the 85%-46% tree (supplementary Figure 1), we found the BiSSE model was also the best-performing model (AICc) for broadcast release by a small margin (Supplementary Table 3), hinting at a possible reduced diversification rate for broadcast releasers, but this was result was not corroborated in the 85%-25% tree (Table 2).

We tested for differences in speciation rates among the 54 species of lampsilines by performing BAMM analyses for 10,000,000 generations on the ultrametric tree (Fig. 4). The mean, model averaged diversification rates estimated along each branch are displayed in Fig. 7a and all four credible rate-shift sets recovered are displayed in Fig. 7b. The best rate-shift configuration (f = 0.44) suggests a static diversification rate across the entire ingroup topology, with no clade-specific differences in diversification rate (Fig. 7a). However, the second, third and fourth most sampled rate-shift configurations (f = 0.22, 0.21 and 0.13), comprising 56% of configurations sampled, indicate an increase in diversification rate on adjacent stem branches of

the 33-species clade containing *Ligumia*, *Epioblasma*, *Obovaria*, *Venustaconcha*, *Hamiota*, *Villosa, Sagittunio, Cambarunio, Leaunio,* and *Lampsilis* spp. (Fig. 7bii-iv).

Discussion

Evolution of Infection Strategies in Lampsiline Mussels

Our genomic phylogeny of Lampsilini represents a robust and comprehensive inferred evolutionary history of this North American unionid tribe. In contrast with earlier mitochondrial phylogenies (Campbell et al., 2005; Zanatta & Murphy, 2006), nodal support throughout the topology (Fig. 3) was generally high: a large majority of nodes displayed support values of 100 and only 15% had values <90. Most of the latter were concentrated within the *Lampsilis* clade, with the exception of the placement of the *Villosa* and *Hamiota* clade, and may stem from either incomplete lineage sorting or hybridization processes (Maddison & Knowles, 2006), but this question requires further investigation. Nevertheless, it is important to emphasize that our genomic phylogeny agrees broadly with those of previous molecular studies both in regard to outgroup/ingroup (Campbell et al., 2005) and among-ingroup (Campbell et al., 2005; Zanatta & Murphy, 2006; Pfeiffer, Breinholt & Page, 2019) relationships.

Our phylogenomic analyses (Fig. 4) indicate that fish host use in the Lampsilini through time is characterized by a high degree of mussel clade specificity for both primary host type and host infection mechanism(s). This result corroborates Haag's (2012) suggestion that host use is highly conserved in this group as well as Hewitt et al's (2019) finding of topological congruence between North American unionids and their hosts. It also implies that lure-based host infection mechanisms are adaptive in origin, being specialized for attracting suitable hosts, as has been observed in the wild for a subset of co-occurring mussels (Haag & Warren, 2003). There are numerous examples of such across our tree topology (Fig. 4), *e.g.*, most *Lampsilis* species target

bass [*Micropterus* spp. - predators that are highly piscivorous when large (Hickley et al., 1994)] as primary hosts using large, conspicuous mantle lures that typically resemble small fishes (Barnhart, Haag & Roston, 2008). Likewise, *Toxolasma* species have a worm-like mantle lure (Fig. 2ii) and predominantly target sunfishes in the genus *Lepomis* that are generalist predators with a diet that includes worms (Parsons & Robinson, 2007). Finally, the clade composed of *Medionidus* spp. (with small, cryptic mantle lures) and *Ptychobranchus* spp. (with small demersal brood lures that typically mimic insect or fish larvae) specialize in darters and sculpins [small, benthic predatory fishes (Haag, 2012; Cummings & Watters, 2017)].

Our ancestral state reconstruction results corroborated Graf & Ó Foighil's (2000) and Zanatta and Murphy's (2006) mt phylogeny-based inferences that lampsiline mantle lures evolved early in this clade, followed by multiple secondary losses. These inferred losses occurred across much of the ingroup topology, apart for the genus *Toxolasma* (characterized by its worm-like mantle lures), and mantle lure loss was associated with the *de novo* gain of either complex brood lures or of broadcast infection strategies (Fig. 5; Supplementary Figure 2). The former occurred independently in two genera [*Ptychobranchus*, and in 3/4 of the *Hamiota* species represented] and involved a change in mimetic lure type: from mimetic mantle lures to mimetic brood lures, although *Hamiota altilis* retains both. The latter cases of mantle lure loss, inferred separately for *Cyrtonaias tampicoensis* and *Glebula rotundata*, and for *Leptodea ochracea, Potamilus ohiensis* and *Truncilla macrodon*, were more radical in that they involved the abandonment of prey mimicry and host deception as a host infection strategy. Haag and Warren (1998) found that population densities of specialist mussels and their fish hosts were correlated for broadcasters, but not so for lure-producing mussels. The evolutionary loss of lures in host specialist mussels would therefore appear counterintuitive, especially for mussels with

low-density fish hosts, but there are potentially mitigating life history traits in some of these taxa that may act to increase their rate of host infection.

One such life history trait is increased larval production: relative to other lampsilines, *Glebula rotundata* females release more larval broods per year (Parker, Hackney & Vidrine, 1984) and the genera *Truncilla* and *Leptodea* have higher fecundities and smaller-sized larvae (Haag, 2013). Another such trait may involve targeting mussel predators as larval hosts, e.g., adult *Aplodinotus grunniens* (freshwater drum) prey on mussels and at least some of the species that use it as a host may engage in a sacrificial strategy whereby infection occurs when gravid females (especially smaller specimens) are consumed (Barnhart, Haag & Roston, 2008; Haag, 2012). Four of five members of the *Leptodea*/*Potamilus*/ *Truncilla* spp. clade (Fig. 4) are *A. arunniens* specialists [the fifth, *L. ochracea*, occurs outside of this fish's range (Page & Burr, 2011)] and, until recently, it was assumed that these three mussel genera lacked mantle lures. However, Sietman et al. (2018) documented the presence of cryptic, nocturnally displayed, mantle lures for one member of each of these genera (including *Truncilla truncata* and *Leptodea fragilis*). In light of these new data, we view the current categorization of *Leptodea ochracea, Potamilus ohiensis* and *Truncilla macrodon* as lacking mantle lures (Figs. 4 & 5) as provisional. For the taxa included here, our topology confirms the topology by Smith et. al. (2020) and our data support their decision to reclassify *Leptodea ochracea* to *Atlanticoncha ochracea*.

Our ancestral state reconstruction of brood lures (Fig. 5b) is consistent with two origins (one each in the genera *Ptychobranchus* and *Hamiota*) of complex, mimetic brood lures, and one additional origin of simple, non-mimetic brood lures in the ancestor of the 33-species, 10-genus, predominantly bass host specialist clade (Fig. 5b). The latter clade contains *Hamiota*, implying that the complex tethered brood lure found in *Hamiota* species (Fig. 2h) may be derived from the

simple brood lures found in most of this clade, including species of its sister genus *Villosa* (Fig. 5b). In contrast, the darter/sculpin specialist clade containing *Ptychobranchus* (Fig. 4) lacks simple, non-mimetic brood lures (Fig. 5b). The evolutionary origins of the *Ptychobranchus* demersal mimetic brood lure (Fig. 2f) may stem from a common ancestor with the genus *Cyprogenia*. Previous mt phylogenies (Campbell et al., 2005; Zanatta & Murphy, 2006) have placed the genus *Cyprogenia*, with its demersal, mimetic baited brood lures (Barnhart, Haag & Roston, 2008), sister to the genus *Ptychobranchus*. Unfortunately, we failed to extract sufficient genomic data for our *Cyprogenia stegaria* sample to corroborate this relationship.

The 10-genus, predominantly bass host specialist clade comprised 33 species (Fig. 4) of which 26 (in the genera *Lampsilis, Villosa, Ligumia, Leaunio, Cambarunio, Sagittunio,* and *Venustaconcha*) produce mantle lures as well as simple brood lures (Fig 5A, 5B). Mantle lures are regarded as their primary method of infecting fish hosts (Haag & Warren, 2000; Barnhart, Haag & Roston, 2008; Gascho Landis et al., 2012) and a gravid female may display hers for weeks to months (Kraemer, 1970; Haag & Warren, 2003). During an elicited host fish attack on mantle lure-displaying *Lampsilis* spp. gravid females, glochidia are extracted (Barnhart, Haag & Roston, 2008) from only a subset of their ~60 marsupium water tubes and displaying females often exhibit a mix of undischarged (i.e., containing larvae) and discharged water tubes for much of the spring/summer host infection season (Haag & Warren, 1999). Lampsiline mussels have evolved bradytictic life cycles in which spawning typically occurs in the late summer and the resulting larvae are brooded overwinter (Graf & Ó Foighil, 2000). Gravid females must therefore release the previous year's brood to facilitate fertilization and retention of their new clutch of eggs and it was initially unclear if the release of simple brood lures in these species represented a default end-season emptying of marsupial water tubes (Barnhart, Haag & Roston, 2008), a stress

response to captivity (Corey, Dowling & Strayer, 2006), or a supplementary host infection strategy (Corey, Dowling & Strayer, 2006; Barnhart, Haag & Roston, 2008; Haag, 2012). Gascho Landis et al. (2012) performed a detailed experimental study of mantle lure display and simple brood lure production in *Ligumia subrostrata* and concluded that the latter clearly represents a secondary bet-hedging infection strategy. Nevertheless, the relative attractiveness of simple brood lures as putative food items to host fishes remains to be established as does their durability in nature: they typically break up quickly after release (Barnhart, Haag & Roston, 2008).

Based on available data, we propose a hypothesized three-step bet-hedging host infection strategy in these mussels (Fig. 8). This would involve A) host attraction and infection via prolonged maternal mantle lure display; B) the secondary release of residual brooded larvae within simple brood lures prior to the onset of seasonal spawning; and C) tertiary broadcast dispersal (in lotic habitats) of individual infective larvae following simple brood lure breakup, although the probability of broadcast larvae encountering a host is likely low (Jansen, Bauer & Zahner-Meike, 2001) unless the latter is locally abundant.

The genus *Epioblasma* is a notable exception to the modal host infection mechanism found in this 10-genus crown clade in that gravid females produce mantle lures only and specialize in darter hosts that they actively trap during the infection process using female-specific shell margin extensions (Barnhart, Haag & Roston, 2008). This genus is highly underrepresented in our study with only one member, *E. triquetra*, included; a shortcoming primarily due to the exceptionally intense extinction pressure the genus has been subjected to over the past century. Of the 28 currently recognized species of *Epioblasma* (Williams et al.,

2017), 13 are listed as extinct on the IUCN Red List and most of the remainder are critically endangered.

Diversification Rates

The BAMM and state-dependent speciation model analyses yielded new insights into lampsiline diversification rates albeit with some methodological and sampling (*e.g.*, the genus *Epioblasma*) caveats. The most supported BAMM result–a single diversification rate regime across the entire Lampsilini clade (Fig. 7bi)– needs to be treated with caution as this methodology is biased towards zero rate shifts in smaller trees that contain fewer than approximately 150 species (Rabosky, Mitchell & Chang, 2017; Kodandaramaiah & Murali, 2018). In contrast, the three next-most supported results (Fig. 7bii-iv) identified inferred rate shift accelerations that were tightly clustered on adjacent stem nodes of the 10-genus/33-species crown clade. This collective topological placement bracketed the inferred origin of the mixed infection strategy predominant in this crown clade that combines the use of mantle lures, a plesiomorphic trait (Fig. 5A), with simple brood lures, a derived trait (Fig. 5B). That topological congruence is broadly consistent with the BiSSE (Table 2; Supplementary Table 3) and MCMC (Fig. 6) modeling results that found evidence for increasing diversification rates among lampsiline species with brood lures. However, it must be emphasized that the majority of these species produce simple brood lures and are likely to rely on mantle lures as their primary host infection strategy (Gascho Landis et al., 2012). Barnhart, Haag & Roston (2008) suggested that species that use both mantle lures and brood lures (conglutinates) could potentially parasitize both large- and small-bodied hosts (the latter being less likely to attack mantle lures). Similarly, a hypothesized three-step bet-hedging strategy (Fig. 8) could potentially generate higher diversification rates by expanding the repertoire of potential host fishes and thereby decreasing

the risk of extinction. However, testing such a hypothesis requires significantly better data on host infection processes in natural populations as well as a more comprehensive phylogeny of unionids. The latter is also required to adequately address another outstanding question: the relative diversification rates of broadcasters and lure-using mussel taxa. It is notable that in a broadly parallel case, the evolution of deceit pollination in orchids apparently did not increase their rate of net diversification (Givnish et al., 2015).

Adaptive Radiation of Lampsilini

Models of adaptive radiation predict that the availability of ecological niches within an environment, and the response of adapting lineages to occupy them, drive and modulate this important evolutionary process. (Schluter, 1996; Gavrilets & Losos, 2009; Losos, 2010; Arbour & López-Fernández, 2016). Our primary phylogenomic result - that lampsiline clades are highly specific in primary fish host type and in host infection mechanism - is consistent with adaptive radiation expectations in regard to their larval ecology, despite the relatively brief duration of this life history stage. Two factors may bear on this ostensibly surprising result. Once lampsiline mussels evolved a high degree of fish host specialization (Haag & Warren, 1998), the number of discrete larval ecological niches potentially available to them, in the form of local host fish species diversity, greatly increased. In addition, successful larval infection and metamorphosis (transformation) on a fish host is a necessary precondition for juvenile mussel recruitment, and therefore for ecological persistence, in wild populations.

Although our data support an adaptive radiation framework operating at the level of lampsiline clades, they lack the fine-grained resolution of specific host data needed to establish if it equally applies to within-clade diversification. For instance, it remains to be established to what degree sympatric, closely related lampsiline species preferentially target different species of

host within the same host guild, consistent with a seamless adaptive radiation paradigm, or rather compete for the same host species, consistent with an evolutionary arms race paradigm (Van Valen, 1977). We anticipate that the balance of these two potential within-clade evolutionary processes may differ among lampsiline lineages according to the range of potential hosts available to them. For example, there are ~200 species of North American darters, many with small ranges (Near et al., 2011), and there may be considerable evolutionary scope for a high degree of host exclusivity and within-clade adaptive radiation among the darter-specialist lampsiline genera such as *Medionidus, Ptychobranchus* and *Epioblasma*. In contrast, there are fewer (~41; (Roe, Harris & Mayden, 2002; Baker, Blanton & Johnston, 2013; Freeman et al., 2015) species of centrarchids in North America than of lampsiline centrarchid specialists (~50; Williams et al., 2017). Although new centrarchids species continue to be described (Baker, Blanton & Johnston, 2013; Freeman et al., 2015), the lower number of potential centrarchid hosts implies that some of these mussel species are more likely to compete directly, when in sympatry, for the same hosts and thereby become entrained in an evolutionary arms race for lure effectiveness. In such cases, coexistence could be modulated by frequency-dependent selection processes (Endler, 1988), in which previously infected host fishes are more likely to engage with unfamiliar/rare lure phenotypes, a process that has also been implicated in the evolution of lure polymorphisms in some lampsiline species (Zanatta, Fraley & Murphy, 2007; Barnhart, Haag & Roston, 2008).

Conclusions

Unionoida is by far the most speciose freshwater bivalve order (Graf & Cummings, 2007) and this richness was especially heightened in southeastern U.S. watersheds, prior to their destructive 20th century industrialization (Lydeard et al., 2004). A record 69 species–the Muscle

Shoals fauna–was recorded in the middle reaches of the Tennessee River (Garner & McGregor, 2001), each of them dependent on successful larval parasitism of fish hosts for their recruitment and survival. There is an emerging consensus among mussel researchers that larval partitioning of ambient fish host resources is common in diverse North American unionoid communities (Barnhart, Haag & Roston, 2008; Haag, 2012; Cummings & Watters, 2017; Hewitt, Wood & Ó Foighil, 2019) and that the presence of discrete larval niches may explain the persistence of species-rich mussel assemblages over ecological timescales (Rashleigh & DeAngelis, 2007). We propose that these larval niches are evolutionary end-products of cryptic adaptive radiation processes, operating in these watersheds over long time scales (Losos, 2010; Arbour & López-Fernández, 2016), but we acknowledge that much more detailed field work is required to build a comprehensive understanding of their extent and scope.

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Table 2.1: Freshwater mussel species included in the phylogenomic analysis, including their host infection strategy, preferred host, total number of illumina reads, total number of clusters, number of consensus reads and total number of loci included in the assembly at an 85% clustering threshold and 25% samples per loci. UF = University of Florida, INHS = Illinois Natural History Survey, and NCS = North Carolina State University.

Species Name	Infection Strategy	General Host	Tissue Source	Museum ID	Raw Reads	Total Clusters	Consensus Reads	Loci in Assembly
Amblema plicata	Broadcast	Generalist	Collected by T. Hewitt	306255	2900279	998124	57759	301
Cambarunio taeniatus	Mantle lure and Simple brood lure	Bass	NCS	29180	1472633	414265	32713	1004
Cyrtonaias tampicoensis	Broadcast	Gar	UF	438173	858098	339345	18540	208
Epioblasma triquetra	Mantle lure and Host Trapping	Darter/Sculpin	INHS	36609	5459944	1469677	64027	1678
Eurynia dilatata	Broadcast	Generalist	Collected by T. Hewitt	306256	790501	323262	21107	96
Glebula rotundata	Broadcast	Sunfish	UF	440636	1070046	557092	25673	303
Hamiota altilis	Mantle lure; tethered, complex brood lure	Bass/Sunfish	From Paul Johnson	306257	5387472	1266412	64930	1827
Hamiota australus	Tethered, complex brood lure	Bass	UF	441239	3109960	1048442	49094	1494
Hamiota perovalis	Tethered, complex brood lure	Bass	From Paul Johnson	306258	5270101	1222099	62362	1826
Hamiota subangulata	Tethered, complex brood lure	Bass	UF	438064	668819	207361	20455	722
Lampsilis bracteata	Mantle lure and Simple brood lure	Bass	UF	439084	2568126	602005	45170	1594
Lampsilis cardium	Mantle lure and Simple brood lure	Bass	Collected by J. Bergner	306259	7216326	2506439	67346	2545
Lampsilis fasciola	Mantle lure and Simple brood lure	Bass	Collected by T. Hewitt	306260	3435913	870542	55060	3816
Lampsilis floridensis	Mantle lure and Simple brood lure	Bass	UF	340525	3303826	1045716	53781	1595
Lampsilis higginsi	Mantle lure and Simple brood lure	Bass	INHS	49425	1009895	330086	13435	512
Lampsilis hydiana	Mantle lure and Simple brood lure	Bass	UF	440994	2000552	504555	44904	1743
Lampsilis ornata	Mantle lure and Simple brood lure	Bass	UF	438031	4893511	1455910	64521	2177
Lampsilis ovata	Mantle lure and Simple brood lure	Bass	UF	438255	1807208	453929	40479	1935
Lampsilis radiata	Mantle lure and Simple brood lure	Bass and perch	UF	439013	800488	170092	26694	1262
Lampsilis satruna	Mantle lure and Simple brood lure	Bass	UF	441167	4904722	916074	63718	2328
Lampsilis siliquoidea	Mantle lure and Simple brood lure	Bass	INHS	25963	2111249	685663	42797	1786
Lampsilis splendida	Mantle lure and Simple brood lure	Bass	UF	438354	1149372	286475	28129	1237
Lampsilis straminea	Mantle lure and Simple brood lure	Bass	UF	383152	4914716	1562952	66297	2123
Lampsilis virescens	Mantle lure and Simple brood lure	Bass	Paul Johnson	306261	4169896	708955	57290	2043
Leaunio umbrans	Mantle lure and Simple brood lure	Sunfish/Sculpin	UF	438189	5607023	1832948	69194	1738
Leaunio vanuxemensis	Mantle lure and Simple brood lure	Sculpin	UF	438796	1120139	366899	18117	504
Lemiox rimosus	Mantle lure	Darter/Sculpin	NCS	47243	1911799	434117	38814	460
Leptodea fragilis	Mantle lure	Drum	INHS	79830	3519359	1143382	54580	484
Leptodea ochracea	Broadcast	white perch	UF	438459	287978	107862	6669	82
Ligumia recta	Mantle lure and Simple brood lure	Walleye	UF	438249	1659317	370364	37676	1382
Medionidus acutissimus	Mantle lure	Darter/Sculpin	Paul Johnson	306262	1851620	349715	41256	475
Medionidus conradicus	Mantle lure	Darter/Sculpin	UF	438914	7718202	1466030	66764	619
Medionidus parvulus	Mantle lure	Darter/Sculpin	Paul Johnson	306263	6651085	2082691	62803	604
Medionidus penicillatus	Mantle lure	Darter/Sculpin	Paul Johnson	306264	7915534	2253442	80037	660
Medionidus simpsonianus	Mantle lure	Darter/Sculpin	Paul Johnson	306265	4362329	1066797	57543	583
Medionidus walkeri	Mantle lure	Darter/Sculpin	Paul Johnson	306266	3139933	559539	49255	559
Obovaria choctawensis	Mantle lure	Darter/Sculpin	UF	441237	1470462	373459	32610	1052
Obovaria subrotunda	Mantle lure	Darter/Sculpin	UF	438391	1672141	601899	32020	1157
Potamilus ohiensis	Mantle lure and Simple brood lure	Drum	UF	438806	2251207	785191	34220	294
Ptychobranchus fasciolarus	Complex brood lure	Darter/Sculpin	UF	438254	2517640	878247	37577	454
Ptychobranchus foremanianus	Complex brood lure	Darter/Sculpin	Paul Johnson	306267	14377252	3961795	78567	659
Ptychobranchus jonesi	Complex brood lure	Darter/Sculpin	UF	441272	1455454	491977	29992	355
Quadrula quadrula	Mantle lure	Catfish	UF	438787	4999562	1569250	58525	148
Sagittunio nasutus	Mantle lure and Simple brood lure	Sunfish and Perch	UF	438285	4608659	1458774	55120	1513
Sagittunio subrostratus	Mantle lure and Simple brood lure	Sunfish	UF	441304	1814864	583195	30748	998

Toxolasma corvunculus	Mantle lure	Sunfish	UF	440843	2924381	1001628	49472	275
Toxolasma cylindrellus	Mantle lure	Sunfish	INHS	49319	11371070	3006669	82040	361
Toxolasma lividum	Mantle lure	Sunfish	UF	438185	779097	307476	16824	113
Toxolasma texasiensis	Mantle lure	Sunfish	UF	438567	1298761	409308	26318	139
Truncilla macrodon	Broadcast	Drum	UF	441301	685468	174606	18594	109
Truncilla truncata	Mantle lure	Drum	UF	438976	950716	250143	25987	303
Venustaconcha ellipsiformis	Mantle lure and Simple brood lure	Darter/Sculpin	INHS	87179	4434860	1022209	62605	1702
Venustaconcha trabalis	Mantle lure and Simple brood lure	Darter/Sculpin	UF	438909	1660491	264191	36956	1469
Villosa amygdala	Mantle lure and Simple brood lure	unknown	UF	441054	2021257	400560	39674	1133
Villosa delumbis	Mantle lure and Simple brood lure	Bass	UF	437984	4433617	1358358	61582	1544
Villosa vibex	Mantle lure and Simple brood lure	Sunfish	UF	438545	1272879	370119	28877	941
Villosa villosa	Mantle lure and Simple brood lure	Bass/Sunfish	UF	441268	2756754	671290	48066	1340

Table 2.2: Displays the AIC, AICc, and log likelihood values for a set of state dependent speciation models performed independently for three different traits: Mantle lure, Brood lure, and broadcast strategy. The four models performed for each trait include a BiSSE model (2 state trait dependent), a HiSSE model (4 state model with two trait states and two hidden states), a 2-state trait independent null model, and a 4 state trait independent null model.

	Mantle Lure			Brood Lure			Broadcast Strategy		
Model Name	AIC	AICc	Log Likelihood	AIC	AICc	Log Likelihood	AIC	AICc	Log Likelihood
2-state CID	**24.88**	**26.13**	**-7.4420**	16.01	17.25	-3.0014	**5.43**	**6.68**	**2.2836**
BiSSE	30.35	31.60	-10.1743	**8.59**	**9.84**	**0.7047**	8.42	9.67	0.7899
4-state CID	30.15	34.24	-6.0748	17.03	21.12	0.4830	11.16	15.26	3.4179
HiSSE	33.74	37.83	-7.8684	16.45	20.54	0.7739	13.85	17.94	2.0739

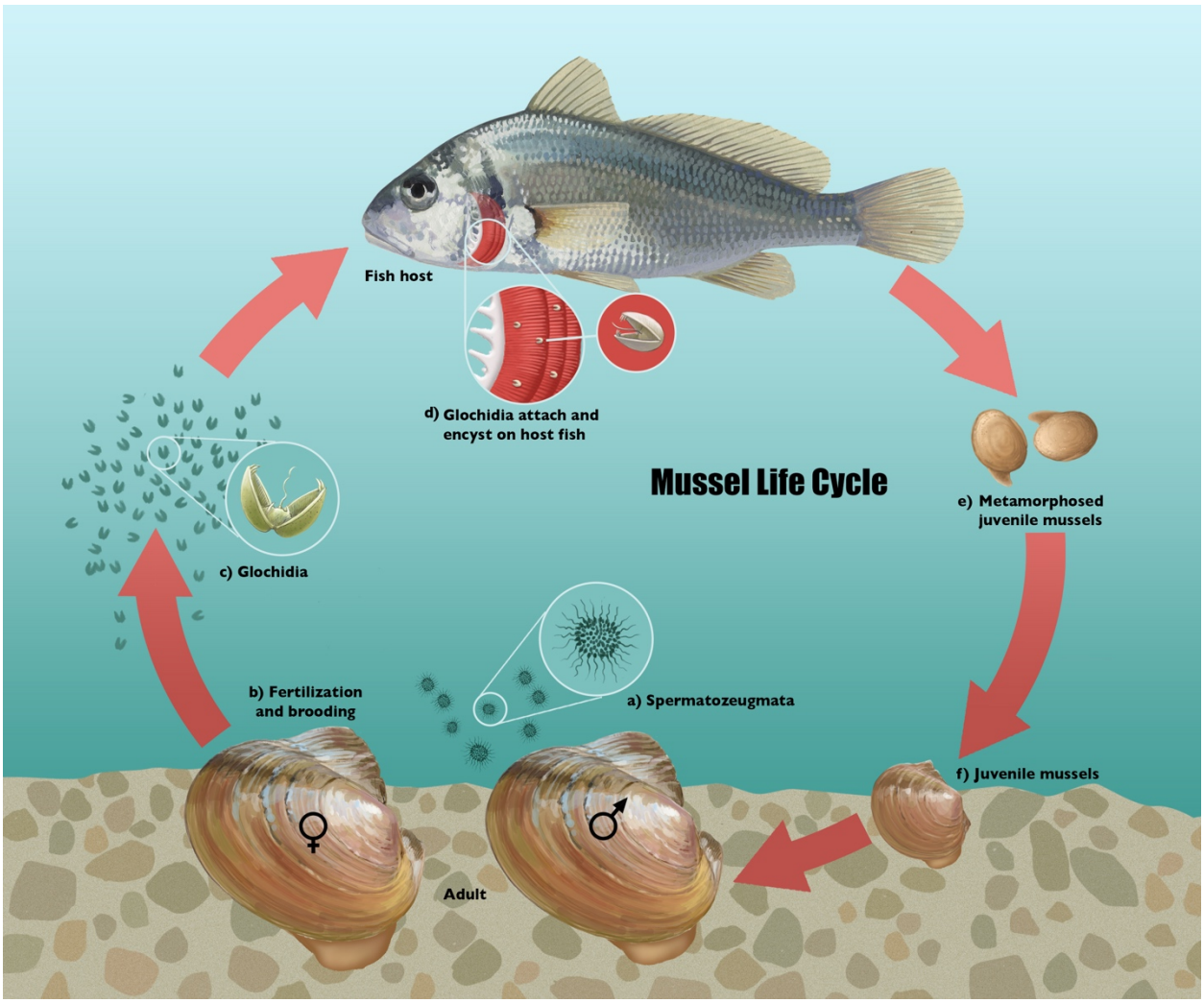

Fig. 2.1: Illustration depicting the general life cycle of unionid mussels using *Potamilus ohiensis* as exemplar. a) male mussels release spermatozoa into the water column, b) spermatozeugmata enter female mantel cavity via incurrent siphon to fertilize brooded eggs, c) parasitic larvae (glochidia) are released into the water column, d) glochidia attach and encyst on host fish *Aplodinotus grunniens*, e) metamorphosed juvenile mussels detach from the host, f) juvenile mussels assume the prolonged benthic phase of the life cycle.

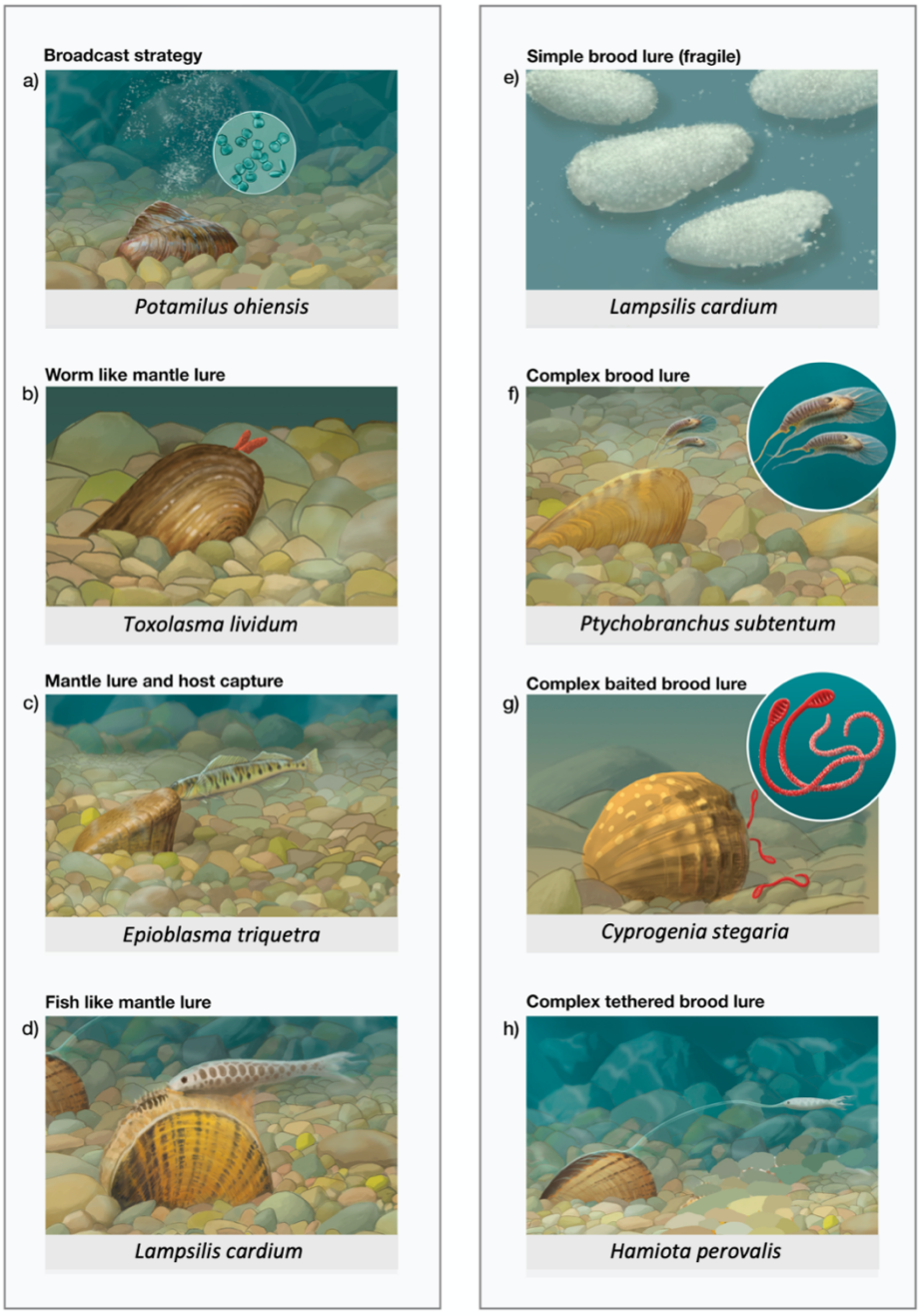

Fig. 2.2: Illustrations representing most of the primary host infection strategies found in the Lampisilini tribe of North American unionid mussels: a) broadcast larval release, found in members of the genera *Cyrtonaias, Glebula*, *Leptodea*, *Potamilus* and *Truncilla*, b) mantle lures in the genus *Toxolasma* – vermiform prey mimic, c) mantle lure (too small to see here) with associated host capture in the genus *Epioblasma*, d) mantle lure in the genus *Lampsilis* - piscine prey mimic, e) simple brood lures, composed of individual marsupia that rapidly break up, released by the genera *Lampsilis, Ligumia, Venustaconcha, Villosa*, *Sagittunio, Cambarunio,* and *Leaunio* f) complex brood lures in the genus *Ptychobranchus* – larval insect mimic, g) baited brood lures (white dots are individual larvae) released by the genus *Cyprogenia* h) tethered complex brood lure in the genus *Hamiota* - piscine prey mimic.

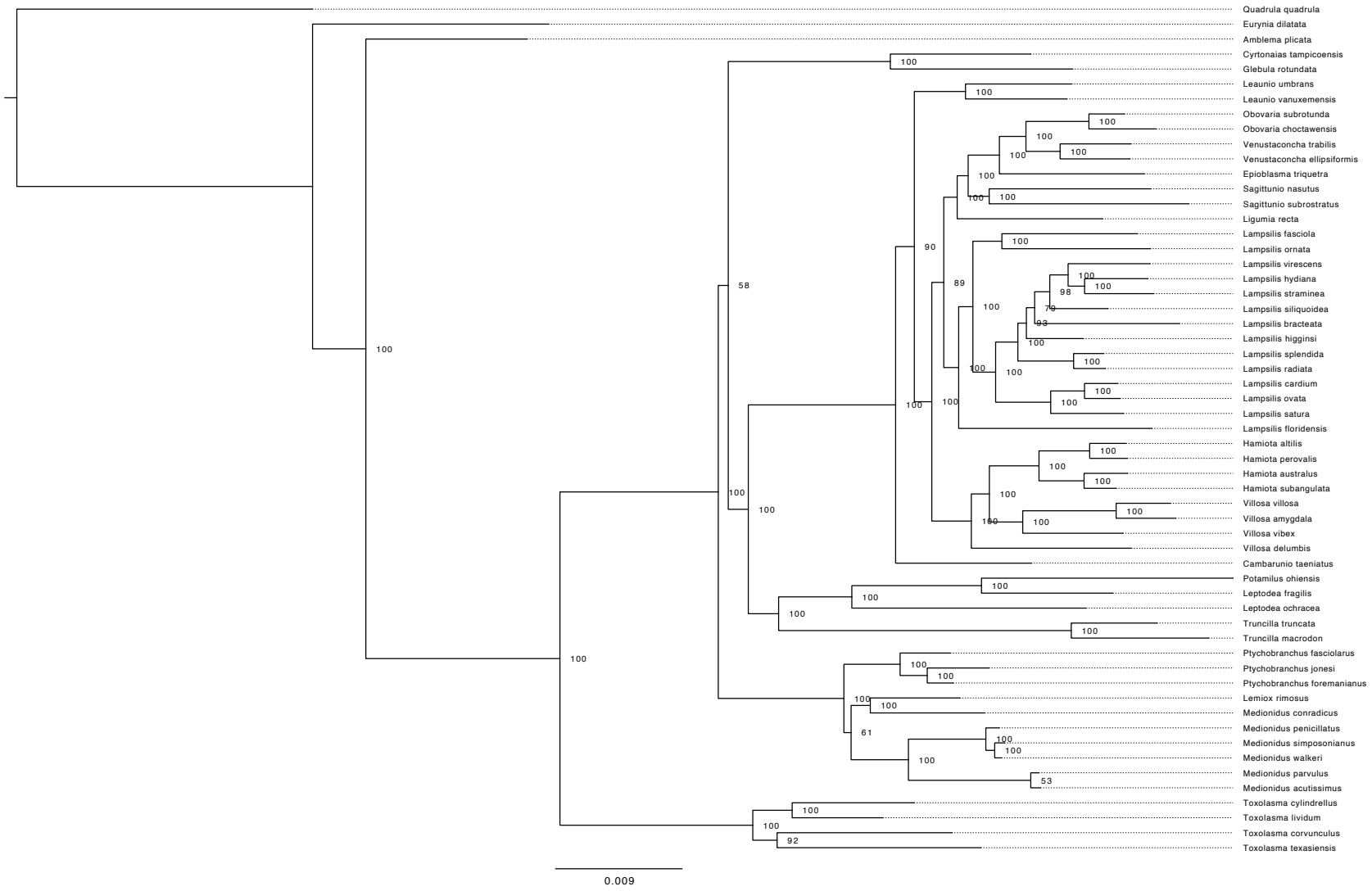

Fig. 2.3. Maximum likelihood phylogeny of North American lampsiline mussels created with RAxML v8.2.8 using a general time reversible model. Support for each node was determined using 100 fast parametric bootstrap replications. Bootstrap values are adjacent to each node. Scale bar represents mean number of base pair substitutions per site.

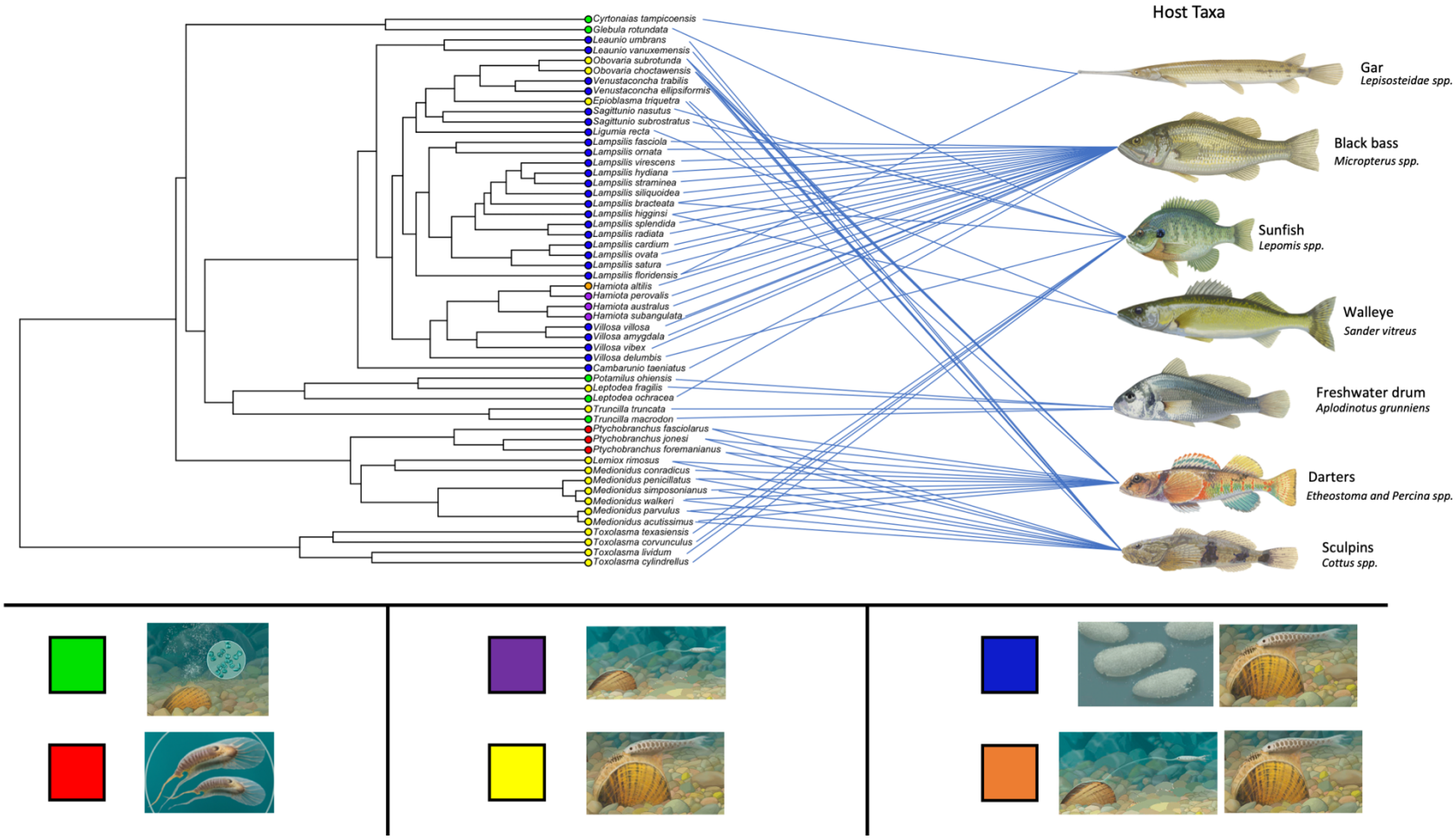

Fig. 2.4. Ultrametric phylogeny created from maximum likelihood phylogeny of lampsiline mussels (Fig. 4) using TreePL. This tree was trimmed to remove outgroups and retain only a single individual per species. Tips are color coded based on the known host infection strategies used by each species: green = broadcast. red = complex brood lure, purple = tethered brood lure, yellow = mantle lure, blue = mantle lure and simple brood lure, and orange = mantle lure and tethered brood lure. See Barnhart et al. (2008) for an in-depth review of host infection strategies. Primary host type for each mussel species is visualized by connecting lines. Sources used for determining host use are found in Supplementary Table 1.

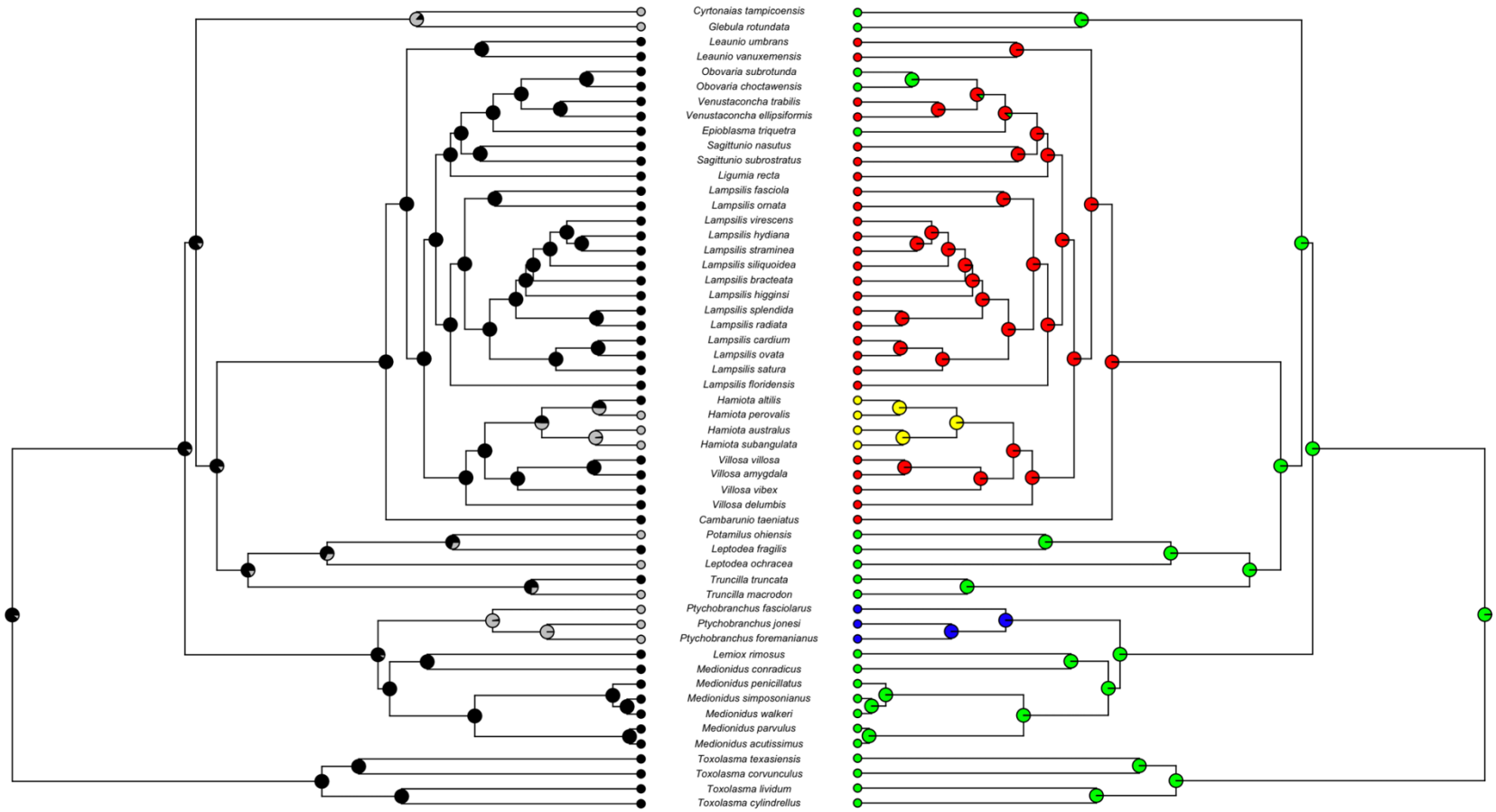

Fig. 2.5. Ultrametric phylogenies created from maximum likelihood phylogeny of Lampsiline mussels (Fig. 3) using TreePL. These trees were trimmed to remove outgroups and retain only a single individual per species. A) Ancestral state reconstruction of mantle lures using a symmetrical rates model: Grey = presence of a mantle lure (fig. 2d), Black = no mantle lure. B) Ancestral state reconstruction of brood lures using a symmetrical rates model: Blue = complex brood lure (fig. 2f), Red = simple brood lure (fig. 2e), Yellow = tethered brood lure (fig. 2h), Green = no brood lure.

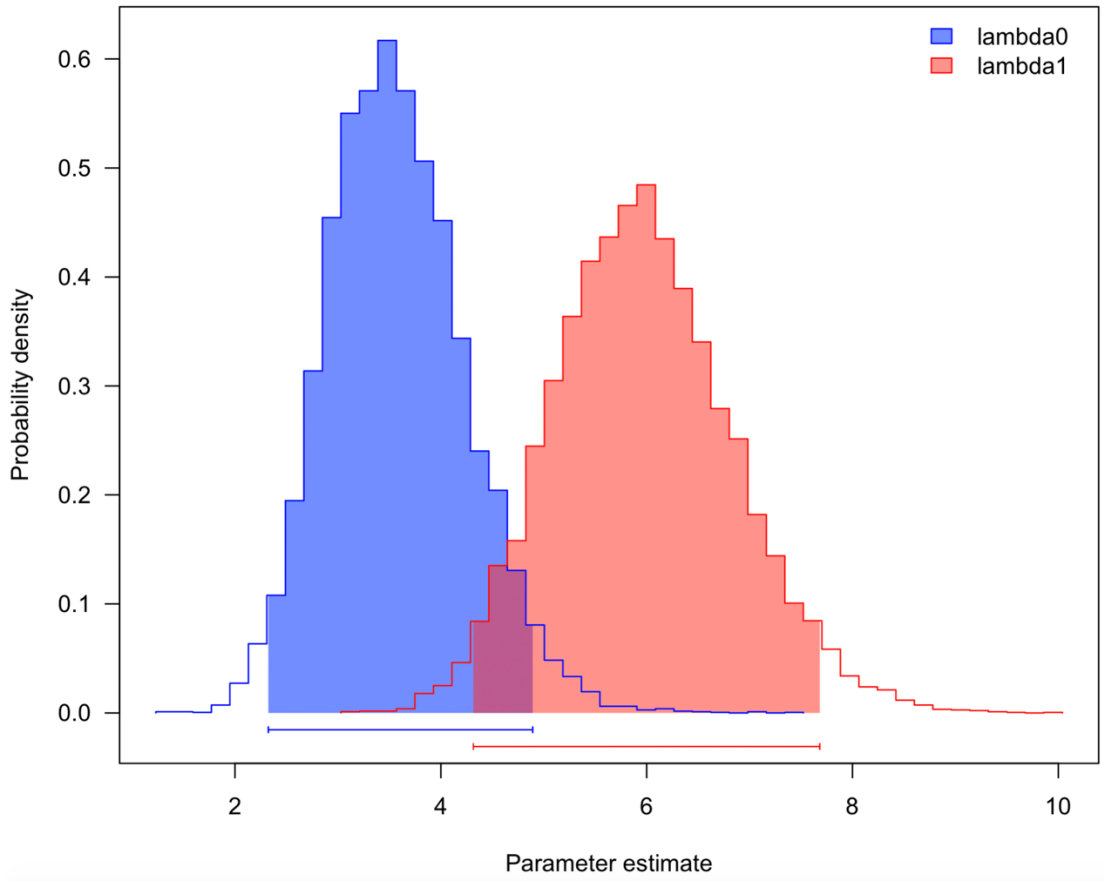

Fig. 2.6: Parameter estimates for net diversification rates between species without a brood lure (lambda0) and species with a brood lure (lambda1). Parameters were estimated using a MCMC approach, implemented in the R package 'diversitree' for 10,000 generations.

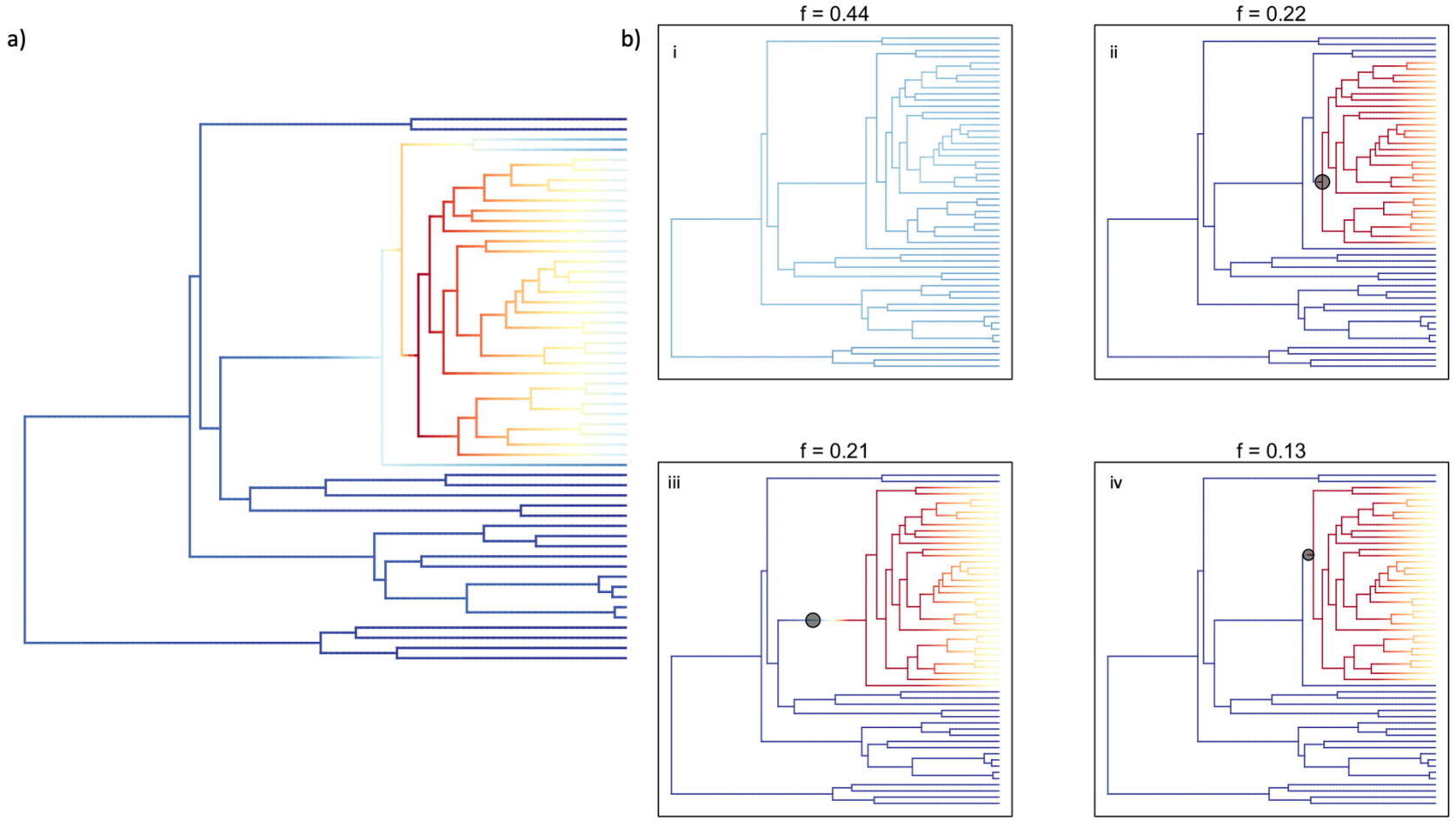

Fig. 2.7. BAMM analyses results showing the best rate regime (a) and the most credible shift sets (b) generated for the Lampsilini ingroup. The four trees shown in b (i-iv) represent the four most frequently sampled rate regimes sampled by BAMM and their respective frequencies are displayed on tip of each tree.

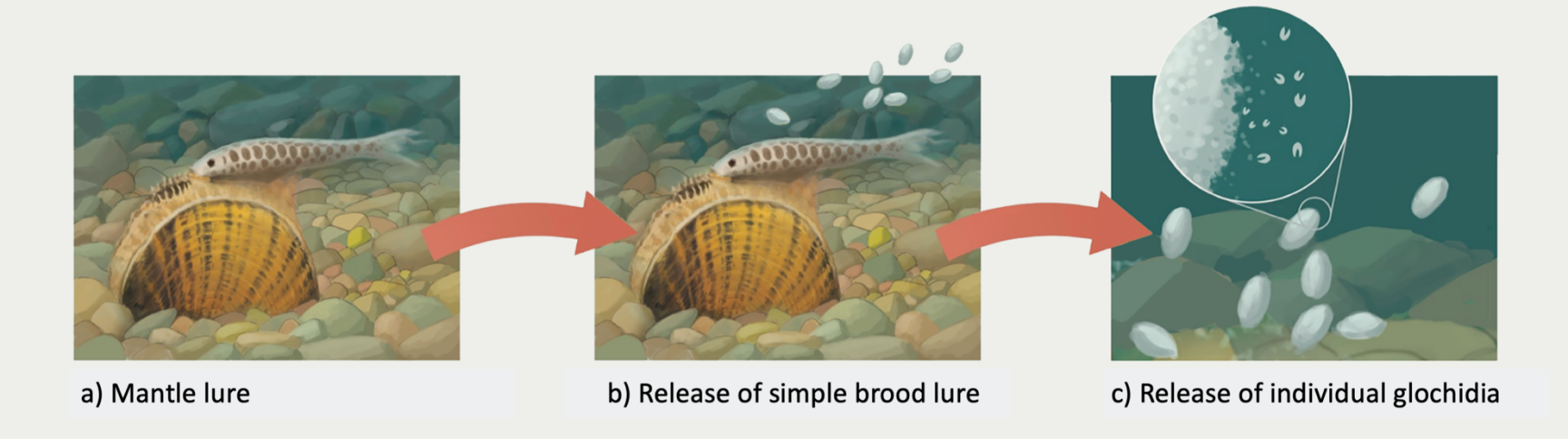

Fig. 2.8. A three-step generalized hypothetical bet-hedging host infection strategy for gravid mussels that produce both a mantle lure and a simple brood lure (genera *Lampsilis, Ligumia, Venustaconcha, Sagittunio, Leaunio, Cambarunio,* and *Villosa*). The first two steps are based on Gascho Landis et al. (2012) and consist of a prolonged mantle lure display (a, the primary strategy) for much of the host infection season, followed by release of residual brooded larvae later in the season within simple brood lures (b, the secondary strategy). Most simple brood lures are fragile and quickly break up releasing larvae (Barnhart et al., 2008). We propose that this latter process represents a tertiary larval broadcast strategy (c) that may occur in more lotic habitats where water movement is sufficient to keep individual larvae in suspension.

CHAPTER 3

Aggressive Mimicry Lure Polymorphisms in the Parasitic Mussel *Lampsilis fasciola* Model Fish or Leech Host Prey and Differ in Morphology and Pigmentation, but not in Display Behavior.

ABSTRACT

Unionoida are free-living apart from a brief, obligately parasitic, larval stage that infects fish hosts and female mussels have evolved a spectrum of strategies to infect hosts with their larvae. In many North American species this involves displaying a mantle lure: a pigmented fleshy extension that acts as an aggressive mimic of a host fish prey, thereby eliciting a feeding response that results in host infection. The mantle lure of *Lampsilis fasciola* is of particular interest because it is apparently polymorphic, with two distinct primary lure phenotypes. One, described as "darter-like", has "eyespots", a mottled body coloration, prominent marginal extensions, and a distinct "tail". The other, described as "worm-like", lakes those features and has an orange and black coloration. We investigated this phenomenon to 1) confirm that it is a true polymorphism; 2) investigate its ecological persistence; 3) identify the range of putative model species targeted by this mimicry system within a river drainage; 4) determine if the mantle lure polymorphim includes a behavioral component. Detection of within-brood lure variation and within-population phylogenomic (ddRAD-seq) analyses of individuals bearing different lures confirmed that this phenomenon is a true polymorphism. It appears stable over ecological

timeframes: the ratio of the two lure phenotypes in a River Raisin (MI) population in 2017 was consistent with that of museum samples collected at the same site 6 decades earlier. Within the River Raisin, four main "darter-like" lure motifs visually approximated four co-occurring darter species (*Etheostoma blennioides, E. exile, E. microperca,* and *Percina maculata*) and the "worm-like" lure resembled a widespread common leech, *Macrobdella decora*. Darters and leeches are typical prey of *Micropterus dolomieui* (smallmouth bass), the primary fish host of *L. fasciola*. *In situ* field recordings were made of the *L. fasciola* "darter" and "leech" lure display behaviors, in addition to the non-polymorphic lure display of co-occurring *L. cardium*. Despite having putatibve models in distinct phyla, both *L. fasciola* lure morphs have similar display behaviors that differ significantly from that of sympatric *L. cardium* individuals. We conclude that the *L. fasciola* mantle lure polymorphism does not include a behavioral component. Discovery of discrete within-brood inheritance of the lure polymorphism implies potential control by a single genetic locus and identifies *L. fasciola* as a promising study system to identify regulatory genes controlling a key adaptive trait of freshwater mussels.

INTRODUCTION

In ecology, mimicry refers to a convergent adaptive trait prevalent in many biological communities: the deceptive resemblance of one organism to another (Pasteur, 1982; Schaefer & Ruxton, 2009; Maran, 2015). It involves three categories of interacting ecological players: mimic (organism displaying the deceptive resemblance), model (organism being mimicked), and receiver (organism being deceived) (Pasteur, 1982; Maran, 2015). Mimicry occurs across a wide variety of ecological contexts and sensory modalities, but conceptually (Jamie, 2017), individual cases can be categorized by the traits being mimicked (signals or cues), as well as by the degree of deceptiveness and the fitness consequences being communicated to the receiver (aggressive, rewarding, Müllerian or Batesian mimicry).

Mimetic systems that are polymorphic (multiple within-species mimic morphs with discrete models) have been particularly influential in uncovering the genetic basis of complex adaptive traits in natural populations (Jay et al., 2018; Palmer & Kronforst, 2020). Such polymorphisms are rare in Nature, with the most well studied examples occurring in papilionid butterflies (Hazel, 1990; Joron & Mallet, 1998; Nijhout, 2003; Jay et al., 2018; Palmer & Kronforst, 2020). For instance, Müllerian mimicry polymorphisms in *Heliconious* species are determined by presence/absence of an introgressed chromosomal inversion 'supergene' (Jay et al., 2018), and alleles of a single ancestral gene (*doublesex*) control female-specific Batesian mimicry polymorphisms in *Papilio* species (Palmer & Kronforst, 2020).

In contrast to papilionid butterflies, the genetics of mimicry trait evolution among unionoid mussels is poorly understood. Unionoida comprise ~75% of the planet's freshwater bivalve species and are free-living apart from a brief, obligately parasitic, larval stage that infects fish hosts (Bogan, 2007; Haag, 2012). Gravid female mussels have evolved a spectrum of

strategies to infect hosts with their larvae (Zanatta & Murphy, 2006; Barnhart, Haag & Roston, 2008; Hewitt, Wood & Ó Foighil, 2019). Females in many species use a mantle lure (Welsh, 1933): a pigmented fleshy extension that acts as an aggressive mimic (Jamie, 2017) of a host fish prey item (a small fish, aquatic invertebrate, egg mass etc.) cue and elicits a feeding response resulting in host infection (Haag & Warren, 1999; Barnhart, Haag & Roston, 2008; Figure 1a). Mimetic mantle lures predominate in Lampsilini, a major clade of North American freshwater mussels recently identified as a cryptic adaptive radiation centered on larval ecologies and specialized host-infection behaviors (Hewitt, Haponski & Ó Foighil, 2021). Notably, mantle lures are not gender specific traits. Ortmann (1911) and Kramer (1970) documented the production of rudimentary lures in juveniles and male lampsilines but noted that formation of fully developed lures is restricted to sexually mature females, and that only gravid females engage in lure display behaviors. Although mimetic mantle lures are a key adaptive trait of freshwater mussel diversification, the genetic regulators underlying their formation (Kramer, 1970), variation (Haag, Warren & Shillingsford, 1999; Zanatta, Fraley & Murphy, 2007; Barnhart, Haag & Roston, 2008), and evolution (Zanatta & Murphy, 2006; Hewitt, Haponski & Ó Foighil, 2021) remain completely unknown. This gap in our knowledge is exacerbated by the stark conservation status of North American freshwater mussels with 2/3rds of species classified as threatened or near-threatened (Lopes-Lima et al., 2018). As with papilionid butterflies (Jay et al., 2018; Palmer & Kronforst, 2020), targeting lampsiline species with polymorphic mantle lures for in-depth study may represent a tractable route to closing that gap.

Lampsilis fasciola, the wavy-rayed lampmussel, is a promising candidate species in that it produces a number of distinct mantle lure phenotypes (Zanatta, Fraley & Murphy, 2007) across its Eastern North America distribution, extending from southern Ontario to northern Alabama

(Parmalee & Bogan, 1998). Two range-wide lure phenotypes predominate in northern populations. The more common of the two, labeled "darter-like" by Zanatta et al. (2007), has "eyespots", a mottled "main body" pigmentation composed of lateral and dorsal spots that can vary substantially in color, numerous and prominent marginal extensions (AKA "appendages" or "tentacles"), and a distinct "tail" region (Kramer, 1970; Zanatta, Fraley & Murphy, 2007)– see Figure 1b. A rarer lure phenotype, labeled "worm-like" by McNichols (2007), lacks the above features and has instead a uniform bright orange coloration underlain with a black basal stripe (Zanatta, Fraley & Murphy, 2007) – see Figure 1c. The latter lure phenotype is highly distinctive within the genus *Lampsilis* where fish-like mantle lures are the norm (Kramer, 1970). Based on the results of laboratory larval infection experiments, and on the degree of ecological overlap, *Micropterus dolomieu* (smallmouth bass), and to a lesser extent *Micropterus salmoides* (largemouth bass), have been identified as *Lampsilis fasciola's* primary fish hosts (Zale & Neves, 1982; McNichols, 2007; Morris et al., 2009; McNichols, Mackie & Ackerman, 2011; VanTassel et al., 2021). Both host species are generalist predators of aquatic invertebrates and vertebrates (Clady, 1974).

There are a number of outstanding, inter-related questions that need to be addressed prior to developing an integrated *Lampsilis fasciola* mantle lure polymorphism study system. First among them is residual uncertainty that the mantle lure morphs represent polymorphisms rather than cryptic species. Zanatta et al. (2007), using microsatellite markers, did not detect evidence of cryptic species but qualified their conclusions due to small sample sizes, and their result requires molecular phylogentic corroboration (Fisheries and Oceans Canada, 2018). Secondly, we lack any data on the relative persistence of the lure polymorphisms in natural populations through time. Thirdly, one set of important ecological players – the respective models of each *L. fasciola* mantle lure mimic – remains to be determined with any specificity. Finally, mantle lure display

behavior is an important component of effective mimicry in freshwater mussels (Welsh, 1933; Jansen, Bauer & Zahner-Meike, 2001; Haag & Warren, 2003; Barnhart, Haag & Roston, 2008), but it is unknown if the morphologically divergent *L. fasciola* mantle lures, that presumably mimic very distinct host prey models, also differ in their display behaviors.

Our study aimed to address these outstanding questions for *L. fasciola* using novel data from two southeastern Michigan river populations [the Raisin (our primary study location), and the Huron], and from a captive brood raised by the Alabama Aquatic Biodiversity Center. Using a phylogenomic (ddRADseq) approach, we corroborated Zanatta et al's (2007) finding that mussels bearing the two mantle lure morphs do not represent cryptic lineages. This was confirmed by our documentation of discrete, within-brood, inheritance of the *L. fasciola* lure polymorphism in the captive Alabama specimens. Availability of mid-20th century museum specimens from a River Raisin population allowed us to determine that the relative frequency of the two morphs has remained broadly stable over 60 years. In addition, availability of a comprehensive Raisin River ichtyofauna survey (Smith, Taylor & Grimshaw, 1981) allowed us to identify four darter species as putative models for the predominant "darter-like" mantle lures in this population. The most palusible model for the "worm-like" *L. fasciola* mantle lure appears to be *Macrobdella decorata* (the American medical leech). Despite having putative models in distinct phyla, detailed video analyses of gravid females in the two drainages revealed that both *L. fasciola* lure morphs have similar display behaviors that differ significantly from that of sympatric *L. cardium* indivuals bearing non-polymorphic fish-mimic lures. We conclude that the *L. fasciola* mantle lure polymorphism does not include a significant behavioral component.

MATERIALS AND METHODS

Tissue Sample Collection

Lampsilis fasciola mantle tissue samples were collected for genotyping purposes by taking non-lethal mantle clip biopsy (Berg et al., 1995) from wild population lure-displaying female mussels during the summers of 2017, 2018, and 2021 from a total of three rivers (Figure 2). Two of the sampling locations were in southeastern Michigan: The River Raisin at Sharon Mills County Park (42.176723, -84.092453; N=30; 24 "darter-like", 6 "worm-like", collectively sampled in 2017, 2018 & 2020), and the Huron River at Hudson Mills Metropark, MI (42.37552, -83.91650; N=13; 7 "darter-like", 6 "worm-like", collectively sampled in 2017, 2018, and 2020); both of these rivers drain into Lake Erie. The third location was in North Carolina: the Little Tennessee River (N=15; 35.32324, -83.52275; N=1-, all 10 were "darter-like" and sampled in 2017); this river is part of the Mississippi drainage. Prior to each biopsy, photographs of the intact, undisturbed, lure display were taken with an Olympus Tough TG-6 underwater camera (Supplementary figure 1).

Captive Brood Tissue Samples

In addition to the wild-sampled specimens, we also obtained tissue samples from 50 members of a captive-raised brood that had been ethanol-preserved a decade earlier. In 2009, the Alabama Aquatic Biodiversity Center (AABC) established a culture facility for endangered freshwater mussels. The Center's inaugural culture attempt, by co-authors Paul Johnson and Michael Buntin, was a proof-of-concept trial involving a single gravid female *Lampsilis fasciola*, a non-endangered species, sourced from a wild Paint Rock River population (N 34˚ 47.733′,W 86˚ 14.396′) in Jackson County, AL (Figure 2) on June 11, 2009. This female *L. fasciola* had a "worm-like" lure: the AABC data sheet for the trial 2009 host infection (Supplemental Figure 1) records that it was "bright orange and black", and it lacked the "eyespots", mottled body coloration, marginal extensions and "tail" of the "darter-like" lure phenotype (Butlin & Johnson,

pers. observ.). On July 13th 2009, ~31K glochidia larvae were extracted from the female's marsupia (Supplemental Figure 1) and used to infect *Micropterus coosae* (Redeye Bass) hosts sourced from the Eastaboga Fish Hatchery (Calhoun County, AL) using standard protocols (Barnhart, Haag & Roston, 2008). The female mussel was then returned to the Paint Rock River Population. Following completion of larval development on the fish hosts, ~ 9.3K metamorphosed juvenile mussels were recovered and reared, first in in pond water cages, then in pond water flow-through tanks, for two years with ~2.2K surviving. In 2011, this proof-of-concept culture experiment was terminated, and the survivors were donated to several research groups with the large majority being used for toxicology experiments (Leonard et al., 2014a,b).

Prior to the brood's termination, Johnson noticed that a few females had attained sexual maturity and were displaying polymorphic lures (Figures 3b, 3c). We aimed to substantiate that 2011 observation by tracking down any remaining brood specimens and were successful in recovering 50 individuals that had been preserved in 95% ethanol and shipped to Nathan Johnson (USGS) in Gainsville, FL in 2011. Because *Lampsilis* spp. males, and immature females, produce a rudimentary mantle lure (Ortmann, 1911; Kramer, 1970), we were able to determine the primary lure phenotype (darter-like" or "worm-like") of all 50 preserved brood members. Using a Leica dissecting microscope, individual photomicrographs were taken of the preserved rudimentary lure structures (Figure 3d,e and Supplementary Fig 2), and their respective lure phenotypes were identified independently by both T. Hewitt and by D. Ó Foighil. In addition, tissue samples were collected from each brood member and included in the downstream phylogenomic analyses.

Phylogenomic analyses

Genomic DNA was extracted from tissue samples using the E.Z.N.A. Mollusk DNA kit (Omega Bio-Tek, Norcross, GA) according to manufacturer's instructions and then stored at -80°C. The quality and quantity of DNA extractions were assessed using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA) and ddRADseq libraries were prepared following the protocols of Peterson et al. (2012). We then used 200 ng of DNA for each library prep. This involved digestion with Eco-RI-HF and MseI (New England Biolabs, Ipswich, MA) restriction enzymes, followed by isolating 294-394 bp fragments using a Pippen Prep (Sage Science, Beverly, MA) following the manufacturer's instructions. Prepared ddRADseq libraries then were submitted to the University of Michigan's DNA sequencing core and run in four different lanes using 150 bp paired-end sequencing on either a Illumina HiSeq 2500 or a Illumina novaseq shared flow cell. Two control individuals of *Lampsilis fasciola* were run in each lane and reads for both individuals clustered together in every analysis with 100% bootstrap support, indicating no lane effects on clustering across individuals. Raw demultiplexed data were deposited at genbank under the bioproject ID PRJNAXXXXXX with accession numbers SAMNXXXXX-SAMNXXXXX.

The alignment-clustering algorithm in ipyrad v.0.7.17 (Eaton, 2014; Eaton & Overcast, 2020) was used to identify homologous ddRADseq tags. Ipyrad is capable of detecting insertions and deletions among homologous loci which increases the number of loci recovered at deeper evolutionary scales compared to alternative methods of genomic clustering (Eaton, 2014). Demultiplexing was performed by sorting sequences by barcode, allowing for zero barcode mismatches (parameter 15 setting 0) and a maximum of five low-quality bases (parameter 9). Restriction sites, barcodes, and Illumina adapters were trimmed from the raw sequence reads (parameter 16 setting 2) and bases with low-quality scores (Phred-score <20, parameter 10

setting 33) were replaced with an N designation. Sequences were discarded if they contained more than 5 N's (parameter 19). Reads were clustered and aligned within each sample at an 85% similarity threshold, and clusters with a depth < 6 were discarded (parameters 11 and 12). We also varied the number of individuals required to share a locus from ~50% to ~75%.

We analyzed the two concatenated ddRAD-seq alignment files (50% and 75% minimum samples per locus) using maximum likelihood in RAxML v8.2.8 (Stamatakis, 2014). A general time-reversible model (Lanave et al., 1984) was used for these analyses that included invariable sites and assumed a gamma distribution. Support was determined for each node using 100 fast parametric bootstrap replications. Lure phenotype information was recorded and mapped on to the phylogenetic tree. Phylogenetic signal of lure phenotype was tested using Pagel's λ (Pagel, 1999) in R (R Core Team, 2018) with the 'phylobase' package (Hackathon et al., 2013).

River Raisin Mantle Lure Phenotype Ratios Over Time

Mid-20th century *Lampsilis fasciola* specimens collected at the Sharon Mills County Park site (Raisin River, MI; Fig. 2a) are preserved as part of the University of Michigan's Museum of Zoology wet mollusk collection. They stem from 8 different collecting events between 1954 and 1962 (Table 2) and their presence afforded an opportunity to assess the stability of the *L. fasciola* "darter/worm" mantle lure polymorphism in that population over a six-decade time intreval. All of the museum specimens, males as well as females, were examined to determine if their fully-formed (female) or vestigial (male) mantle lures were "darter-like" or "worm-like". For females, this could be achieved by simple visual examination but male lure classification required a dissecting microscope. The ratio of mantle lure phenotypes observed in the Sharon Mills County Park population was compared among mid-20th century (UMMZ

preserved females and males) and 2017 (field photographs and videos of displaying females) samples using a Fisher's exact test, implemented in R.

Putative Lure Mimicry Models

Population-specific putative model species for the *Lampsilis fasciola* mantle lure mimicry system were investigated at the River Raisin Sharon Mills County Park study site (Figure 2) in part because of the availability of a comprehensive ecological survey of Raisin River fishes (Smith, Taylor & Grimshaw, 1981). "Darters" – members of the speciose North American subfamily Etheosomatidae – have been implictly identified as models for the predominant "darter-like" mantle lure phenotype (Zanatta, Fraley & Murphy, 2007)and they are preyed upon by *Micropterus dolomieu* (Surber, 1941; Robertson & Winemiller, 2001; Murphy et al., 2005), *L. fasciola's* primary fish host (Zale & Neves, 1982; McNichols, 2007; Morris et al., 2009; McNichols, Mackie & Ackerman, 2011; VanTassel et al., 2021). Ten species of Etheosomatidae occur in the River Raisin, as does *M. dolomieu* (Smith, Taylor & Grimshaw, 1981).

River Raisin gravid female *Lampsilis fasciola* engage in mantle lure displays from May-August and during the summer of 2017 a total of 27 different displaying females were photographed along a 150m stretch downstream of the dam at Sharon Mills county park using an Olympus Tough TG-6 underwater camera. The lures were first categorized into broad groupings based on visual similarity (in terms of morphology and coloration; Supplementary Figure 1) and these groupings were then used to identify putative host prey fish model species, present in the River Raisin drainage (Smith, Taylor & Grimshaw, 1981), in terms of convergent size, shape and coloration. Putative model species were further assessed based on their relative local abundance (Smith *et al.*, 1981) and on their range overlap with both mimic and receiver. Geographic ranges of *L. fasciola,* the primary host *M. dolomieu*, and each prospective model species were carefully

produced by hand in Arcgis software, and the overlap between *L. fasciola, M. dolomieu,* and each putative model species were assessed using Arcgis software.

Behavioral Analyses

Standardized video recordings of 30 mantle lure-displaying female *L. fasciola* were recorded using a Go Pro Hero 6 camera in the Summer of 2018 at the two different southeastern Michigan study sites: Sharon Mills County Park (River Raisin) and Hudson Mills Metropark (Huron River). An additional 4 video recordings of the lure behavior of the co-occuring congener *Lampsilis cardium* were collected from the Sharon Mills site to assess interspecific variability in lure behavior. Recordings were captured from a top down perspective during daylight hours using a standardized frame that included a metric ruler and a Casio TX watch to record date, time, and water temperature data within the video frame. For each displaying female, videos of the lure movements were recorded for 10 minutes at 120 frames-per-second. Analysis of the videos involved manually recording mantle lure movements for 20,000 frames, starting at 5,000 frames to to avoid any initial setup effects on mussel display behavior. The frame numbers when an undividual movement began and ended were notated and movements of the left and the right mantle lure flaps were assessed seperately.

To qualitatively assess behavioral differences among samples, gait analysis diagrams were created in R for each individual displaying mussel. Averages and standard deviations for the time interval between lure undulations were calculated for each individual, as well as the speed of undulation and the proportion of movements synchronized. A Kruskal-Wallis test was used to test for overall differences among lure groups (*L. fasciola* "darter-like", *L. fasciola "*worm-like", and *L. cardium*), and pairwise Wilcoxon Signed rank tests were used to compare groups directly with a Bonferroni *P* value adjustment to correct for multiple tests. A Spearman

correlation was used to test for the effect of water temperature on the time interval between lure undulation.

To further explore differences in lure behavior among groups, we used a General Linear Mixed Model (GLMM), with sample ID as a random factor, to test for differences in lure movement intervals. The GLMM appoach, unlike simple mean comparisons, allows the inclusion of all lure movements for all individuals in the model. Because displaying mussels all varied in the number of lure movements recorded over the 20,000 frames analyzed, a dataset of 1000 random bootstrap values was constructed for each individual. Models were fitted using the 'lmerTest' package in R and Satterthwaite's Method (Satterthwaite, 1946; Kuznetsova, Brockhoff & Christensen, 2017). was used to test for significance of fixed effects of lure phenotype on the interval between lure undulations.

RESULTS

Captive Brood

Mantle lure microphotographs (Figure 3d, e) of all 50 available AABC-cultured and 95% ethanol-preserved *Lampsilis fasciola* individuals, members of the same maternal brood, are individually presented in Supplementary Figure 2. These specimens were 2 years post-metamorphosis when preserved in 2011 and independent classification of their mantle lure phenotypes concurred that the ratio of "worm-like" to "darter-like" phenotypes was respectively 17/33 for this brood subsample.

ddRAD-seq and Phylogenomic analyses

Genomic sequencing returned raw reads ranging from 258,664 to 13,366,692 per individual across the 108 unionid specimens included in the analyses comprising samples of the

ingroup *Lampsilis fasciola*, sourced from 4 different populations, and of the outgroups *Lampsilis cardium* and *Sagittunio nasuta*. Mean coverage depth, for the 85% clustering threshold, ranged from 2.03 (L_fasciola_AL_mom_2) to 14.25 (L_fasciola_Raisin_16; Table 1). Between 28,725 and 16,161 homologous loci were identified across the two best ddrad datasets (85%-50% and 85%-75% respectively) and the number of loci recovered was generally consistent among all samples.

The maximum likelihood tree produced by RAxML (Supplementary Figure 3) recovered the following ingroup/outgroup topology: (*S. nasuta* (*L. cardium, L. fasciola*)) with outgroup branch lengths greatly exceeding those of the ingroup. To optimize the legibility of ingroup relationships, a compressed, color-coded graphic excluding *S. nasuta* was constructed (Figure 4). A nested series of phylogenetic relationships was recovered for the four *L. fasciola* fluvial populations with the two Michigan drainages being paraphyletic: (Little Tennessee River (Paint Rock River (River Raisin (River Raisin, Huron River))). The ingroup topology also showed evidence of within-population genealogical relationships with all Paint Rock River brood members forming an exclusive clade (Figure 4).

The respective primary mantle lure phenotypes – "darter-like" or "worm-like" – of all 92 *Lampsilis fasciola* ingroup individuals is indicated in Figure 4. Note that 3/4 population samples – Little Tennessee River, River Raisin and Huron River – were exclusively composed of mantle-lure displaying wild females and that the latter two samples were polymorphic in mantle lure composition. Regarding the Paint Rock River sample, polymorphic lures were restricted to the 50 captive raised AABC brood members (primarily of indeterminate gender) sourced from a gravid, wild female in 2009 (not included in the analyses). The ingroup phylogeny (Figure 4) contained two polymorphic mantle lure clades, one composed of both Michigan populations

(River Raisin and Huron River), the other consisting only of the AABC brood, and both clades had individuals of either lure phenotype interspersed across their respective topologies. Pagel's λ was calculated to assess the degree of phylogenetic signal associated with either primary mantle lure phenotype, and little phylogenetic signal was detected ($\lambda = 0.21$; $P = 0.13$).

Phenotypic Ratios Over Time

Table 2 summarizes the gender, and primary lure phenotypes, of 57 mid-20th century *Lampsilis fasciola* female and male specimens collected from 1954-1962 at the River Raisin Sharon Mills County Park study site (Figure 2a) and preserved in the University of Michigan Museum of Zoology's wet mollusk collection (Figure 5b, c). These historical samples had a collective "darter-like" to "worm-like" ratio of 48:9, with 15.8% of individuals having the less common "worm-like" mantle lure phenotype. Figure 5a contrasts the mid-20th century lure phenotype ratios with a contemporary (2017) estimate in that same population, based on photographic recordings of 27 displaying females. The contemporary estimate was 23:4, with 14.8% of individuals having the less common "worm-like" mantle lure phenotype. A Fisher's exact test of these two temporal estimates of relative mantle lure phenotype frequencies in the River Raisin Sharon Mills County Park *L. fasciola* population yielded an estimated P value = 1, confirming that they did not differ significantly.

Putative Raisin River Lure Mimicry Models

The mantle lure field photographs of 27 displaying female *L. fasciola* in the Raisin River Sharon Mills County Park population in 2017 (Supplementary Figure 1) were categorized into either "darter-like" (Zanatta, Fraley & Murphy, 2007) or "worm-like" (McNichols, 2007), as summarized in the Materials & Methods section. In addition to the specific features that separate these two primary mantle lure phenotypes (presence/absence of "eyespots" mottled

pigmentation, marginal extensions and a "tail"), "darter-like" lures exhibited a much higher degree of variation than did "worm-like" lures, both within populations and across the species' range. The latter lure phenotype exhibited a relatively simple, uniform morphology combined with a bright orange coloration underlain with a black basal stripe phenotype in Michigan (Figure 6f-h), in Alabama (Fig. 6i, j), and in North Carolina populations (Fig. 2A in Zanatta et al, 2007). In contrast, Raisin River "darter-like" mantle lures exhibited individual-level variation that was sometime quite marked, especially in details of their pigmentation, and to a more limited degree in their marginal extensions (Figures 6a-d; Supplementary Figure 1). Among individual variation was most pronounced for inter-population camparisons, e.g., see the much larger "tail" in the lure displaying Paint Rock River, Alabama specimen shown in Figure 6e, and also the wider range of phenotypes present in North Carolina populations (Fig. 2b-d in Zanatta et al, 2007).

Despite the considerable individual variation among the 24 photographed Raisin River "darter-like" mantle lures (Supplementary Figure 1), it was possible to identify some shared phenotypic motifs, especially in pigmentation pattern, and to informally categorize 23/24 mantle lures with those shared motifs into 4 general groupings. Group 1 "darter-like" mantle lures were characterized by prominent, chevron-like, darker pigmented blotches, spaced regularily along the flanks of the lure, over a lighter background coloration (Figure 6a). This general pattern occurred in 7/24 Raisin River "darter-like" lures examined. Group 2 was rarer (3/24 individuals) and consisted of a darker background coloration with large orange blotches spaced regularily along the lure flanks, some divided into "dorsal" and "ventral" elements (Figure 6b). Group 3 (9/24 individuals) lures were characterized by prominent dark lateral maculation spatially divided into a "ventral" pattern of larger, regularly spaced blotches and a "dorsal" pattern of more numerous,

irregular blotches of different sizes (Figure 6c). Finally Group 4 (3/24 individuals) lures were characterized by an evenly-dispersed, fine grained freckling of numerous pigmented spots over a lighter background (Figure 6d).

To explore putative model species for the four *Lampsilis fasciola* Raisin River "darter-like" mantle lure groupings (Figure 6a-d), potential matches (in terms of size, shape and coloration) were sought among the 10 species of Etheosomatidae that occur in the River Raisin (Smith *et al.*, 1981), many of which display pronounced sexual dimorphism in body coloration (Kuehne and Barbour, 2014). The best apparent matches, depicted in Figure 7, are as follows: Group 1 (Fig. 6a)-*Etheostoma blennioides* (female coloration*)*, Group 2 (Fig. 6b)-*Etheostoma exile* (male coloration), Group 3 (Fig. 6c)-*Percina maculata* (male and female coloration) and Group 4 (Fig. 6d)-*Etheostoma microperca* (female coloration).

The distinctive color combination of the *Lampsilis fasciola* "worm-like" lure - solid orange with a black underlay (Figure 6 f-j) - did not match that of any Raisin River darter, or other Raisin River fishes (Smith, Taylor & Grimshaw, 1981). It does, however, match the coloration and size/shape, of the common North American leech, *Macrobdella decora*, which is widespread and abundant in eastern North America watersheds and typically feeds on aquatic vertebrates (Klemm, 1982; Munro et al., 1992). *M. dolomieu*, *L. fasciola*'s primary host fish, is a generalist predator with a diet of aquatic invertebrates, including leeches, in addition to small fishes (Clady, 1974) and recreational fishers frequently use live and/or artifical leeches as bait to catch this species (Cooke et al., 2022). Based on the available data, it seems that *Macrobdella decora* may be the best model species candidate for the "worm-like" (McNichols, 2007) *L. fasciola* mantle lure phenotype.

The geographic range of the mimic, *Lampsilis fasciola*, is a subset of that of its receiver/host *M. dolomieu* (Figure 2), and its degrees of range overlap with all 5 putative River Raisin mantle lure models were calculated using Arcgis (Table. 3) and are shown in Figure 8. Three of the five putative models -*Etheostoma blenniodes, Percina maculata* and *Macrobdella decorata* - have a large degree of overlap with *L. fasciola*'s range, but *E. exile* and *E. microperca* are restricted to northern portions.

Behavioral Analyses

Lure movement data was extracted from video recordings of 30 *L. fasciola* and 4 *L. cardium.* Qualitatively, *L. fasciola* and *L. cardium* have very different mantle lure display behaviors. Gait analysis diagrams show a clear distinction between both primary *L. fasciola* lure phenotypes ("darter" and "leech") and *L. cardium*. A representative individual for each group is shown in Figure 9, and gait analyses for all individuals can be found in Supplementary Figure 4. *L. cardium* consistently exhibited a synchronized lure undulation of both mntle lure flaps, whereas *L. fasciola* samples frequently moved left and right mantle flaps independently. Gait analysis diagrams also qualitatively showed that while *L. fasciola* behavior is charcterized by a high level of variability in undulation interval, *L. cardium* is comparitavely much more regular in undulation interval with a steady beat frequency (Figure 9a-c).

Table 4 details the time, date, location, temperature and summary statistics of all 34 lure display field recordings. Due to the independent movements of the left and right mantle flaps in *L. fasciola*, movements were recorded for both sides seperately. Boxplots for means and standard deviations of left lure movement intervals are displayed in Figure 10a,b. Boxplots of mean length of lure movements (L) are shown in Figure 10c and the proportion of synchronized lure movements are displayed in Figure 10d. Only left mantle flap movements are displayed in Figure

10a-c, however the distributions for the right mantle flap movements are highly similar. Kruskal-Wallis tests suggest highly significant differences between groups for average undulation interval and for standard deviation of undulation interval (P = 0.001 and 0.005 respectively). Pairwise Wilcoxon test revealed highly significant differences in lure movement interval (left) between *L. cardium* and both *L. fasciola* groups (P = 0.007 and 0.013 for "darter" and "leech" respectively) and between both primary *L. fasciola* mantle lure phenotypes as well (P = 0.031). Pairwise Wilcoxon test also revealed significant differences in the standard deviation of left movement interval between *L. cardium* and both *L. fasciola* groups (P = 0.007 and 0.013 for "darter" and "leech" respectively) but not between the two *L. fasciola* mantle lure groups (P = 0.2). These results suggest that, in addition to the qualitative differences between *L. cardium* and *L. fasciola* lure behavior, the *L. cardium* lures are beating at a faster rate and with greater regularity. There also appears to be a slight difference in the rate of lure beats between the two *L. fasciola* mantle lure morphs, with the "darter" lure beating slightly slower, but with no differences in variability. No significant relationship was observed between lure movement interval (left) and water temperature (Supplementary Figure 5; P = 0.428).

The General Linear Mixed Model (GLMM)'s were used as an alternative analytical approach that included a large, bootstrapped dataset of lure movements. GLMM results were similar to that of the mean comparisons, with *L. cardium* individuals having faster movement frequency than either *L. fasciola* lure morphs (an estimated 0.21 seconds for *L. cardium* versus 3.2 and 1.0 seconds respectively for L. fasciola "darter" and "leech" lures). However, these fixed effects are not significant.

DISCUSSION

Two new lines of data, phylogenomic and genetic, corroborated Zanatta et al's. (2007) preliminary finding that the primary mantle lure morphs in *Lampsilis fasciola* (Figure 1b, c) represent a within-population polymorphism rather than cryptic taxa. In the phylogenomic analyses, all three polymorphic population samples (Huron, Raisin, and Paint Rock Rivers), collectively spanning the species range (Figure 2a-c), produced tip clades that were comprehensively polyphyletic regarding lure morph type (Figure 4) and the "darter vs leech" dichotomy yielded a low estimate of phylogenetic signal ($\lambda = 0.21$). However, the phylogenomic data did reveal clear evidence of geographic structuring (Figure 4), even among regional populations with a continuous freshwater connection, *e.g*., the Huron and Raisin drainages empty in Western Lake Erie and the Little Tennesse and Paint Rock drainages empty into the Tennessee River, see also VanTassel et al. (2021). The Paint Rock River (AL) population was sister to the Michigan populations (Figure 4), a result consistent with phylogeographic associations of multiple other North American species, including unionid mussels and *Micropterus dolomieu*, attributed to hypothesized glacial refugia in the southern Appalachian mountains (Soltis et al., 2006; Borden & Krebs, 2009; Zanatta & Harris, 2013; Hewitt et al., 2018).

Discovery of within-brood mantle lure heterogeneity (Figure 3), apparently the first such record for unionids, confirms that the *Lampsilis fasciola* "darter-like" and "leech-like" mantle lures are true polymorphisms and provides initial, although limited, genetic insights into lure phenotype inheritance. Of the 50 available offspring, the maternal "leech" phenotype was inherited by 17, the remaining 33 had the "darter" phenotype, but none exhibited a recombinant phenotype, *e.g.*, "leech" coloration with "darter" marginal extensions or "darter" coloration without marginal extensions. Evidence of discrete, within-brood segregation of the mantle lure polymorphism implies potential control by a single genetic locus and expression of the maternal

phenotype in ~1/3rd of the offspring is inconsistent with a dominant hypothetical "leech" allele. Additional pedigree insights are currently inhibited by not knowing the number of sires that contributed to the brood: the dam was a wild-mated Paint Rock River individual. Freshwater mussel broods frequently have multiple paternity (Ferguson et al., 2013; Wacker et al., 2018) and this may well be the case also for the AABC *L. fasciola* brood (Figure 3) although additional analyses of its RADseq dataset are needed to resolve that issue.

There are well-known cases of a single genetic locus controlling a mimic polymorphism in other systems. In butterflies, polymorphic mimicry in wing pigmentation is controlled by an introgressed mimicry supergene in *Heliconius* species (Jay et al., 2018) and by mimicry alleles of the transcription factor *doublesex* (*dsx*) in some *Papilio* species (Palmer & Kronforst, 2020). Note, however, that the *Lampsilis fasciola* mantle lure mimicry polymorphism differs in important ways from these butterfly systems. It is more complex, because it involves putative models (darters and leeches) from disparate phyla rather than from similar morphospecies (other butterflies), thereby requiring polymorphic trait differentiation in pigmentation **and** in morphology (Figure 1b, c). It is also a case of aggressive mimicry (Jamie, 2017), different from the Müllerian mimicry of *Heliconius* (Kronforst & Papa, 2015) or the Batesian mimicry of *Papilio* (Kunte, 2009).

Persistence of the *Lampsilis fasciola* mantle lure polymorphism across a broad geographic scale (Michigan to Alabama, Figure 2) is notable although the mechanism responsible for its widespread maintenace is unclear. One hypothesized mechanism for the persistence of polymorphisms in a species or population is frequency-dependent selection where rare phenotypes are selected for, causing the ratio of phenotypes to vary over time (Ayala & Campbell, 1974). Frequency-dependent selection has been observed in other polymorphic

mimicry systems (Shine, Brown & Goiran, 2022) and it has been suggested as a possible mechanism for the persistence of the *L. fasciola* polymorphism (Zanatta, Fraley & Murphy, 2007; Barnhart, Haag & Roston, 2008; Hewitt, Haponski & Foighil, 2021). One criterion for frequency-dependent selection is that the phenotype ratios oscillate over time as initially rare phenotypes become more successful. However, the historical (1954-1962) and contemporary (2017) data from Sharon Mills County Park (Figure 5) did not show evidence of such oscillation: the frequency of the rarer "leech" lure - 15.8% vs. 14.8% - and the ratio of "leech" to "darter" lures - 9:48 vs. 4:23 - remained essentially the same for both time windows, although we lack data for the intervening years. Theoretically, there are other mechanisms for balancing selection to maintain polymorphisms over long time-scales, including heterozygote advantage, or opposing selection pressures favoring different alleles at polymorphic loci (Prout, 2000; Mérot et al., 2020), but the underlying genetics of the *L. fasciola* polymorphism is unknown at this time and more data is clearly needed.

An integrative outline of the *Lampsilis fasciola* mimetic system at the River Raisin study site was assembled containing 1 "leech" and 4 "darter" mantle lure phenotypes together with their putative model species (Figure 7). The four putative River Raisin darter model species – *Etheostoma blennioides*, *E. exile*, *E. microperca* and *Percina maculata* – are all common and widespread members of the drainage's ichthyofauna with 300-900 specimens/species recovered from 30-100 sampling locations by the Smith et al. (1981) ecological survey. The relative uniformity of the "leech" mantle lure phenotype in the River Raisin and throughout the *L. fasciola* range (Figure 6f-j) stands in sharp contrast with much higher degree of local and range-wide variation shown by "darter" lures (Figure 6a-e). That phenotypic lure disparity mirrors the collective phenotypic variability of darters vs. *Macrobdella decora*, darters being the second-

most diverse fish clade in North America with ~170 species (Warren & Burr, 1994; Stein & Morse, 2000). Another possibility is that at least some *L. fasciola* "darter-like" lures across the mussel's range are composite mimics of visual elements from more than one member of their local darter fauna, but that remains to be established.

While the behavior of mantle lures in *Lampsilis* mussels has been documented and studied for many decades (Ortmann, 1911; Kramer, 1970; Haag & Warren, 1999), detailed analysis of lure undulation behavior is currently lacking, and the relative importance of behavior versus coloration and morphology is not well understood. The lure undulation for both *L. cardium* and *L. fasciola* starts about 2/3rds of the way to the "posterior" ("tailed") side of the lure, and then travels forward toward the "eyespot"-bearing "anterior". This is quite different from the oscilatory "S" shaped routine swimming movements used by many fishes (Liao, 2007; Smits, 2019), however, it shares some resemblence to the "C" start behavior that many fishes use as an escape mechanism (Witt, Wen & Lauder, 2015). The unusual motion of the mantle lures may therefore be mimicing an escape behavior to some extent to entice the host to strike.

The respective mantle lure display behaviors of *Lampsilis fasciola* and *L. cardium* differ significantly in important qualitative and quantitative details. Our putative model for River Raisin *L. cardium* mantle lures is a species of pelagic minnow, *Pimephales notatus* (Figure 7) whose swimming behavior and ecology differs markedly from that of darters (Burress et al., 2017). Darters are primarily benthic in habit, have lost or greatly reduced their swim bladder (Demski, Gerald & Popper, 1973; Zeyl et al., 2016), and they swim by "hopping" across the river bed in a manner that is much more erratic and intermittent than the pelagic swimming behavior of most minnows (Winn, 1958). This conforms to a general difference observed between *L. cardium* and *L. fasciola* lures: *L. cardium* moves faster and more regular in a highly

synchronized way in contrast with the erratic, often left-right-unsynchronized movements of *L. fasciola* lures. Qualitatively, there isn't any major differences in lure behavior between the "darter" and "leech" lures of *L. fasciola*, although "darter" lures beat at a slightly slower rate (Fig. 9a). Both *L. fasciola* morphs have a similar erratic motion, despite the polymorphism modeling taxa from disparate phyla. Leeches swim by a dorsoventeral bending wave moving from head to tail, caused by a central pattern generator (CPG) made from interneurons in the ventral nerve cord (Jordan, 1998). This swimming behavior is very different from the lure undulations observed in the leech-like *L. fasciola* lures. Despite small differences in overall lure beat frequency between "darter" and "leech" mimics, it seems as though the polymorphic mimicry is only skin deep. The rhythmic movements of lure undulations are likely caused by a CPG, but we currently have no information about how these motor patterns are activated or modulated (Marder et al., 2005).

The *Lampsilis fasciola* mantle lure polymorphism is complex, each morph containing correlated morphological and pigmentation distinctions, however, the polymorphism does not appear to be linked to any large differences in lure behavior, or in the CPG that controls lure behavior. The regulators of some polymorphic mimetic traits have been found to have relatively simple genetics (Jay et al., 2018; Palmer & Kronforst, 2020). Timmermans et al. (2020) used SNP data from *Papilio dardanus* to discover a genomic inversion associated with a mimetic polymorphism, and this approach is likely also tractable for *Lampsilis fasciola* if a full genome can be sequenced for RADseq loci to be mapped onto. Mantle lures are a remarkable and highly important trait in Lampsiline evolution (Hewitt, Haponski & Foighil, 2021), and *Lampsilis fasciola* presents the best model system to begin discovering the genetics of lure development and variation.

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Table 3.1: Raw reads, total clusters, and total loci in assembly from the ddRAD sequencing are displayed for each genotyped sample of *Lampsilis fasciola* and of the outgroup taxa. Individual *Lampsilis fasciola* lure phenotype designation followed Zanatta et al. (2007).

Sample Name	Lure Phenotype	Raw reads	Total clusters	Average clustering depth	Loci in assembly
L_fasciola_AL_brood_1	Worm-like	258664	97681	2.14	483
L_fasciola_AL_brood_2	Darter-like	5201836	1120710	3.28	25686
L_fasciola_AL_brood_3	Worm-like	5492519	1126749	3.4	25703
L_fasciola_AL_brood_4	Darter-like	2429494	632254	2.84	21398
L_fasciola_AL_brood_5	Worm-like	3152003	760260	3.02	23761
L_fasciola_AL_brood_6	Darter-like	3212851	810898	2.87	23434
L_fasciola_AL_brood_7	Darter-like	3649891	593765	4.22	25363
L_fasciola_AL_brood_8	Darter-like	4869307	1462723	2.29	19089
L_fasciola_AL_brood_9	Worm-like	3158818	718169	3.08	23033
L_fasciola_AL_brood_10	Darter-like	4000321	915881	3.12	24916
L_fasciola_AL_brood_11	Worm-like	5679854	1171842	3.35	25770
L_fasciola_AL_brood_12	Darter-like	4212783	979265	3.04	24693
L_fasciola_AL_brood_13	Worm-like	1300563	399134	2.51	12145
L_fasciola_AL_brood_14	Darter-like	4100372	1043360	2.79	23521
L_fasciola_AL_brood_15	Darter-like	5804293	1412102	2.91	25570
L_fasciola_AL_brood_16	Worm-like	1555906	427061	2.7	14099
L_fasciola_AL_brood_17	Darter-like	2073968	598680	2.59	13668
L_fasciola_AL_brood_18	Worm-like	6919783	1574429	3.08	25811
L_fasciola_AL_brood_19	Darter-like	3434210	829507	2.94	23708
L_fasciola_AL_brood_20	Darter-like	4778853	994416	3.35	25500
L_fasciola_AL_brood_21	Worm-like	2462560	590095	2.91	20588
L_fasciola_AL_brood_22	Worm-like	6600876	1406451	3.26	26080
L_fasciola_AL_brood_23	Darter-like	7090859	1628965	3.06	25932
L_fasciola_AL_brood_24	Worm-like	4546435	1061394	3	24174

L_fasciola_AL_brood_25	Worm-like	5379577	1135906	3.35	25703
L_fasciola_AL_brood_26	Worm-like	5592652	1501130	2.67	23965
L_fasciola_AL_brood_27	Worm-like	4893957	825855	4.09	25924
L_fasciola_AL_brood_28	Darter-like	2596873	519103	3.59	22103
L_fasciola_AL_brood_29	Darter-like	3401334	883485	2.87	21377
L_fasciola_AL_brood_30	Worm-like	3876395	1014133	2.8	22072
L_fasciola_AL_brood_31	Worm-like	5391442	1246528	3.07	25009
L_fasciola_AL_brood_32	Darter-like	4365005	1084596	2.85	23030
L_fasciola_AL_brood_33	Darter-like	5116507	1117916	3.16	24667
L_fasciola_AL_brood_34	Darter-like	7480755	1601100	3.19	26163
L_fasciola_AL_brood_35	Darter-like	8121426	1825135	3.02	25972
L_fasciola_AL_brood_36	Darter-like	5521997	1414238	2.78	24163
L_fasciola_AL_brood_37	Darter-like	6562641	1579514	2.88	25476
L_fasciola_AL_brood_38	Darter-like	6303766	1596624	2.76	24448
L_fasciola_AL_brood_39	Darter-like	6206795	1488925	2.91	24648
L_fasciola_AL_brood_40	Darter-like	8630897	1891164	3.11	26176
L_fasciola_AL_brood_41	Darter-like	7293683	1716571	2.95	25604
L_fasciola_AL_brood_42	Darter-like	4896252	1193262	2.88	22829
L_fasciola_AL_brood_43	Darter-like	6098052	1471714	2.9	25074
L_fasciola_AL_brood_44	Darter-like	7495994	1698871	3.04	25701
L_fasciola_AL_brood_45	Darter-like	3937758	670698	4.06	24947
L_fasciola_AL_brood_46	Darter-like	6370942	1343655	3.26	25855
L_fasciola_AL_brood_47	Darter-like	5542864	1318463	2.96	24550
L_fasciola_AL_brood_48	Darter-like	6313913	1469606	2.98	24983
L_fasciola_AL_brood_49	Darter-like	3163000	789239	2.9	24776
L_fasciola_AL_brood_50	Darter-like	1728370	548529	2.35	17837
L_fasciola_Huron_5	Darter-like	953302	259898	2.8	10996
L_fasciola_Huron_6	Worm-like	1682931	362706	3.31	16809
L_fasciola_Huron_7	Worm-like	746944	157212	3.29	10644

L_fasciola_Huron_8	Worm-like	1899689	402515	3.25	16584
L_fasciola_Huron_9	Darter-like	1213655	293090	2.97	11818
L_fasciola_Huron_10	Darter-like	7775910	1275602	3.87	22035
L_fasciola_Huron_11	Darter-like	1533281	295767	3.55	15386
L_fasciola_NC_1	Darter-like	1308813	254002	3.61	11873
L_fasciola_NC_2	Darter-like	4862573	852380	3.77	18321
L_fasciola_NC_3	Darter-like	663874	165869	2.95	9960
L_fasciola_NC_4	Darter-like	2610453	465228	3.76	13790
L_fasciola_NC_5	Darter-like	6927947	1459334	3.05	20804
L_fasciola_NC_6	Darter-like	1051195	202171	3.27	12415
L_fasciola_NC_7	Darter-like	1948092	382878	3.61	17101
L_fasciola_NC_8	Darter-like	3475751	669278	3.69	20683
L_fasciola_NC_9	Darter-like	5693936	1634946	2.46	22325
L_fasciola_NC_10	Darter-like	2175381	464794	3.38	17094
L_fasciola_NC_11	Darter-like	2189933	516643	3.05	17580
L_fasciola_Redo_1	Darter-like	1455864	327622	2.62	13478
L_fasciola_Redo_2	Darter-like	1839020	436418	2.43	13181
L_fasciola_Raisin_2	Darter-like	8235827	1716137	3.29	25555
L_fasciola_Raisin_3	Darter-like	6032935	1488448	2.85	25006
L_fasciola_Raisin_4	Darter-like	12947164	3587458	2.45	25245
L_fasciola_Raisin_1	Darter-like	6639384	1086218	3.97	23458
L_fasciola_Raisin_5	Darter-like	10059843	1997619	3.41	25363
L_fasciola_Raisin_6	Darter-like	8019689	1847955	3.01	25769
L_fasciola_Raisin_7	Darter-like	3816242	681697	3.95	24606
L_fasciola_Raisin_8	Darter-like	6117037	1282299	3.27	22439
L_fasciola_Raisin_9	Worm-like	5170380	775979	4.64	25798
L_fasciola_Raisin_10	Darter-like	761451	176858	3.14	11477
L_fasciola_Raisin_11	Worm-like	7140657	1670143	2.97	25519
L_fasciola_Raisin_12	Darter-like	890521	203114	2.91	10582

L_fasciola_Raisin_13	Darter-like	1071361	225030	3.47	13512
L_fasciola_Raisin_14	Darter-like	3644379	946273	2.82	21995
L_fasciola_Raisin_15	Darter-like	3578043	482446	5.04	17514
L_fasciola_Raisin_16	Darter-like	2351544	114072	14.25	516
L_fasciola_Raisin_17	Darter-like	5272816	1304726	2.87	23305
L_fasciola_Huron_1	Worm-like	13366692	4050829	2.26	17555
L_fasciola_Huron_2	Darter-like	2819896	928226	2.24	20205
L_fasciola_Huron_3	Darter-like	662275	186602	2.66	7653
L_fasciola_Huron_4	Darter-like	4792093	855457	3.88	24512
L_fasciola_AL_mom_1	Darter-like	8095030	1840917	2.95	25420
L_fasciola_AL_mom_2	Darter-like	10329331	3504027	2.03	24488
L_fasciola_AL_mom_3	Darter-like	10384477	2987559	2.34	25056
L_fasciola_Huron_12	Worm-like	6906349	1672394	2.87	25281
L_fasciola_Huron_13	Worm-like	6955496	1670627	2.88	25593
L_fasciola_Raisin_18	Worm-like	5506215	1301878	3	25373
L_fasciola_Raisin_19	Worm-like	6611596	1524682	3.03	25604
L_fasciola_Raisin_20	Worm-like	4894495	1276608	2.74	24931
L_fasciola_Raisin_21	Worm-like	8396562	1736736	3.26	25490
L_cardium_1		6864226	1710220	2.8	14625
L_cardium_2		4898330	1091622	3.11	13433
L_cardium_3		7109883	2005565	2.5	14563
L_cardium_4		4637077	997208	3.27	13860
S_nasuta_1		4544989	1169260	2.55	10441

Table 3.2: Summary of the gender and lure phenotypes of all 57 University of Michigan Museum of Zoology *Lampsilis fasciola* individuals present in 8 separate mid-20th century collections made from the River Raisin at Sharon Mills County Park (Fig. 2a).

Collection Date	Male darter-like	Male worm-like	Female darter-like	Female worm-like	Total darter-like	Total worm-like	Total
9/30/54	2	1	5	1	7	2	9
5/21/58	0	0	4	0	4	0	4
7/28/59	0	0	2	0	2	0	2
4/24/62	3	0	3	0	6	0	6
5/10/62	5	1	4	1	9	2	11
6/19/62	4	2	1	0	5	2	7
6/25/62	3	1	2	0	5	1	6
7/20/62	5	1	5	1	10	2	12

Table 3.3: The 5 broad categories of lure phenotypes (Groups a-e) observed at the River Raisin Sharon Mills County Park population of *Lampsilis fasciola* (Fig. 2a), as well as this mussel species'estimated geographic range overlap with its 5 Raisin River putative model species.

Type	Proposed Model	Range overlap (km^2)
Group a	*Etheostoma blennioides*	480,731
Group b	*Etheostoma exile*	87,796
Group c	*Percina maculata*	525,772
Group d	*Etheostoma microperca*	164,539
Groud e	*Macrobella decora*	419,259

Table 3.4: Summary data on individual mantle lure display field recordings. Video recordings were taken during the summer of 2018 at Sharon Mills (Fig. 2a) and Hudson Mills (Fig. 2b). Average movement length and interval was calculated from frame number from a 120fps video recording using a Go Pro Hero 6.

File	Average Movement length (L)	Average Movement Length (R)	Average Interval (L)	Average Interval (R)	Interval Standard Deviation (L)	Interval Standard Deviation (R)	Proportion of Movements Synchronized	Lure Phenotype	Time	Temperature °C	Date	Site
GH010073	0.18	0.18	1.50	1.99	1.28	1.65	0.44	“leech”	11:26	16.4	7/10/18	Hudson Mills
GH010074	0.14	0.14	1.72	1.69	1.73	1.73	0.56	“leech”	14:09	18.5	7/9/18	Sharon Mills
GH010599	0.22	0.23	13.17	8.71	20.34	19.34	0.04	“darter”	11:29	18.3	7/10/18	Hudson Mills
GH010601	0.21	0.22	1.02	1.25	0.52	0.33	0.48	“leech”	NA	NA	7/10/18	Hudson Mills
GH010602	0.17	0.17	1.19	1.52	0.62	0.69	0.32	“leech”	13:36	20.6	7/10/18	Hudson Mills
GH010603	0.23	0.21	1.20	1.14	1.22	1.10	0.24	“darter”	13:57	20.5	7/10/18	Hudson Mills
GH010075	0.15	0.15	1.77	1.58	1.79	1.78	0.27	“leech”	2:48	18.2	7/9/18	Sharon Mills
GH010598	0.27	0.27	1.80	1.75	1.19	1.17	0.80	“darter”	11:18	18.2	7/10/18	Hudson Mills
GH010597	0.31	0.30	1.30	1.27	0.57	0.50	0.93	“leech”	11:06	18.1	7/10/18	Hudson Mills
GH010595	0.22	0.22	1.51	1.99	1.17	1.04	0.42	“leech”	10:34	17.6	7/10/18	Hudson Mills
GH010056	0.24	0.24	3.12	3.00	1.89	2.26	0.38	“darter”	12:59	21.3	6/11/18	Sharon Mills
GH010077	0.19	0.22	1.83	1.66	1.91	1.76	0.18	“darter”	11:26	16.4	7/10/18	Hudson Mills
GH010055	0.29	0.29	2.34	2.34	1.32	1.31	0.60	“darter”	11:59	21.8	6/11/18	Sharon Mills
GH010016	0.35	0.37	1.83	1.81	0.50	0.47	0.53	“darter”	NA	NA	6/12/18	Sharon Mills
GH010057	0.30	0.26	9.87	7.40	11.22	8.65	0.30	“darter”	13:16	21.4	6/11/18	Sharon Mills
GH010593	0.15	0.15	0.99	0.88	0.58	0.59	0.39	“leech”	14:09	20.7	7/9/18	Sharon Mills
GH010062	0.14	0.14	2.27	2.77	1.76	1.84	0.24	“darter”	10:21	19.2	7/4/18	Hudson Mills
GH010064	0.17	0.18	1.10	1.10	0.42	0.42	0.98	“leech”	NA	20.8	7/4/18	Hudson Mills
GH010065	0.12	0.13	1.21	1.66	0.92	0.83	0.45	“darter”	14:23	21	7/4/18	Hudson Mills
GH010063	0.14	0.14	1.05	1.05	0.29	0.31	0.82	“darter”	11:13	19.6	7/4/18	Hudson Mills
GH010579	0.12	0.12	0.88	1.29	0.55	0.47	0.26	“leech”	11:02	20	7/4/18	Hudson Mills
GH010580	0.13	0.13	1.56	1.82	1.13	1.43	0.67	“leech”	11:52	21.3	7/4/18	Hudson Mills
GH010581	0.18	0.18	2.17	2.51	5.16	5.67	0.54	“leech”	13:36	23.3	7/4/18	Hudson Mills
GH010582	0.10	0.11	1.94	2.16	2.76	2.79	0.19	“darter”	14:18	23.4	7/4/18	Hudson Mills
GH010583	0.11	0.12	0.99	1.12	0.91	0.82	0.60	“darter”	14:52	NA	7/4/18	Hudson Mills
GH010068	0.12	0.11	2.79	2.73	2.71	2.37	0.41	“darter”	NA	NA	6/12/18	Sharon Mills
GH010048	0.15	0.14	8.05	7.67	5.94	5.60	0.39	“darter”	NA	NA	6/7/18	Sharon Mills
GH010060	0.17	0.17	0.28	0.28	0.01	0.01	1.00	cardium	NA	NA	6/5/20	Sharon Mills
GH010163	0.23	0.23	0.55	0.55	0.12	0.12	1.00	cardium	NA	NA	6/5/20	Sharon Mills
GH010620	0.25	0.25	0.48	0.48	0.05	0.05	1.00	cardium	NA	NA	5/31/21	Sharon Mills
GH010618	0.25	0.25	0.41	0.41	0.10	0.10	1.00	cardium	NA	NA	6/1/21	Sharon Mills

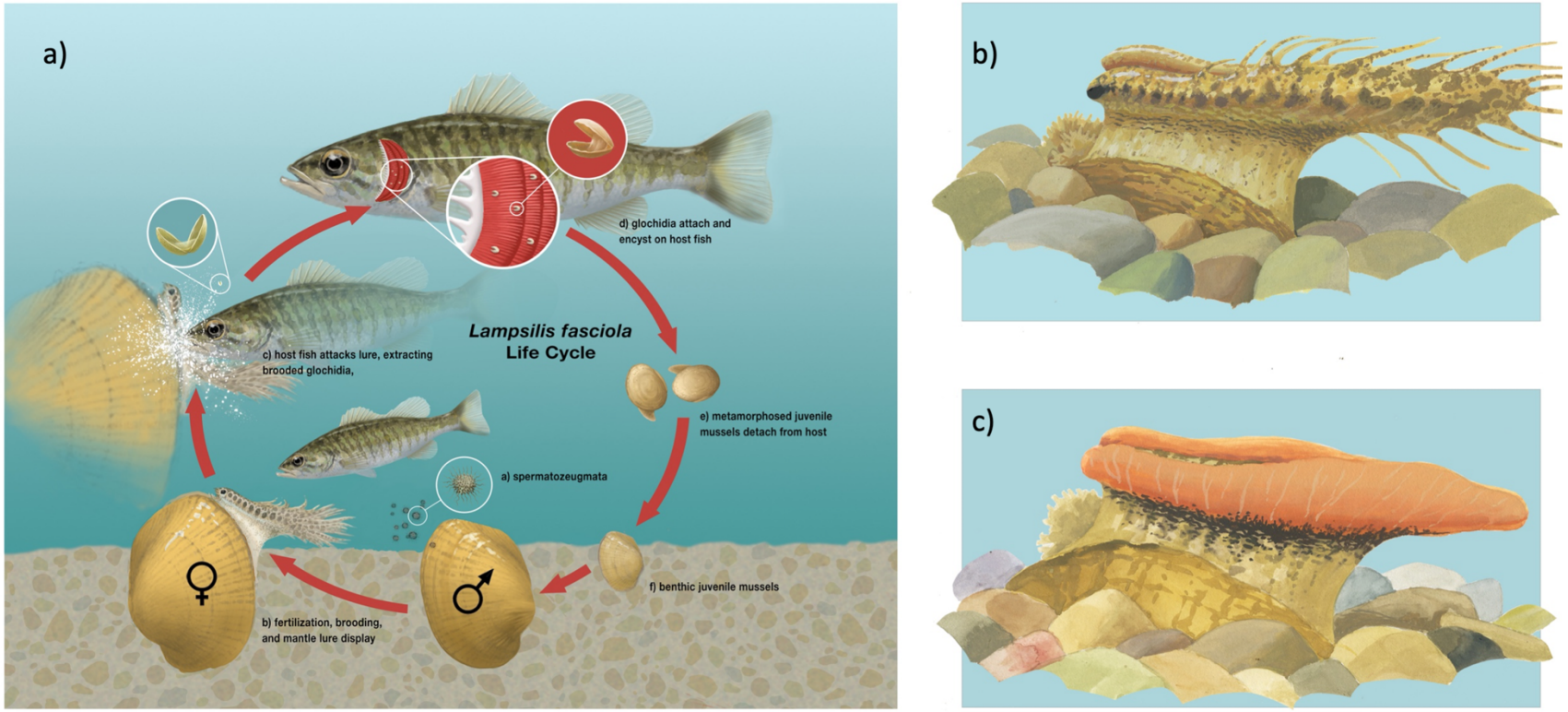


Figure 3.1: a) Summary diagram representing the life cycle of the freshwater mussel *Lampsilis fasciola.* After fertilization, the gravid female mussel displays a mantle lure, here a darter mimic, to the primary fish host, *Micropterus dolomieu*. This elicits an attack through which the host is infected by mussel parasitic larvae (glochidia). After a short infective period (~2 weeks), the parasitic larvae metamorphose into juvenile mussels that detach from the host and fall to the sediment. Panels b ("darter-like") and c ("worm-like") depict the two primary phenotypes of lure observed in *L. fasciola*. The former (b) has "eyespots", a mottled "main body" pigmentation composed of lateral and dorsal spots that can vary substantially in color, numerous and prominent marginal extensions and a distinct "tail" region, whereas the latter lacks those features and has instead a uniform bright orange coloration underlain with a black basal stripe

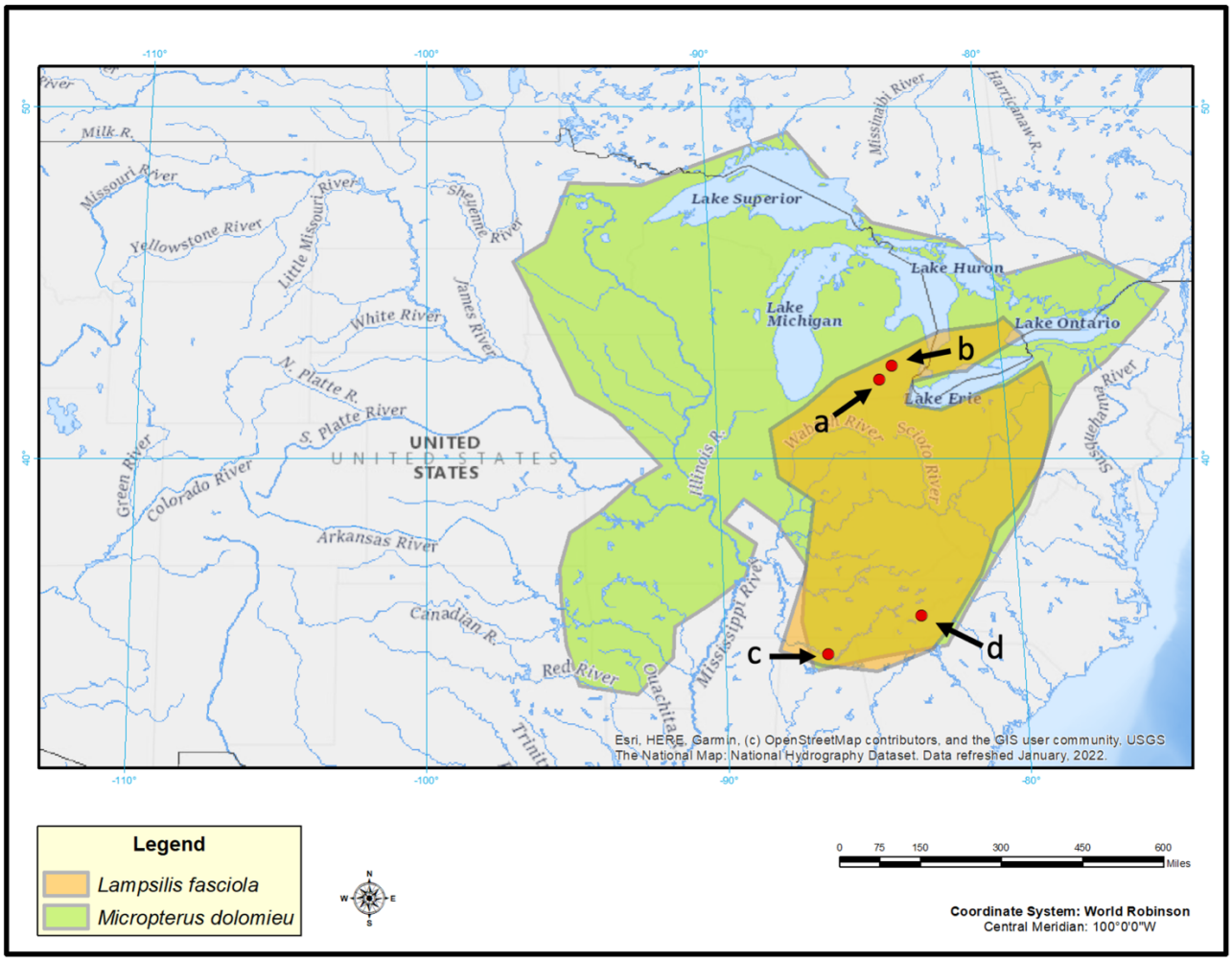


Figure 3.2: Map of Eastern North America showing the sampling sites of *Lampsilis fasciola* and the estimated ranges of this freshwater mussel species and of its primary host fish *Micropterus dolomieu*. A indicates the Raisin River sampling site at Sharon Mills County Park and B the Huron River sampling site at Hudson Mills Park, both in southeastern Michigan. Site C indicates the Paint Rock River sampling site in Alabama, and site D is the Little Tennessee River site in North Carolina.

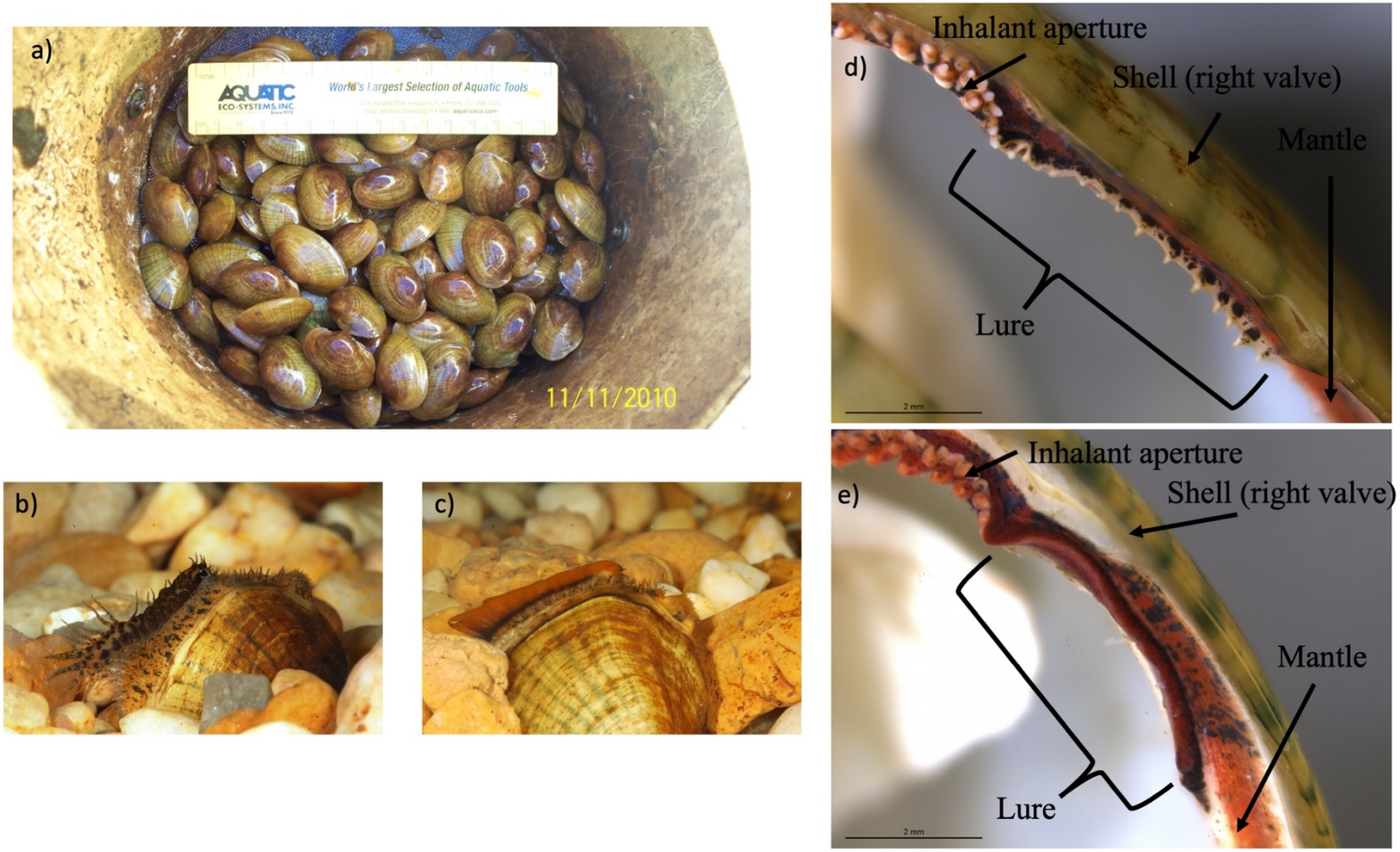


Figure 3.3: The *Lampsilis fasciola* brood raised at the Alabama Aquatic Biodiversity Center from a wild, gravid female, with a "worm-like" mantle lure (Supplemental Figure 1), sampled from the Paint Rock River (Figure 2c) in June 2009. Panel a) shows juvenile members of the brood after ~16 months in culture. Panels b) and c) show single, sexually maturing, females after ~2 years of culture. The young female in b) displayed a developing "darter-like" mantle lure (with "eyespots", mottled lateral coloration, marginal extensions, and a "tail") whereas her full- or half-sibling in c) displayed a "worm-like" mantle lure (lacking the "darter" characteristics and having orange pigmentation with a black underlay). Panels d) and e) respectively show photomicrographs, taken with a dissecting microscope, of 95% ethanol-preserved rudimentary "darter-like" and "worm-like" lures from two additional brood members, part of a 50-individual subsample preserved in 2011.

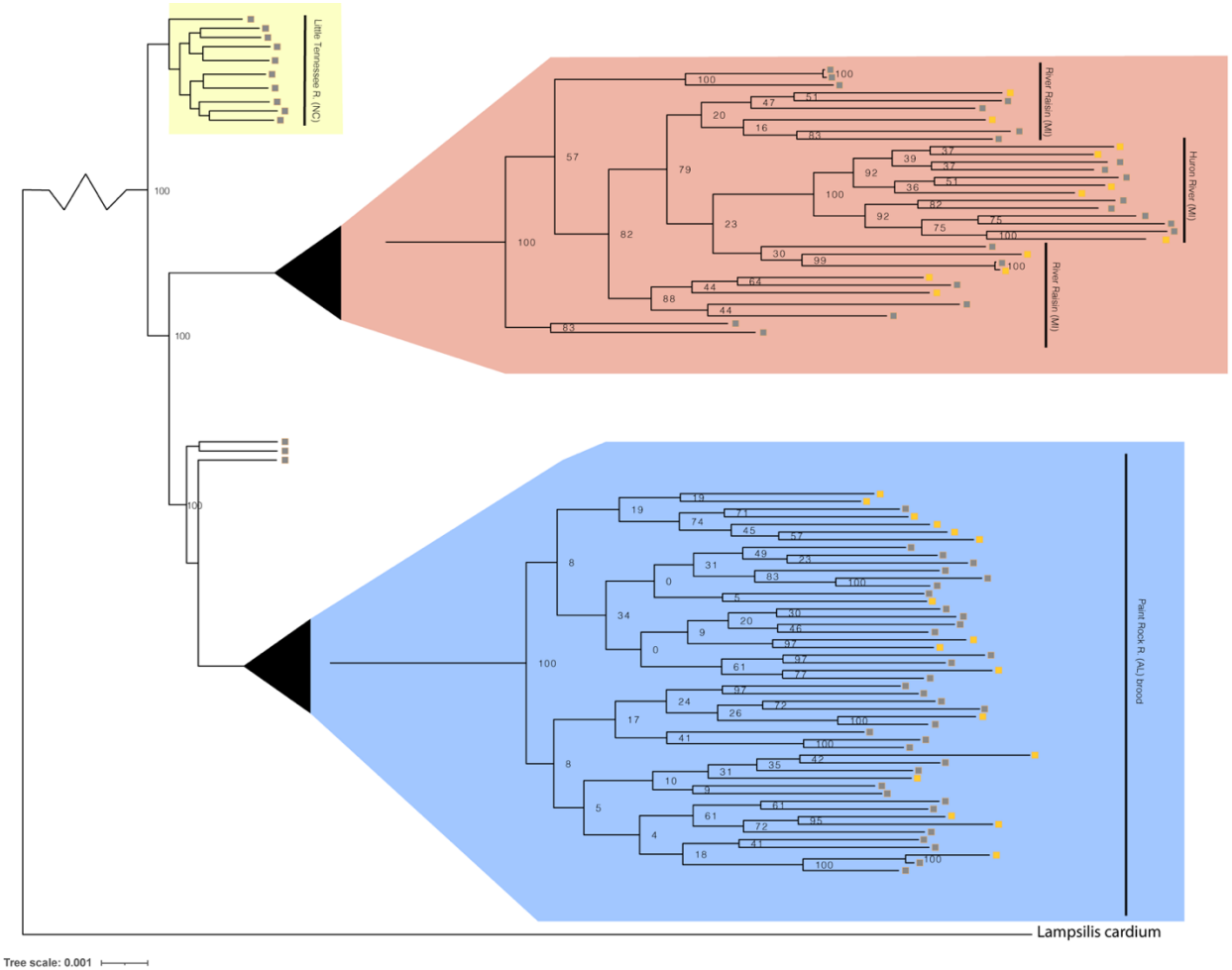


Figure 3.4: Phylogenomic tree of 96 *Lampsilis fasciola* individuals created in RAxML using 28,735 concatenated ddRAD-seq loci. Gravid, lure-displaying females sampled from two Michigan drainages, River Raisin (Fig. 2a) and Huron River (Fig. 2b), are respectively highlighted in peach and pink. Specimens sampled from the Paint Rock River, Alabama (Fig. 2c) are highlighted in blue and consisted of three gravid, lure-displaying females, in addition to 50 larval brood members raised at the Alabama Aquatic Biodiversity Center in the zoomed-in tip clade. Gravid, lure-displaying females sampled from the Little Tennessee River in North Carolina (Fig. 2d) are highlighted in yellow. The respective primary mantle lure phenotypes – "darter-like" or "worm-like" – of all *L. fasciola* individuals is indicated

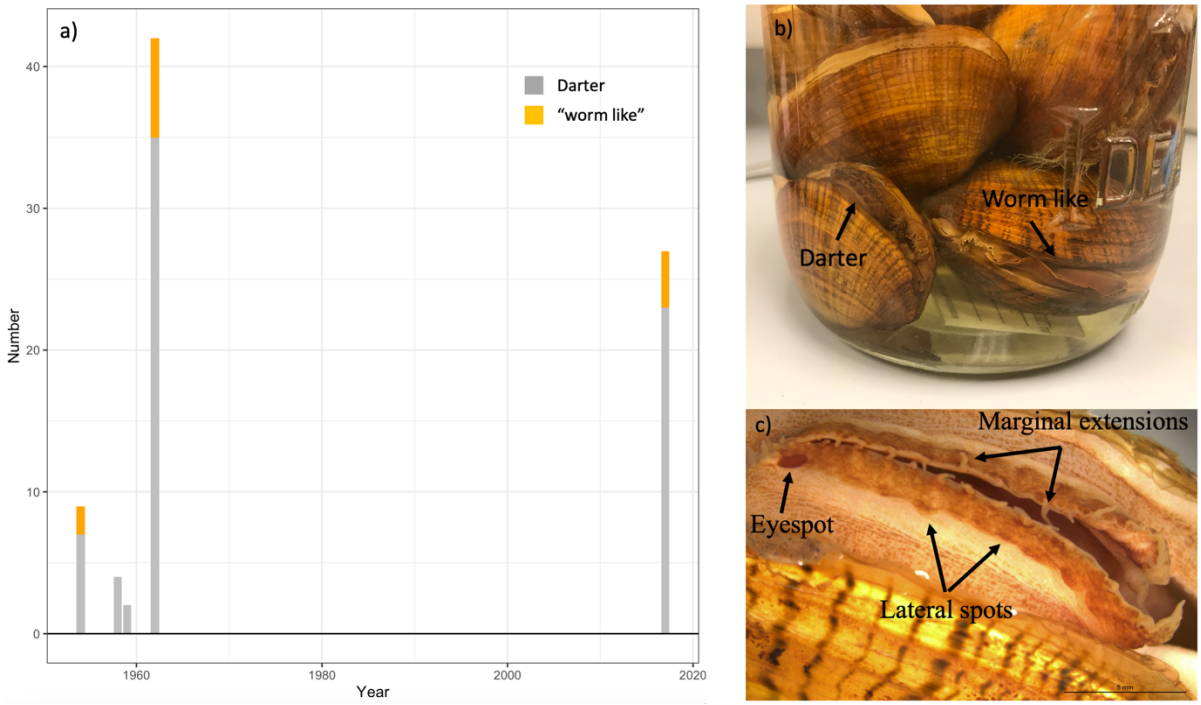


Figure 3.5: a) The observed frequency of River Raisin *Lampsilis fasciola* primary mantle lure phenotypes ("darter-like" vs "worm-like") at the Sharon Mills County Park study site during two different time periods. The 1954-1962 data were obtained from the University of Michigan Museum of Zoology (UMMZ) collection specimens, both female and male. The 2017 data were based on field observations of displaying females. b) a jar of preserved UMMZ Sharon Mills specimens showing a "darter-like" and a "worm-like" mantle lure. c) a "eyespot", lateral pigmented blotches, and marginal extensions.

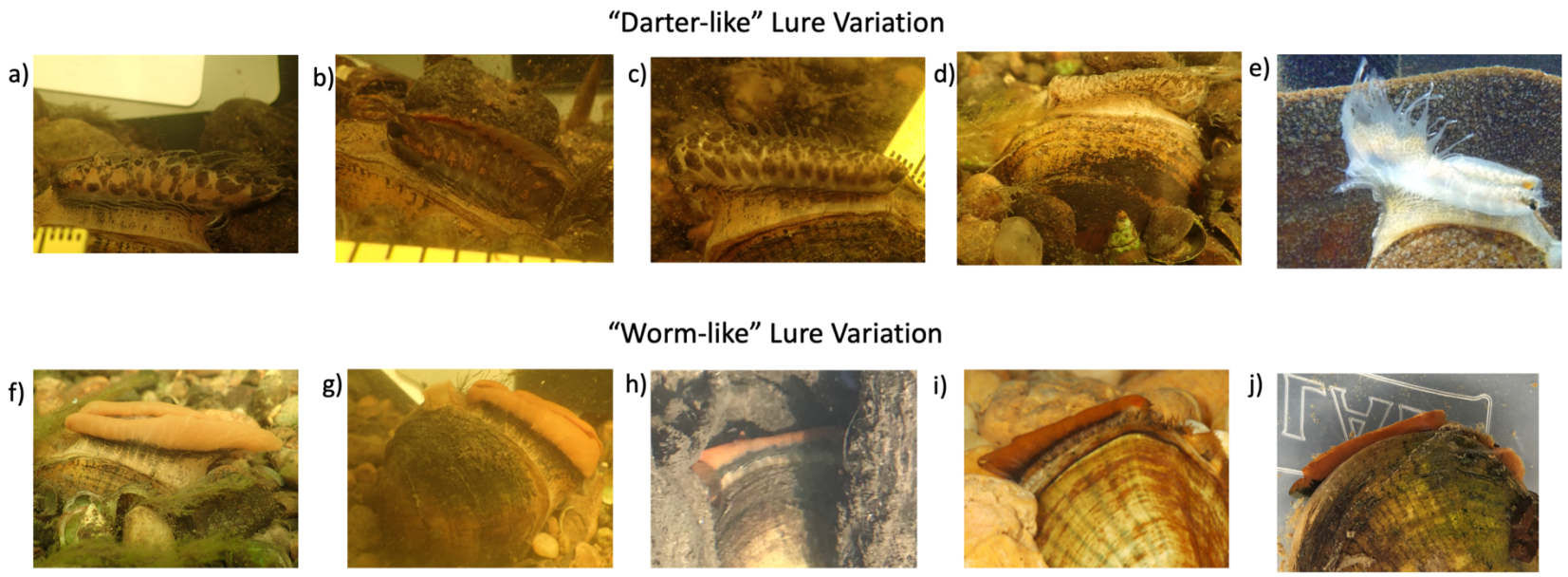


Figure 3.6: This panel displays some of the variability in lure phenotype, both within a population and across the range of *Lampsilis fasciola*. a-d are "darter-like" Raisin River (MI) lures photographed in the field at Sharon Mills County Park. e) depicts a "darter-like" lure displayed by a Paint Rock River (AL) female. f-h show field photographs of "worm-like" lures displayed by three Sharon Mills females, with specimen h being a younger adult. i,j are photographs of two captive AABC specimens, with "worm-like" lures, sourced from the Paint Rock River. The former photo (i), taken in 2011, shows a young (2-year old) female, a member of the captive brood, displaying her lure, and the latter photo (j) is of a female field-sampled in 2022, and showing a partially retracted mantle lure.

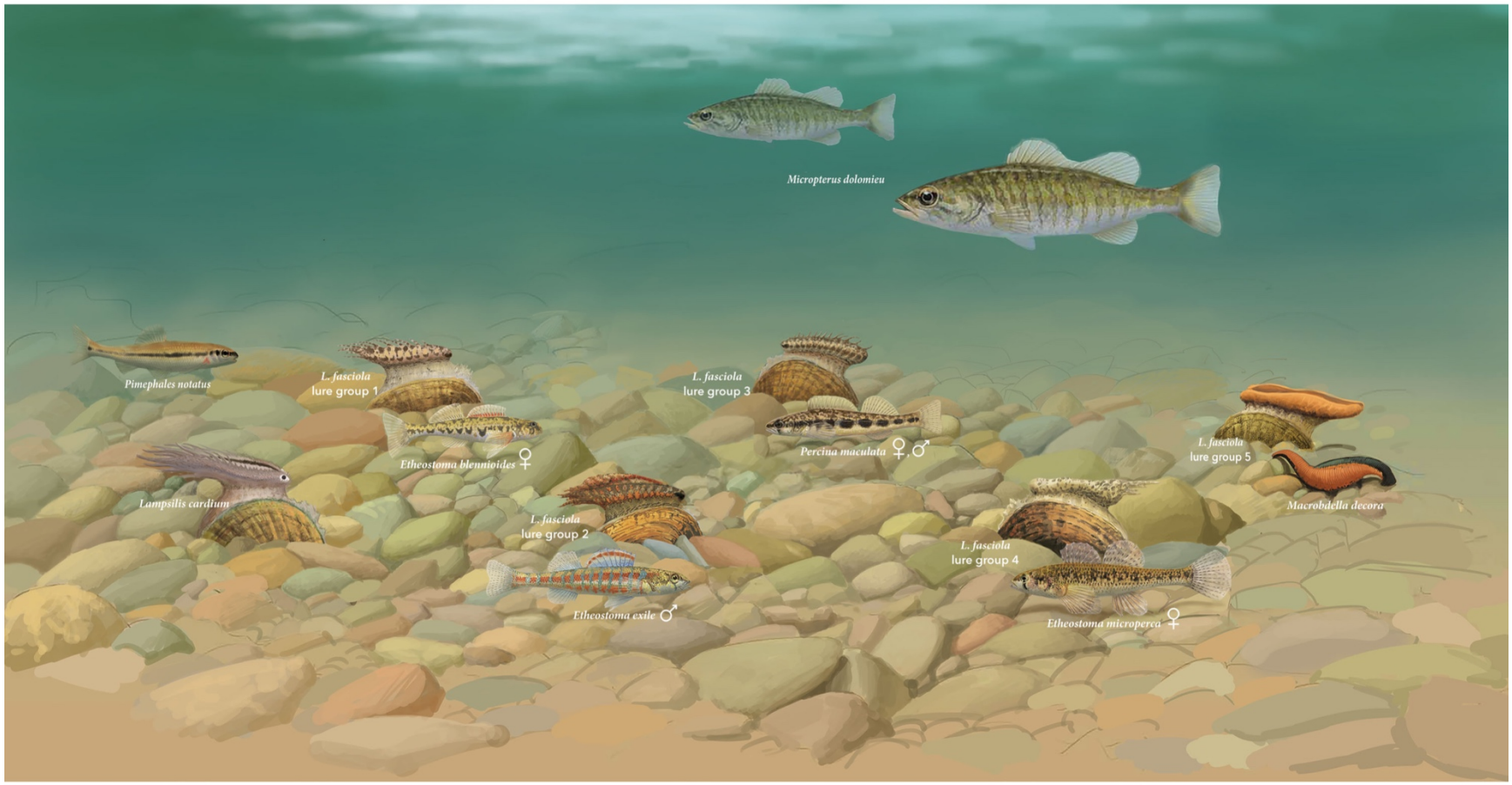


Figure 3.7: A hypothetical Raisin River (Michigan) benthic assemblage showing displaying exemplars of the putative 5 main *Lampsilis fasciola* mimetic mantle lure groups (Figure 6a-d, f) present at the Sharon Mills County Park study site, together with their respective model species, and their primary receiver/fish host, *Micropterus dolomieu*. Also shown is a displaying *Lampsilis cardium* with a "small minnow" mimetic mantle lure (Patterson et al., 2018) and its putative model, *Pimephales notatus*, the most common fish species in the River Raisin (Smith, Taylor & Grimshaw, 1981).

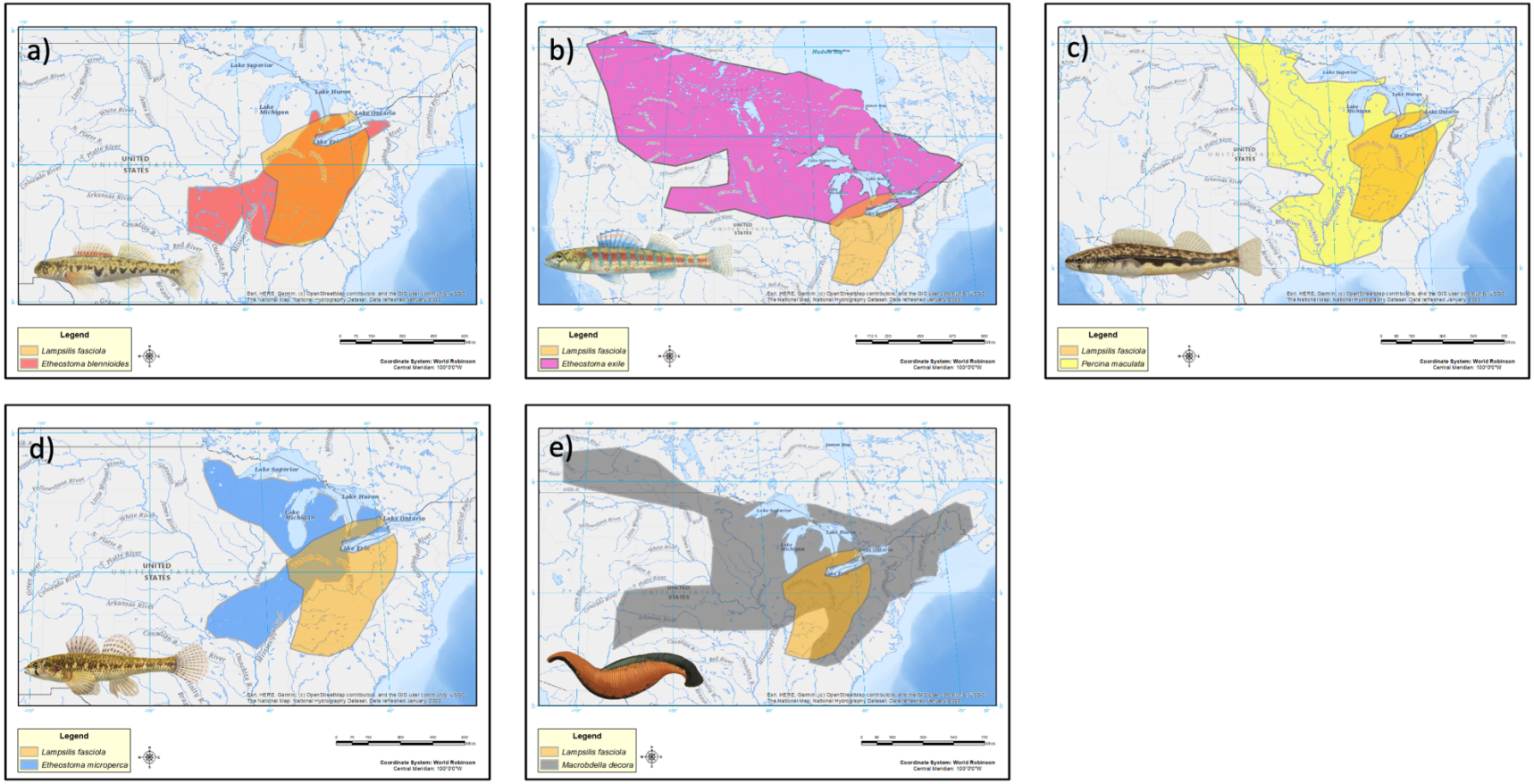


Figure 3.8: Estimated range maps for 5 proposed models for *Lampsilis fasciola* lures. Range maps for each proposed model is compared to the estimated geographic range of *Lampsilis fasciola*. a) *Etheostoma blennoides*, b) *Etheostoma exile*, c) *Percina maculata*, d) *Etheostoma microperca*, and e) *Macrobdella decora.*

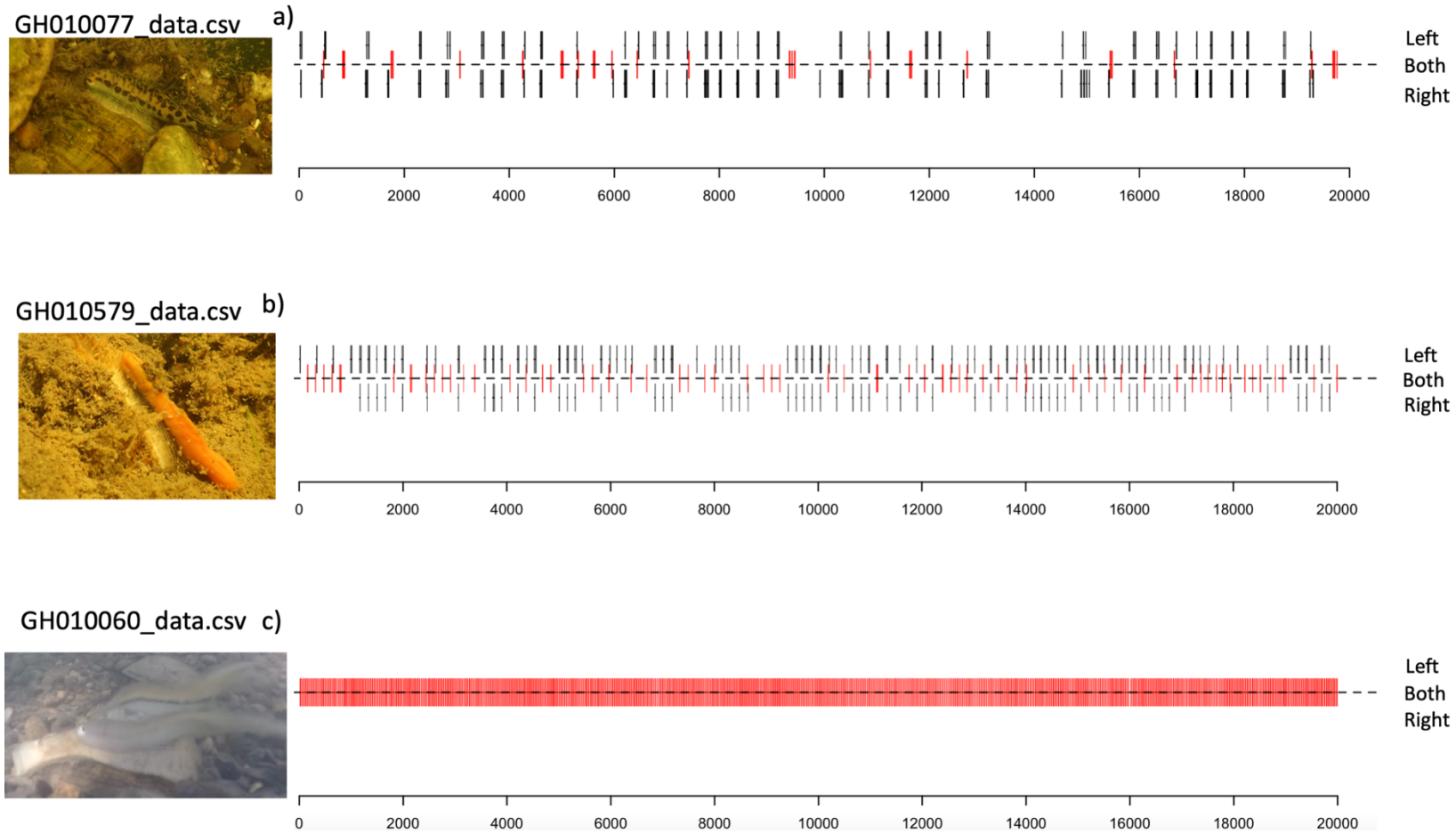


Figure 3.9: Mantle lure gait diagrams for three representative individuals that were field recorded using a Go Pro Hero 6 in 2018. Panel a shows a *Lampsilis fasciola* "darter" lure sample, Panel b displays a *Lampsilis fasciola* "leech" lure sample, and Panel c shows a *Lampsilis cardium* sample. Red center lines indicate synchronized lure movement for both left and right mantle flaps, and black lines above and below the center line indicate independent left and right movements respectively. The X axis denotes frame number (120fps).

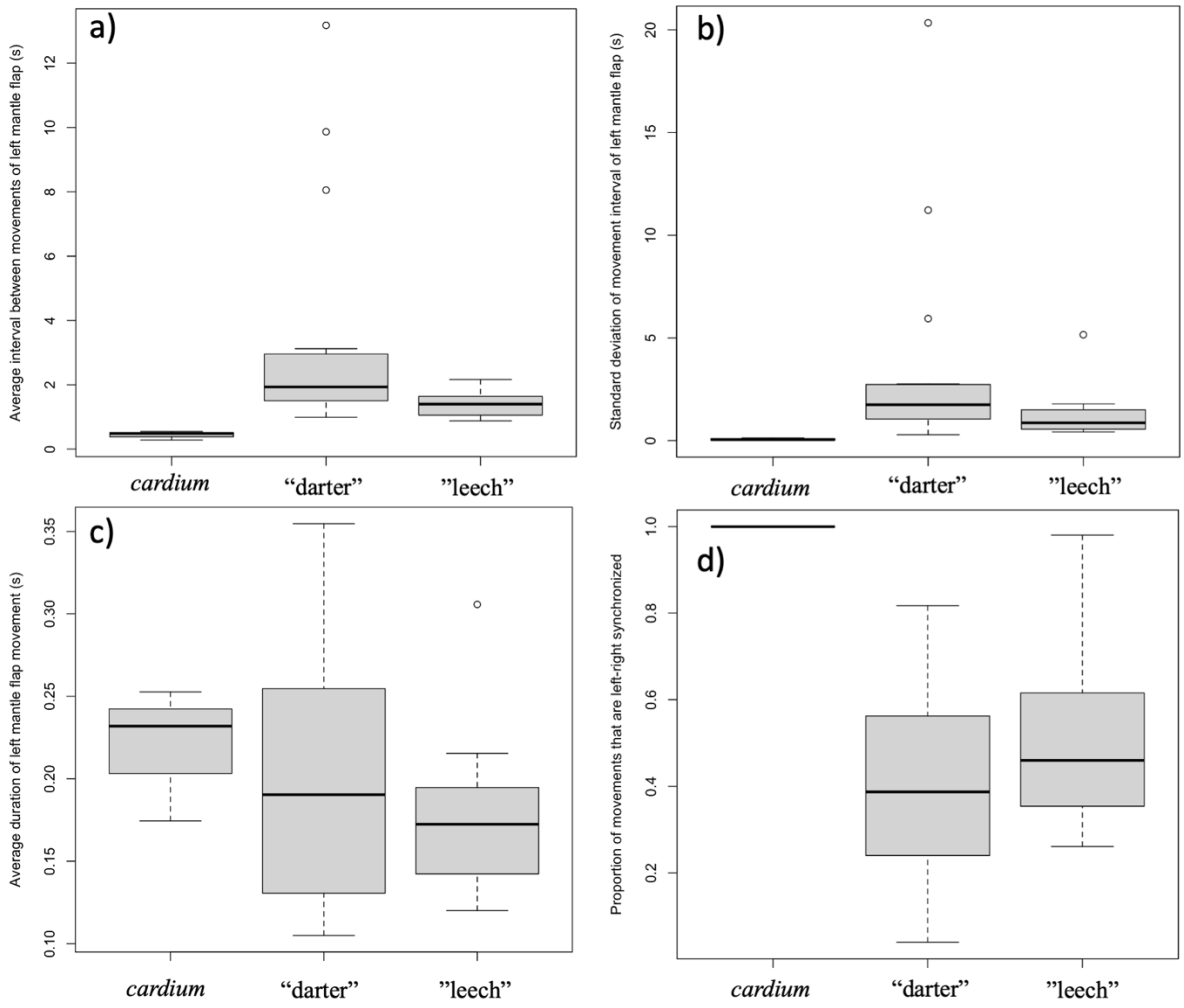


Figure 3.10: Summary boxplots from behavioral analyses of the two primary *Lampsilis fasciola* mantle lure phenotypes (“darter” vs. “leech”) and of *Lampsilis cardium*. Panel a) comparison of the mean interval between lure movements. Panel b) the mean standard deviation in lure movement interval as a proxy of variability. Panel c) the mean length of lure movement. Panel d) the proportion of movements that are left-right synchronized. Panels a-c show means for left mantle flap movements only.

CONCLUSION

The overall goal of my thesis was to test the hypothesis that the unique larval ontogeny and ecology of unionid freshwater mussels has contributed to the extraordinary diversification of this group relative to other freshwater bivalve lineages. This question was addressed at three different levels: macro-, meso-, and microevolutionary scales. New insights were obtained at all three levels that are in-part consistent with the hypothesis, but much more ecological and genomic data are required to fully address this question, and a variety of new lines of research are identified.

My first chapter focused on macroevolutionary and ecological aspects of the North American unionid fauna using a combined analysis of known mussel-fish host interactions (based on lab infections and field observations) and available mussel and fish phylogenies. I found that despite the very brief duration of their parasitic phase, mussels exhibited the right-skewed distribution of host specialization that is typical of parasitic taxa, and that closely related mussel species are more likely to share host fishes. Competition for fish hosts is therefore likely to be a major influence on unionid evolution and diversification. In addition to ecological factors that explain shared host use, I found that number of citations was a major factor, which has two implications: species of mussel that are less well studied are likely have underestimated host-parasite interactions, and some highly studied species likely overestimate the number of hosts that are important in an ecological context. Going forward, we clearly need better, and much more extensive, high-resolution data on mussel-host interactions to build useful models of this complex and dynamic evolutionary system. That will require in-depth analyses of exemplar

natural populations, incorporating field observations and sampling, as well as complementary lab infection experiments.

The second chapter of my dissertation used a phylogenomic approach to reconstruct the evolution of host infection strategies and estimate evolutionary rates in the lampsilines, a highly imperiled and diverse clade of North American freshwater mussels. I found evidence for the early evolution of mantle lures, and multiple independent evolution of brood lures: one complex mimetic brood lure in the genus *Ptychobranchus* and a simple, non-mimetic brood lure in the core *Lampsilis* group. The functionality of simple brood lures is not known, but I hypothesized a bet-hedging strategy in this core *Lampsilis* clade where the simple brood lures are released after the primary mantle lure strategy fails to attract a host. I then argued that lampsiline mussels represents a cryptic adaptive radiation, where ecological diversification occurs primarily during the brief parasitic larval stage, and the morphological divergence criteria for adaptive radiations is primarily observed in gravid females in the form of diverse host infection mechanisms. That hypothesis is fully consistent with larval development and ecology being a driver of diversification in the group. The core *Lampsilis* clade, characterized by both mantle lures and a simple brood lure is associated with slight evidence of a rate shift, but a much broader phylogeny, ideally including the remaining ~240 species of North American unionids, is needed to comprehensively address the role of host infection strategies in their diversification.

My final chapter was an integrative study of the evolution, behavior, and ecology of a critical host-infection trait in *Lampsilis fasciola,* its mantle lure. This species was chosen because it shows exceptional variability in its mantle lure phenotypes. I was able to confirm that this variation represents a true polymorphism using phylogenomic data as well as documenting within-brood polymorphisms in a captive female, the first such record in a unionid. The two

main mantle lure phenotypes differ greatly in morphology and coloration, and my goals were to identify model species for the respective lure phenotypes, and to establish if the lure polymorphism also includes a behavioral component. In the Raisin River study site, I identified four co-occurring darter species as putative models for the one of the primary lure phenotypes, and the leech *Macrobdella decora* as the putative model for other. The leech lure phenotype is largely monomorphic across its range, which is a strong contrast to the high variability of the darter lure phenotype. Field recordings of lure display behaviors show that both the *L. fasciola* lure phenotypes differed significantly that from a co-occurring congener, but not from each other, and that the lure display polymorphism does not display a significant behavioral component. The discrete nature of *Lampsilis fasciola* mantle lure polymorphism within-brood inheritance suggests a relatively simple genetic mechanism for regulating lure phenotype ontogeny in this species. Discovering a gene or transcription factor associated with this polymorphism is the first step in understanding the genetic basis of mantle lure development in Unionoida. Any candidate gene associated with the lure polymorphism in *L. fasciola* can then be further investigated from an evolutionary context in other mantle lure producing mussels.

Each chapter of my dissertation analyzes the role of larval ontogeny on freshwater mussel biodiversity from a different perspective. In summary, this research has obtained considerable supporting evidence for the importance of the larval life-history phase in the diversification of freshwater mussels, although it has also highlighted the need for more data at every level of investigation. The insights obtained with this work emphasize where future investigation of freshwater mussel evolution may be most promising. Elucidating the factors influencing the disparity in freshwater bivalve biodiversity may provide insight into the evolutionary dynamics of host-parasite systems in general. However, the complexity of the unionid-fish relationship

limits the utility of a macroevolutionary approach until better data is obtained. Creating a comprehensive and robust phylogeny for all North American unionid mussels is a highly tractable area for future research and will complement unionid studies on multiple levels of investigation. One of the most promising areas of future research involves identifying the regulatory genes underlying key adaptations enabling the infection of fish hosts *e.g.,* mantle lure development in *L. fasciola.* Ideally, this approach can be further applied to other infection strategies including brood lures.

Appendix A:
Supporting Information for

CHAPTER 1

Ecological Correlates and Phylogenetic Signal of Host Use in North American Unionid Mussels

Supplementary Table S1.1: List of Mussel taxa included in this study, as well as the host infection strategies that were assigned to each species.

Mussel	Strategy	Infection strategy
Actinonaias ligamentina	Passive	Broadcast
Lampsilis siliquoidea	Active	Active Mantel Flap
Lampsilis teres	Active	Active Mantel Flap
Ligumia recta	Active	Active Mantel Flap
Villosa iris	Active	Active Mantel Flap
Actinonaias pectorosa	Passive	Broadcast
Lampsilis ornata	Active	Active Mantel Flap
Lampsilis cardium	Active	Active Mantel Flap
Hamiota altilis	Active	Active Mantel Flap
Hamiota perovalis	Active	Active Mantel Flap
Hamiota subangulata	Active	Active Mantel Flap
Epioblasma brevidens	Active	Active Mantel Trap
Epioblasma capsaeformis	Active	Active Mantel Trap
Epioblasma florentina	Active	Active Mantel Trap
Epioblasma triquetra	Active	Active Mantel Trap
Obovaria unicolor	Active	Active Mantel Flap
Obovaria olivaria	Active	Active Mantel Flap
Villosa villosa	Active	Active Mantel Flap
Ellipsaria lineolata	Active	Active Valve Gaping
Truncilla truncata	Active	Active Valve Gaping
Cyprogenia aberti	Active	Elaborate Conglutinate
Cyprogenia stegaria	Active	Elaborate Conglutinate
Dromus dromas	Active	Elaborate Conglutinate
Lemiox rimosus	Active	Active Mantel Flap
Medionidus conradicus	Active	Active Mantel Flap
Ptychobranchus fasciolaris	Active	Elaborate Conglutinate
Venustaconcha ellipsiformis	Active	Active Mantel Flap
Leptodea fragilis	Active	Female Sacrifice
Leptodea leptodon	Active	Female Sacrifice
Potamilus alatus	Active	Female Sacrifice
Potamilus purpuratus	Active	Female Sacrifice
Glebula rotundata	Passive	Broadcast
Obliquaria reflexa	Active	Conglutinate
Toxolasma parvus	Active	Worm Like Caruncles
Toxolasma texasiensis	Active	Worm Like Caruncles
Amblema plicata	Passive	Broadcast

Fusconaia ebena	Active	Conglutinate
Elliptio arca	Passive	Mucous
Elliptio crassidens	Passive	Mucous
Elliptio dilatata	Passive	Mucous
Pleurobema collina	Active	Conglutinate
Fusconaia cerina	Active	Conglutinate
Fusconaia flava	Active	Conglutinate
Quincuncina burkei	Passive	Broadcast
Fusconaia cor	Active	Conglutinate
Fusconaia cuneolus	Active	Conglutinate
Pleurobema decisum	Active	Conglutinate
Pleurobema clava	Active	Conglutinate
Pleurobema oviforme	Active	Conglutinate
Pleurobema rubellum	Active	Conglutinate
Pleurobema strodeanum	Active	Conglutinate
Pleurobema georgianum	Active	Conglutinate
Pleurobema pyriforme	Active	Conglutinate
Pleurobema cordatum	Active	Conglutinate
Pleurobema rubrum	Active	Conglutinate
Pleurobema sintoxia	Active	Conglutinate
Elliptoideus sloatianus	Passive	Broadcast
Cyclonaias tuberculata	Active	Mantel Magazines
Quadrula quadrula	Active	Mantel Magazines
Quadrula nobilis	Active	Mantel Magazines
Tritogonia verrucosa	Passive	Broadcast
Quadrula metanevra	Active	Mantel Magazines
Megalonaias nervosa	Passive	Mucous
Lasmigona holstonia	Passive	Mucous
Lasmigona costata	Passive	Mucous
Strophitus subvexus	Passive	Mucous
Pyganodon grandis	Passive	Mucous
Cumberlandia monodonta	Passive	Broadcast
Margaritifera margaritifera	Passive	Broadcast

Appendix B:
Supporting Information for

CHAPTER 2

Evolution of Diverse Host Fish Infection Mechanisms Delineates an Adaptive Radiation of Lampsiline Freshwater Mussels Centered on their Larval Ecology

Supplementary Table S2.1: Summary of samples used in our analyses including; where samples were obtained, host infection mechanism used, primary host fish, and sources cited for determining host use and host infection mechanisms. NCS = North Carolina State University, UF = University of Florida, INHS = Illinois Natural History Survey, and AABC = Alabama Aquatic Biodiversity Center.

Sample name	Species name	Infection Strategy	infection citations	Tissue Source	Museum ID	Host	host citation
Aplic	Amblema plicata	Broadcast (larval threads)	Haag, 2012	T. Hewitt (River Raisin, MI).	306255	Generalist	Haag, 2012
TH_32	Cambarunio taeniata	Mantle lure and simple brood lure	Barnhart et al., 2008; Haag, 2012	NCS	29180	Bass	Gordon et al., 1994
TH131	Cyrtonaias tampicoensis	Broadcast	Zanatta and Murphy, 2007	UF	438173	Gar	Howells, 1997
TH132	Cyrtonaias tampicoensis	Broadcast	Zanatta and Murphy, 2007	UF	438173	Gar	Howells, 1997
TLH59	Epioblasma triquetra	Host Trapping	Barnhart et al., 2008	INHS	36609	Darter/Sculpin	Barnhart et al., 2008; Haag, 2012
Edila2	Eurynia dilatata	Broadcast (larval threads)	Zanatta and Murphy, 2007; Barnhart et al., 2008	T. Hewitt (River Raisin, MI).	306256	Generalist	Ford and Oliver, 2015
Edila1	Eurynia dilatata	Broadcast (larval threads)	Zanatta and Murphy, 2007; Barnhart et al., 2008	T. Hewitt (River Raisin, MI).	306256	Generalist	Ford and Oliver, 2015
TH125	Glebula rotundata	Broadcast	Zanatta and Murphy, 2007	UF	440636	Sunfish	Parker et al., 1984
TH126	Glebula rotundata	Broadcast	Zanatta and Murphy, 2007	UF	440636	Sunfish	Parker et al., 1984
TH_36	Hamiota altilis	Mantle Lure and tethered brood lure	Barnhart et al., 2008	From Paul Johnson (AABC)	306257	Bass/Sunfish	Haag and Warren, 1999
TH_7	Hamiota australus	Tethered brood lure	Barnhart et al., 2008	UF	441239	Bass	Haag, 2012
TH_37	Hamiota perovalis	Tethered brood lure	Barnhart et al., 2008	From Paul Johnson (AABC)	306258	Bass	Haag and Warren, 1997; Haag, 2012
TH152	Hamiota subangulata	Tethered brood lure	Barnhart et al., 2008	UF	438064	Bass	O'Brien and Box, 1999; Haag, 2012
TLH9	Lampsilis bracteata	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	439084	Bass/Sunfish	Haag, 2012
LCFL68	Lampsilis cardium	Mantle lure and simple brood lure	Barnhart et al., 2008	J. Bergner (Flatt River, MI).	NA	Bass	Haag, 2012
LCEE70	Lampsilis cardium	Mantle lure and simple brood lure	Barnhart et al., 2008	T. Hewitt (Eel River, IN).	306259	Bass	Haag, 2012
LCEE71	Lampsilis cardium	Mantle lure and simple brood lure	Barnhart et al., 2008	T. Hewitt (Eel River, IN).	306259	Bass	Haag, 2012
LCFL69	Lampsilis cardium	Mantle lure and simple brood lure	Barnhart et al., 2008	J. Bergner (Flatt River, MI).	NA	Bass	Haag, 2012
LFre2	Lampsilis fasciola	Mantle lure and simple brood lure	Barnhart et al., 2008	T. Hewitt (Huron River, MI).	306260	Bass	Mummert et al., 2003; Haag, 2012
TH_2	Lampsilis fasciola	Mantle lure and simple brood lure	Barnhart et al., 2008	T. Hewitt (River Raisin, MI).	NA	Bass	Mummert et al., 2003; Haag, 2012
TH_77	Lampsilis fasciola	Mantle lure and simple brood lure	Barnhart et al., 2008	T. Hewitt (Huron River, MI).	306260	Bass	Mummert et al., 2003; Haag, 2012
LFHM04	Lampsilis fasciola	Mantle lure and simple brood lure	Barnhart et al., 2008	T. Hewitt (Huron River, MI).	306260	Bass	Mummert et al., 2003; Haag, 2012
LFHM07	Lampsilis fasciola	Mantle lure and simple brood lure	Barnhart et al., 2008	T. Hewitt (Huron River, MI).	306260	Bass	Mummert et al., 2003; Haag, 2012
TLH4	Lampsilis floridensis	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	340525	Bass/Gar	Keller and Ruessler, 1997; Haag, 2012
TH_45	Lampsilis higginsi	Mantle lure and simple brood lure	Barnhart et al., 2008	INHS	49425	Bass/Walleye	Waller and Holland, 1988; Haag, 2012
TH_87	Lampsilis hydiana	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	440994	Bass	Draxler et al., 2006; Haag, 2012
TH_88	Lampsilis hydiana	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	440994	Bass	Draxler et al., 2006; Haag, 2012
TH_5	Lampsilis ornata	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	438031	Bass	Haag and Warren, 2003; Haag, 2012
TH_96	Lampsilis ovata	Mantle lure and simple brood lure	Barnhart et al., 2008; Haag and Warren, 2003	UF	438255	Bass	Williams et al., 2008; Haag, 2012
TH_94	Lampsilis ovata	Mantle lure and simple brood lure	Barnhart et al., 2008; Haag and Warren, 2003	UF	438255	Bass	Williams et al., 2008; Haag, 2012
TH_95	Lampsilis ovata	Mantle lure and simple brood lure	Barnhart et al., 2008; Haag and Warren, 2003	UF	438255	Bass	Williams et al., 2008; Haag, 2012
TH117	Lampsilis radiata	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	439013	Bass and perch	Eads et al., 2015
TH_8	Lampsilis satura	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	441167	Bass	Haag, 2012
TLH51	Lampsilis siliquoidea	Mantle lure and simple brood lure	Barnhart et al., 2008	INHS	25963	Bass	Keller and Ruessler, 1997
TH_23	Lampsilis splendida	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	438354	Bass	Haag, 2012
TLH6	Lampsilis straminea	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	383152	Bass	Keller and Ruessler, 1997; Haag, 2012
TH_38	Lampsilis virescens	Mantle lure and simple brood lure	Barnhart et al., 2008	Paul Johnson (AABC)	306261	Bass	Williams et al., 2008; Haag, 2012
TH_22	Leaunio umbrans	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	438189	Sunfish/Sculpin	Williams et al., 2008
TLH26	Leaunio vanuxemensis	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	438796	Sculpin	Haag, 2012
TH_34	Lemiox rimosus	Mantle lure	Zanatta and Murphy, 2007	NCS	47243	Darter/Sculpin	Haag, 2012
TH_57	Leptodea fragilis	Mantle lure	Sietman et al., 2018	INHS	79830	Drum	Haag, 2012
TH133	Leptodea ochracea	broadcast	Haag et al., 2012	UF	438459	white perch	Wick and Huryn, 2003
TH_91	Ligumia recta	Mantle lure and simple brood lure	Barnhart et al., 2008; Corey et al., 2006	UF	438249	Walleye	Haag, 2012
TH_39	Medionidus acutissimus	Mantle lure	Haag and Warren, 2003	Paul Johnson (AABC)	306262	Darter/Sculpin	Haag and Warren, 1997; Haag and Warren, 2003; Haag, 2012
TH_40	Medionidus conradicus	Mantle lure	Zanatta and Murphy, 2007	UF	438914	Darter/Sculpin	Zale and Neves, 1982; Haag, 2012
TH_41	Medionidus parvulus	Mantle lure	Haag, 2012	Paul Johnson (AABC)	306263	Darter/Sculpin	Haag, 2012
TLH28	Medionidus parvulus	Mantle lure	Haag, 2012	Paul Johnson (AABC)	306263	Darter/Sculpin	Haag, 2012
TH_16	Medionidus penicillatus	Mantle lure	Haag, 2012	Paul Johnson (AABC)	306264	Darter/Sculpin	O'brien and Williams, 2002; Haag, 2012

TLH20	Medionidus simposonianus	Mantle lure	Haag, 2012	Paul Johnson (AABC)	306265	Darter/Sculpin	Haag, 2012
TH_17	Medionidus walkeri	Mantle lure	Haag, 2012	Paul Johnson (AABC)	306266	Darter/Sculpin	Johnson et al., 2016; Haag, 2012
TLH18	Obovaria choctawensis	Mantle lure	Haag, 2012	UF	441237	Darter/Sculpin	Haag, 2012
TH146	Obovaria subrotunda	Mantle lure	Haag, 2012	UF	438391	Darter/Sculpin	Haag, 2012
TH145	Obovaria subrotunda	Mantle lure	Haag, 2012	UF	438391	Darter/Sculpin	Haag, 2012
TH144	Potamilus ohiensis	broadcast	Zanatta and Murphy, 2007; Barnhart et al., 2008	UF	438806	Drum	Haag, 2012
TH143	Potamilus ohiensis	broadcast	Zanatta and Murphy, 2007	UF	438806	Drum	Haag, 2012
TH142	Ptychobranchus fasciolarus	complex brood lure	Barnhart et al., 2008	UF	438254	Darter/Sculpin	Haag, 2012
TH141	Ptychobranchus fasciolarus	complex brood lure	Barnhart et al., 2008	UF	438254	Darter/Sculpin	Haag, 2012
TLH42	Ptychobranchus foremanianus	complex brood lure	Barnhart et al., 2008	Paul Johnson (AABC)	306267	Darter/Sculpin	Haag, 2012
TH153	Ptychobranchus jonesi	complex brood lure	Barnhart et al., 2008	UF	441272	Darter/Sculpin	Haag, 2012
TH_89	Quadrula quadrula	Mantle lure	Barnhart et al., 2008	UF	438787	Catfish	Haag, 2012
TLH21	Sagittunio nasuta	Mantle lure and simple brood lure	Barnhart et al., 2008; Haag, 2012	UF	438285	Sunfish and Perch	Eads et al., 2015
TH147	Sagittunio subrostrata	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	441304	Sunfish	Stern and Felder, 1978
TH148	Sagittunio subrostrata	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	441304	Sunfish	Stern and Felder, 1978
TH_19	Toxolasma corvunculus	Manlte lure	Barnhart et al., 2008	UF	440843	Sunfish	Haag, 2012
TLH44	Toxolasma cylindrellus	Manlte lure	Barnhart et al., 2008; Haag et al. 2012	INHS	49319	Sunfish	Haag, 2012
TH130	Toxolasma lividum	Manlte lure	Roe, 2002; Barnhart et al., 2008	UF	438185	Sunfish	Haag, 2012
TH129	Toxolasma lividum	Manlte lure	Roe, 2002; Barnhart et al., 2008	UF	438185	Sunfish	Haag, 2012
TH128	Toxolasma texasiensis	Manlte lure	Zanatta and Murphy, 2007; Barnhart et al., 2008	UF	438567	Sunfish	Haag, 2012
TH127	Toxolasma texasiensis	Manlte lure	Zanatta and Murphy, 2007; Barnhart et al., 2008	UF	438567	Sunfish	Haag, 2012
TH119	Truncilla macrodon	broadcast	Haag et al., 2012	UF	441301	Drum	Haag, 2012
TH120	Truncilla macrodon	broadcast	Haag et al., 2012	UF	441301	Drum	Haag, 2012
TH122	Truncilla truncata	Manlte lure	Sietman et al., 2018	UF	438976	Drum	Haag, 2012
TH121	Truncilla truncata	Manlte lure	Sietman et al., 2018	UF	438976	Drum	Haag, 2012
TH_63	Venustaconcha ellipsiformis	Mantle lure and simple brood lure	Hove and Anderson, 1997; Barnhart et al., 2008	INHS	87179	Darter/Sculpin	Haag, 2012
TLH25	Venustaconcha trabalis	Mantle lure and simple brood lure	Barnhart et al., 2008	Paul Johnson (AABC)	NA	Darter/Sculpin	Haag, 2012
TH_43	Venustaconcha trabalis	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	438909	Darter/Sculpin	Haag, 2012
TH124	Venustaconcha trabalis	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	438909	Darter/Sculpin	Haag, 2012
TH123	Venustaconcha trabalis	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	438909	Darter/Sculpin	Haag, 2012
TH_11	Villosa amygdala	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	441054	Presumed Bass	
TLH12	Villosa delumbis	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	437984	Bass	Johnson et al., 2002
TH_24	Villosa vibex	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	438545	Sunfish	Haag et al., 1997
TH_13	Villosa vibex	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	438021	Sunfish	Haag et al., 1997
TLH14	Villosa villosa	Mantle lure and simple brood lure	Barnhart et al., 2008	UF	441268	Bass/Sunfish	Keller and Ruessler, 1997

Supplementary Table S2.2: Summary of the final number of ddRAD-seq loci for each individual at the 85% and 90% clustering similarity threshold and for 25% and 46% minimum samples per loci.

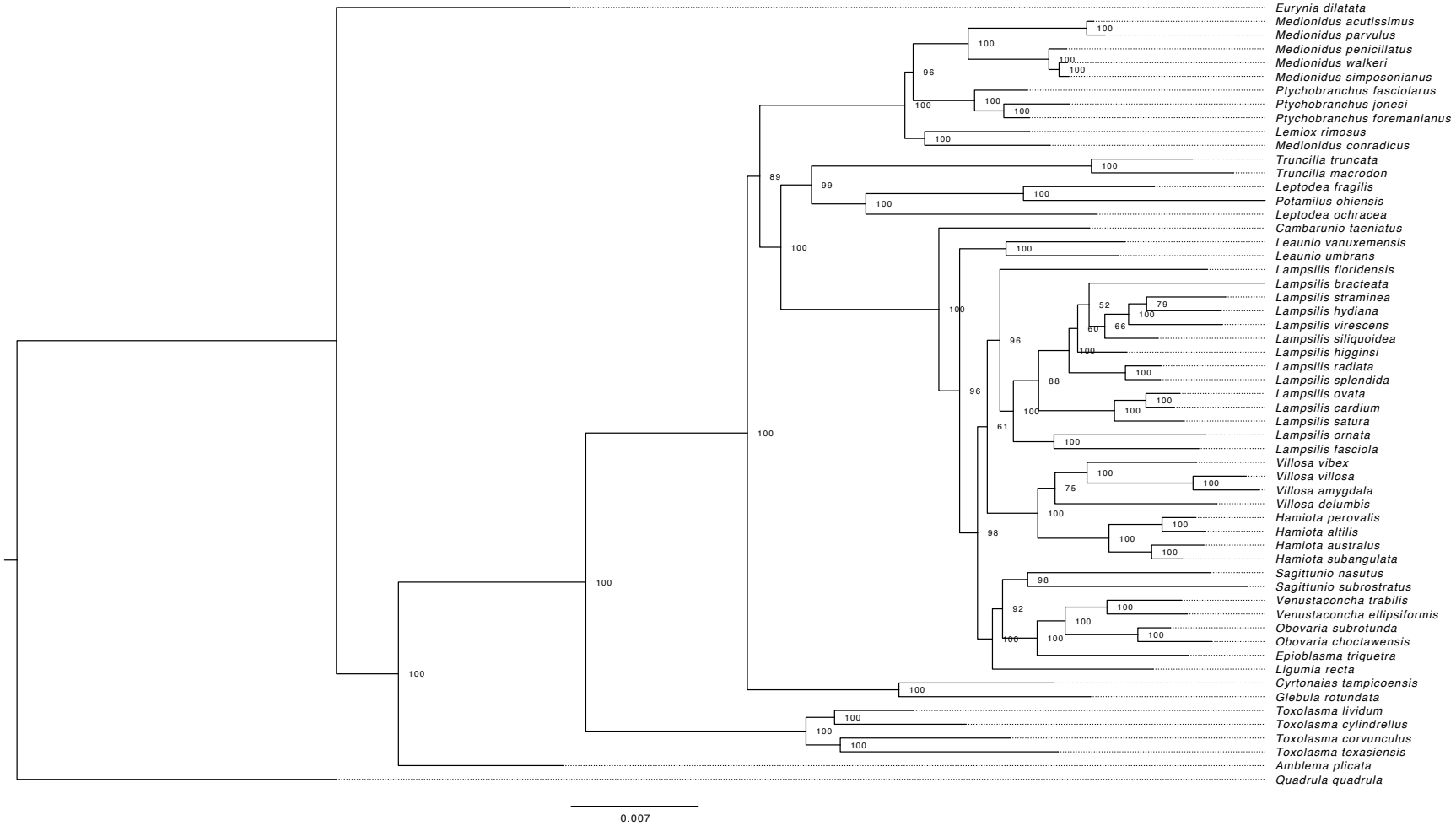
			85% Similarity		90% Similarity	
Sample name	Species name	Raw Reads	25%	46%	25%	46%
Aplic	Amblema plicata	2900279	301	134	19	8
TH131	Cyrtonaias tampicoensis	858098	208	113	10	3
TH132	Cyrtonaias tampicoensis	1506226	366	170	22	7
TLH59	Epioblasma triquetra	5459944	1678	479	129	18
Edila2	Eurynia dilatata	1726245	203	107	9	4
Edila1	Eurynia dilatata	790501	96	55	5	3
TH125	Glebula rotundata	1070046	303	140	11	4
TH126	Glebula rotundata	2490529	537	216	27	8
TH_36	Hamiota altilus	5387472	1827	497	134	23
TH_7	Hamiota australus	3109960	1494	459	115	19
TH_37	Hamiota perovalis	5270101	1826	513	144	24
TH152	Hamiota subangulata	668819	722	255	62	10
TLH9	Lampsilis bracteata	2568126	1594	476	151	19
LCFL68	Lampsilis cardium	7216326	2545	589	213	21
LCEE70	Lampsilis cardium	6965414	2562	597	215	24
LCEE71	Lampsilis cardium	4974228	2424	590	214	22
LCFL69	Lampsilis cardium	4710408	2470	573	213	23
LFre2	Lampsilis fasciola	1929585	2863	496	163	21
TH_2	Lampsilis fasciola	2188128	3296	560	165	18
TH_77	Lampsilis fasciola	3435913	3816	622	186	24
LFHM04	Lampsilis fasciola	1931502	3403	586	164	18
LFHM07	Lampsilis fasciola	1561315	3216	562	156	17
TLH4	Lampsilis floridensis	3303826	1595	473	107	22
TH_45	Lampsilis higginsi	1009895	512	173	66	12
TH_87	Lampsilis hydiana	2000552	1743	480	175	18
TH_88	Lampsilis hydiana	2183348	1945	519	175	21
TH_5	Lampsilis ornata	4893511	2177	514	161	21
TH_96	Lampsilis ovata	3235994	2320	575	206	23
TH_94	Lampsilis ovata	1807208	1935	526	177	17
TH_95	Lampsilis ovata*	3933699	3232	604	184	23
TH117	Lampsilis radiata	800488	1262	426	120	16
TH_8	Lampsilis satruna	4904722	2328	570	208	23
TLH51	Lampsilis siliquoidea	2111249	1786	498	166	19
TH_23	Lampsilis splendida	1149372	1237	390	129	18

TLH6	Lampsilis straminea	4914716	2123	531	195	22
TH_38	Lampsilis virescens	4169896	2043	527	190	23
TH_34	Lemiox rimosus	1911799	460	200	17	8
TH_57	Leptodea fragilis	3519359	484	204	32	9
TH133	Leptodea ochracea	287978	82	49	4	2
TLH21	Sagittunio nasuta	4608659	1513	452	106	20
TH_91	Ligumia recta	1659317	1382	412	88	15
TH147	Sagittunio subrostrata	1814864	998	352	100	19
TH148	Sagittunio subrostrata	2530822	1184	381	106	20
TH_39	Medionidus acutissimus	1851620	475	205	31	10
TH_40	Medionidus conradicus	7718202	619	226	36	10
TH_41	Medionidus parvulus	9957817	633	233	45	12
TLH28	Medionidus parvulus	6651085	604	228	35	11
TH_16	Medionidus penicillatus	7915534	660	242	43	11
TLH20	Medionidus simposonianus	4362329	583	235	35	12
TH_17	Medionidus walkeri	3139933	559	221	26	8
TLH18	Obovaria choctawensis	1470462	1052	374	98	20
TH146	Obovaria subrotunda	1790649	1238	399	105	18
TH145	Obovaria subrotunda	1672141	1157	383	102	18
TH144	Potamilus ohiensis	2454656	310	148	26	9
TH143	Potamilus ohiensis	2251207	294	142	24	9
TH142	Ptychobranchus fasciolarus	2517640	454	184	28	9
TH141	Ptychobranchus fasciolarus	2576902	494	188	29	9
TLH42	Ptychobranchus foremanianus	14377252	659	224	37	10
TH153	Ptychobranchus jonesi	1455454	355	155	21	7
TH_89	Quadrula quadrula	4999562	148	87	5	3
TH_19	Toxolasma corvunculus	2924381	275	131	15	8
TLH44	Toxolasma cylindrellus	11371070	361	158	27	8
TH130	Toxolasma lividum	817733	43	24	1	0
TH129	Toxolasma lividum	779097	113	65	6	4
TH128	Toxolasma texasiensis	1298761	139	84	6	4
TH127	Toxolasma texasiensis	1454598	204	117	13	5
TH119	Truncilla macrodon	878924	269	152	13	8
TH120	Truncilla macrodon	685468	109	64	10	7
TH122	Truncilla truncata	950716	303	158	18	9
TH121	Truncilla truncata	1613852	421	182	19	8
TH_63	Venustaconcha ellipsiformis	4434860	1702	501	141	20
TLH25	Venustaconcha trabalis	5295838	1825	520	169	21
TH_43	Venustaconcha trabalis	5121294	1829	529	173	23
TH124	Venustaconcha trabalis	2642641	1744	520	166	22

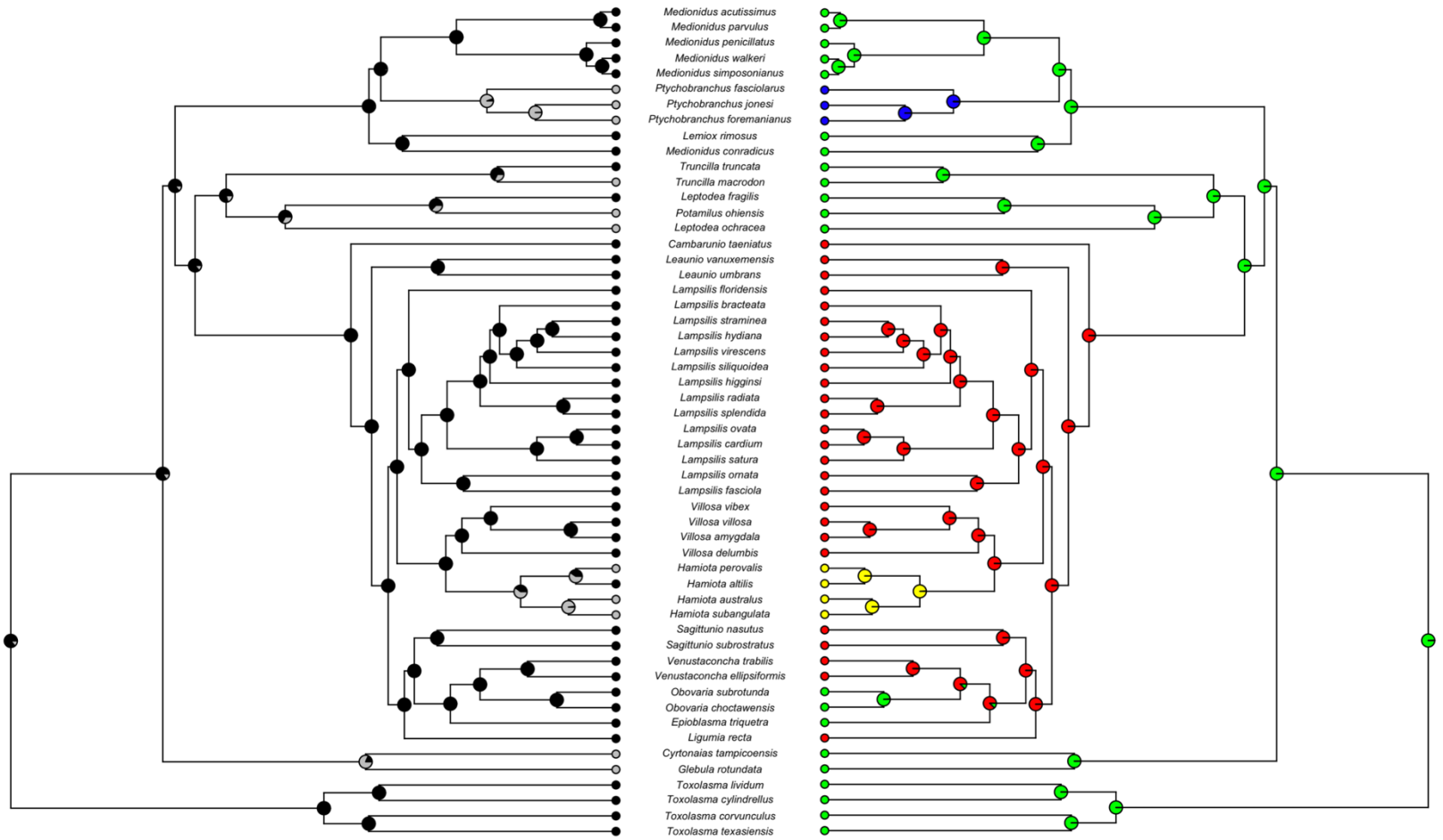
TH123	Venustaconcha trabalis	1660491	1469	495	137	19
TH_11	Villosa amygdala	2021257	1133	377	93	19
TLH12	Villosa delumbis	4433617	1544	447	106	22
TH_32	Cambarunio taeniata	1472633	1004	328	68	16
TH_22	Leaunio umbrans	5607023	1738	492	126	20
TLH26	Leaunio vanuxemensis	1120139	504	188	31	8
TH_24	Villosa vibex	1272879	941	322	74	14
TH_13	Villosa vibex	2427081	1424	454	102	21
TLH14	Villosa villosa	2756754	1340	427	103	22

Supplementary Table S2.3: Displays the AIC, AICc, and log likelihood values for a set of state dependent speciation models performed independently for three different traits: Mantle lure, Brood lure, and broadcast strategy. The four models performed for each trait include a BiSSE model (2 state trait dependent), a HiSSE model (4 state model with two trait states and two hidden states), a 2-state trait independent null model, and a 4 state trait independent null model. Analysis Performed with the topology recovered using 85% clustering threshold and 46% minimum samples per locus.

	Mantle Lure			Brood Lure			Broadcast Strategy		
Model Name	AIC	AICc	Log Likelihood	AIC	AICc	Log Likelihood	AIC	AICc	Log Likelihood
2-state CID	**544.17**	**545.4**	**-267.0859**	527.25	528.47	-258.8246	518.18	519.43	-254.0909
BiSSE	549.3	550.5	-269.6641	**525.25**	**526.47**	**-257.6246**	**516.72**	**517.98**	**-253.3635**
4-state CID	549.2	553.2	-265.6379	530.2	534.2	-256.0995	517.84	521.93	-249.9179
HiSSE	550.12	554.12	-266.058	532.16	536.16	-257.0816	516.94	521.03	-249.469



Supplementary Figure S2.1: Maximum likelihood phylogeny of North American lampsiline mussels created with RAxML v8.2.8 using a general time reversible model from the 85% clustering threshold with 46% minimum samples per locus dataset. Support for each node was determined using 100 fast parametric bootstrap replications. Bootstrap values are adjacent to each node. Scale bar represents mean number of base pair substitutions per site.



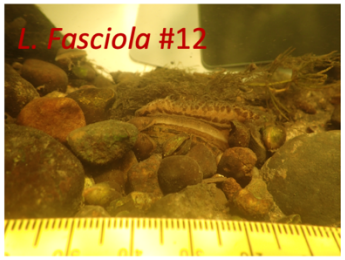
Supplementary Table S2.2: Ultrametric phylogenies created from maximum likelihood phylogeny of Lampsiline mussels (Fig. S1; 85%-46%) using TreePL. These trees were trimmed to remove outgroups and retain only a single individual per species. A) Ancestral state reconstruction of mantle lures using a symmetrical rates model: Grey = presence of a mantle lure (fig. 2d), Black = no mantle lure. B) Ancestral state reconstruction of brood lures using a symmetrical rates model: Blue = complex brood lure (fig. 2f), Red = simple brood lure (fig. 2e), Yellow = tethered brood lure (fig. 2h), Green = no brood lure.

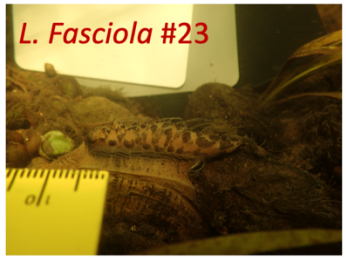
Appendix C:
Supporting Information for

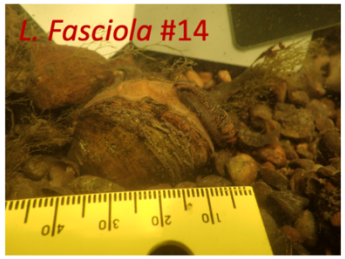
CHAPTER 3

Aggressive Mimicry Lure Polymorphisms in the Parasitic Mussel *Lampsilis fasciola* Model Fish or Leech Host Prey and Differ in Morphology and Pigmentation, but not Behavior

Group 1







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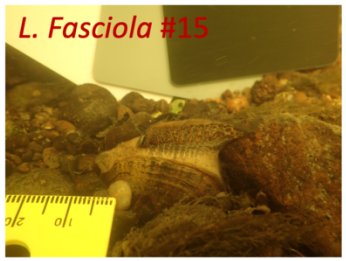
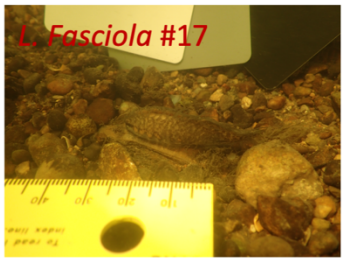
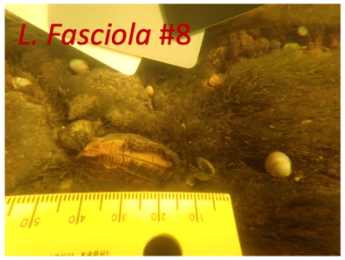
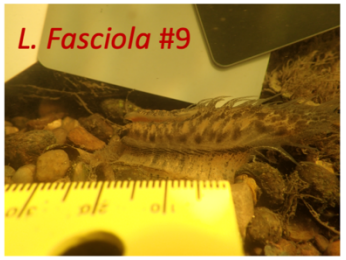




Group 3


L. Fasciola #5


L. Fasciola #8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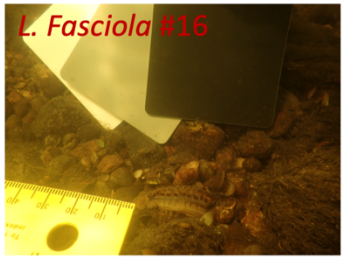
L. Fasciola #13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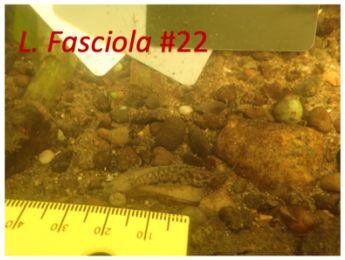
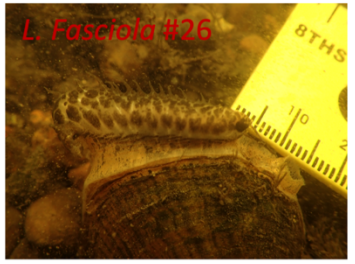
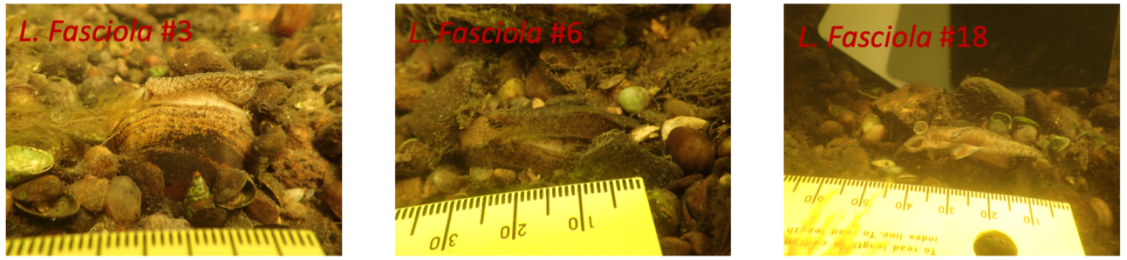

L. Fasciola #22


L. Fasciola #24

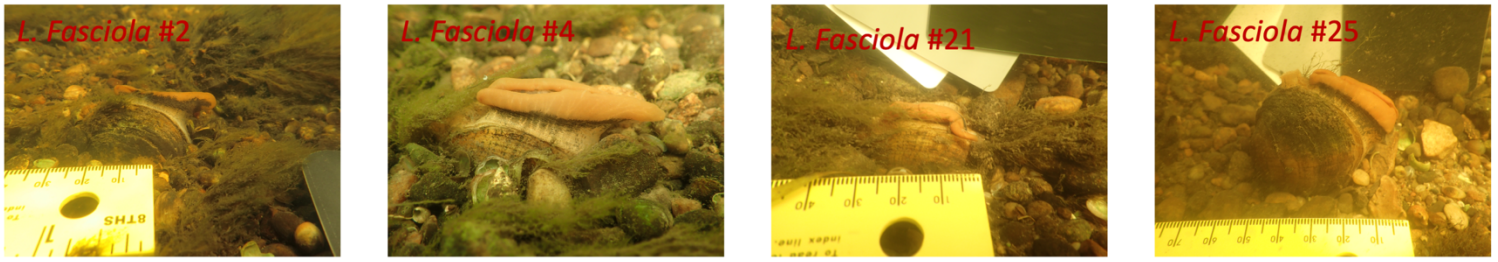

L. Fasciola #26


L. Fasciola #27

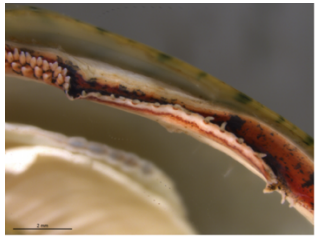
Group 4



Group 5

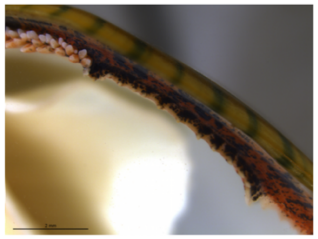


Supplementary Figure S3.1: Photographs from 27 *Lampsilis fasciola* lures taken at Sharon Mills (Fig. 2a) in Summer of 2017. Groups are defined by morphological similarity and individual numbers refer to order in which photographs were taken.



Darter_01

Darter_05



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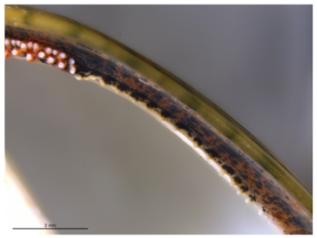
Darter_13

Darter_02

Darter_06

Darter_10



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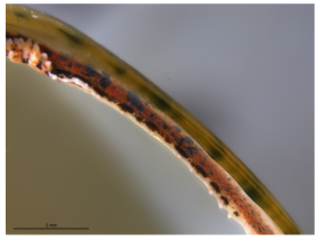
Darter_03



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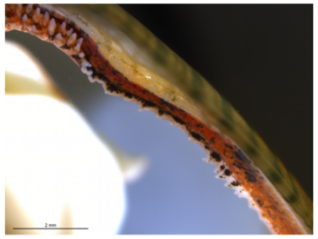

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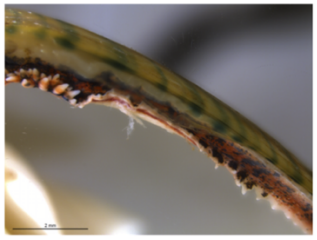

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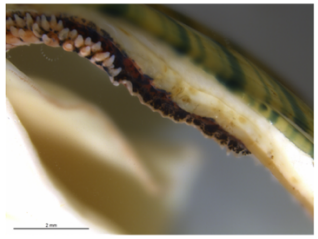

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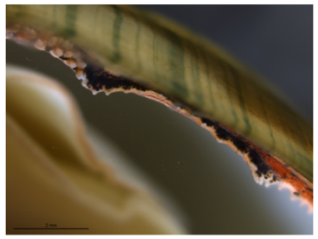

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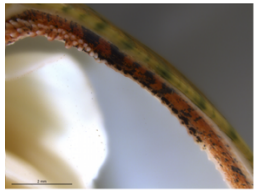
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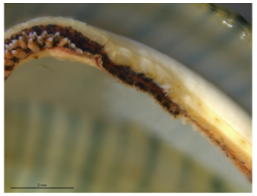
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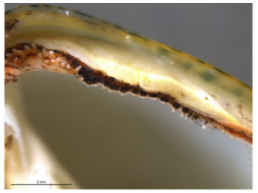
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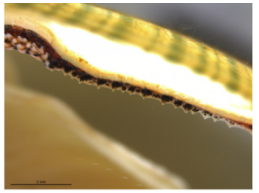
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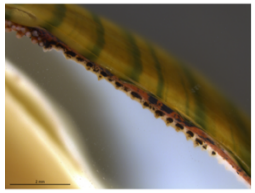
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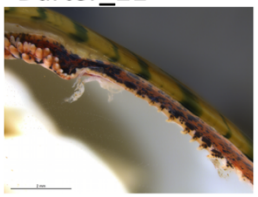
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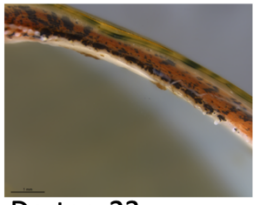
Darter_26


Darter_30


Darter_34


Darter_19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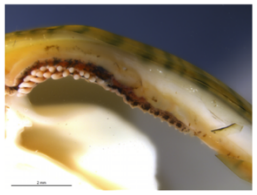
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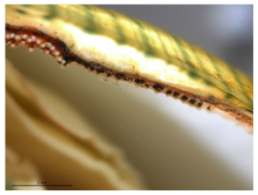
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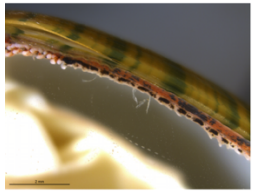
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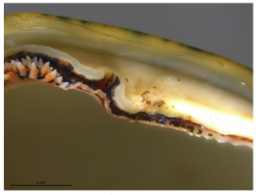
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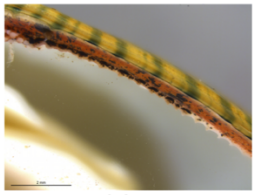
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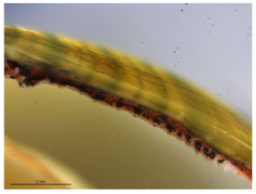
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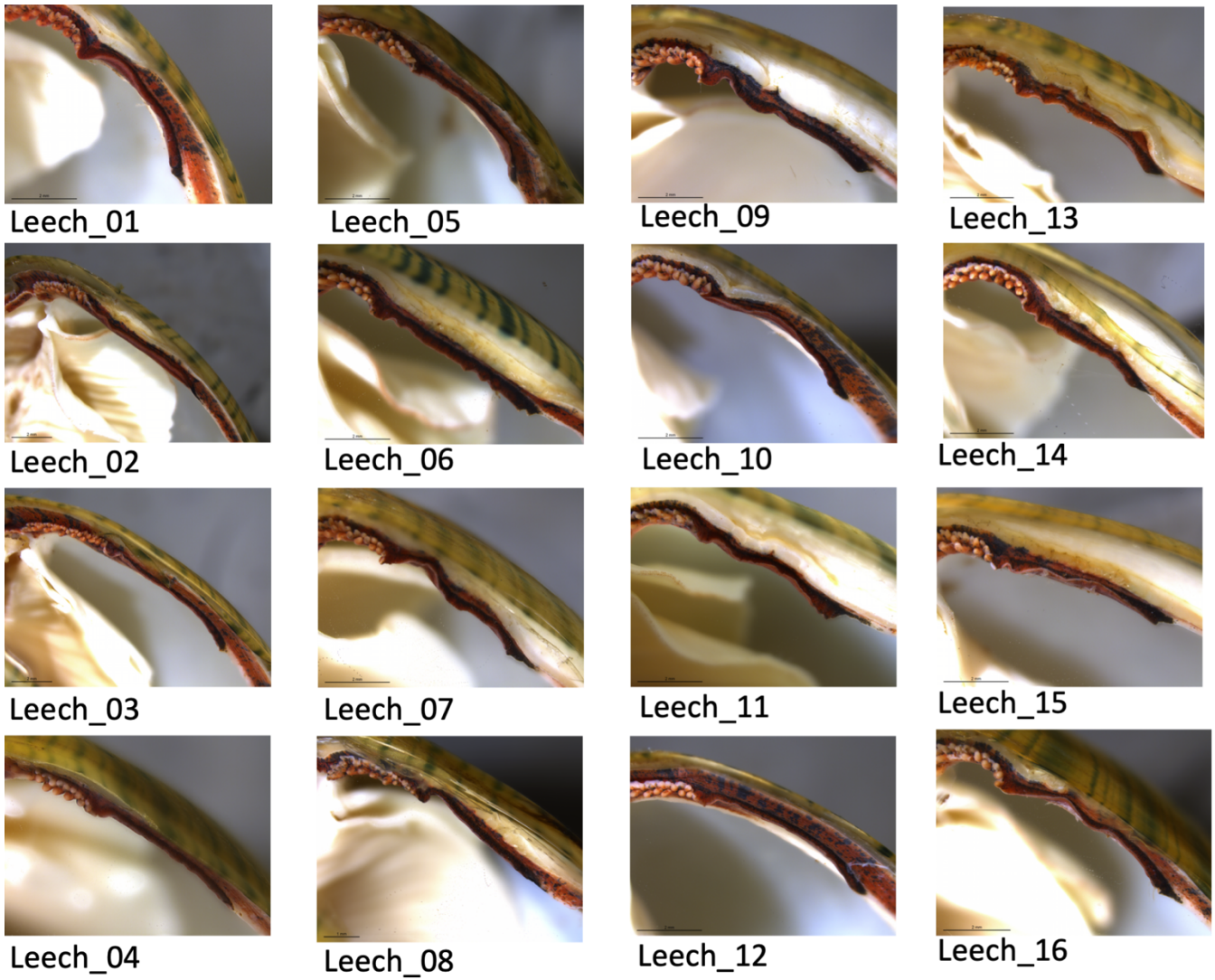
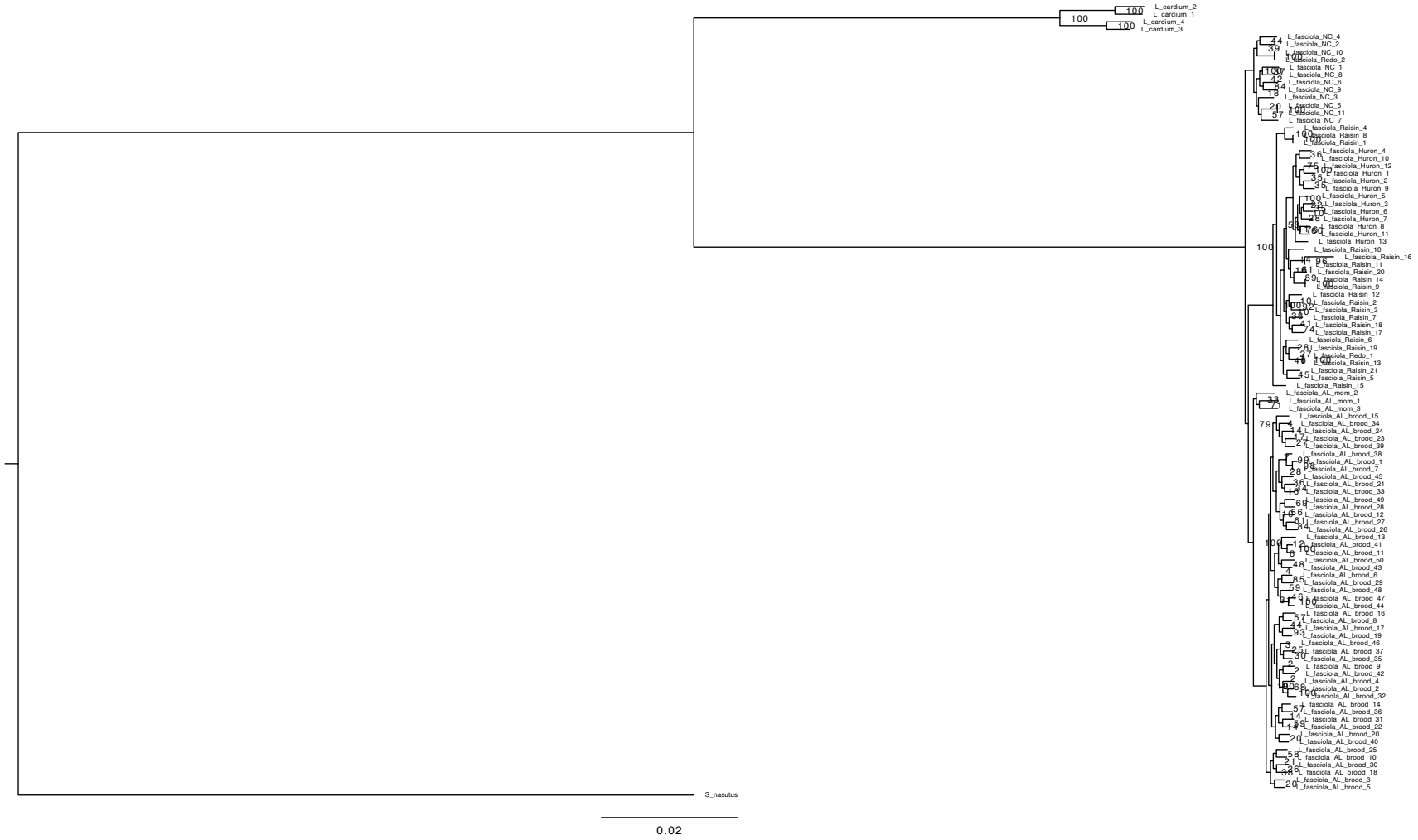


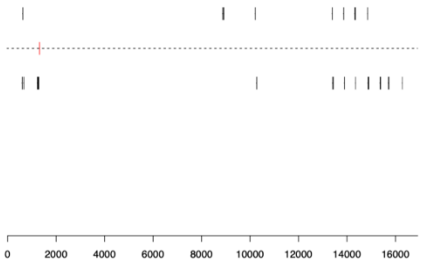
Supplementary Figure S3.2: Photographs of *Lampsilis fasciola* lure structure taken from 50 full or half-siblings raised from a single gravid female at the Alabama Aquatic Biodiversity Center in 2009. Each individual is categorized based on whether it has a darter-like or worm-like lure phenotype.



Supplementary Figure S3.3: Maximum likelihood phylogeny of *Lampsilis fasciola* mussels created with RAxML v8.2.8 using a general time reversible model. Support for each node was determined using 100 fast parametric bootstrap replications. Bootstrap values are adjacent to each node. Scale bar represents mean number of base pair substitutions per site.

GH010599

Darter



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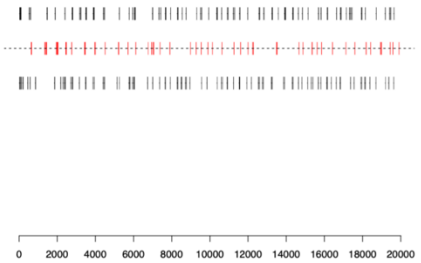
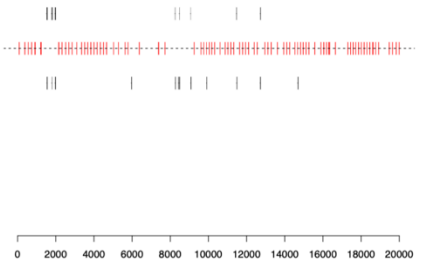
Darter



GH010598

Darter



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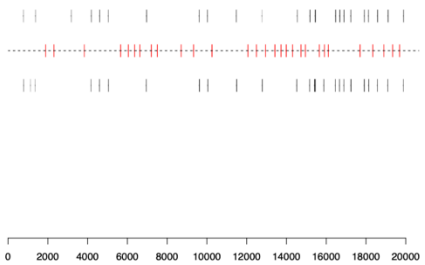

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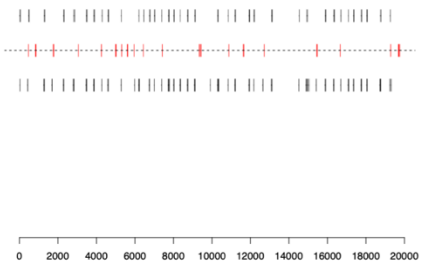

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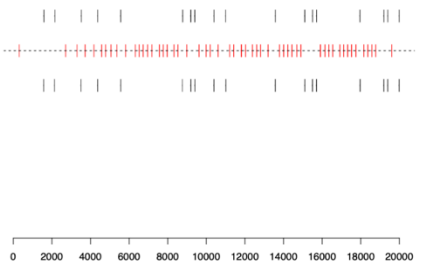

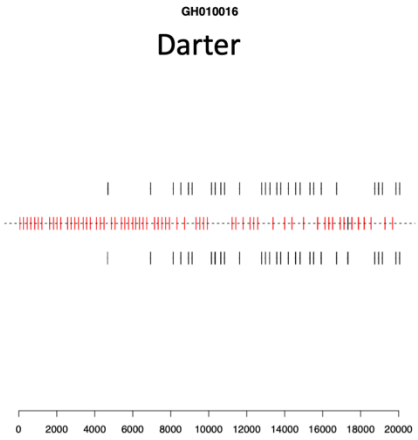
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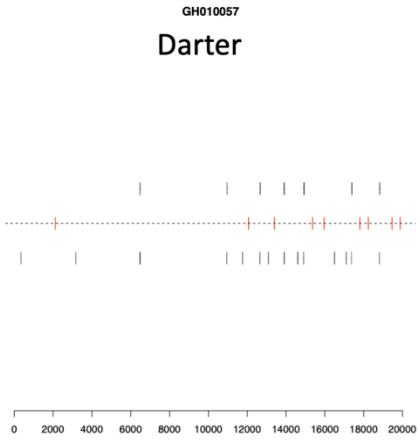
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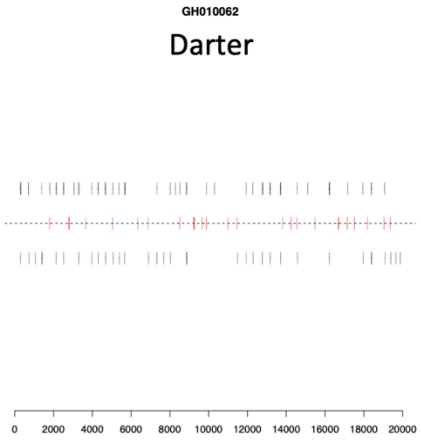
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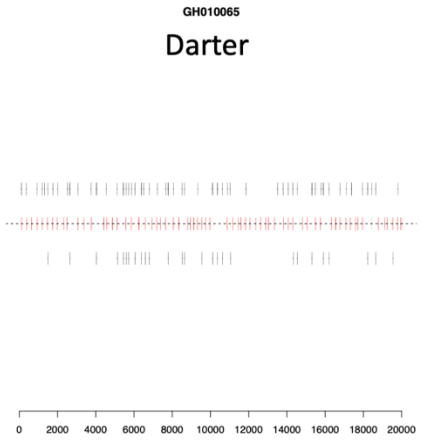
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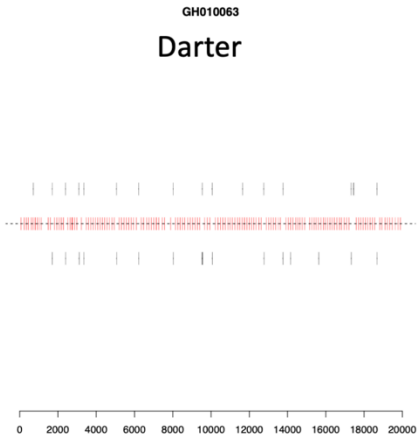
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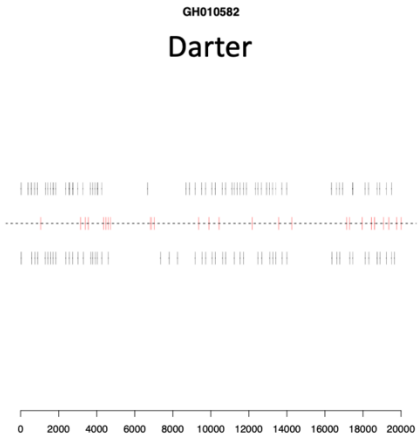
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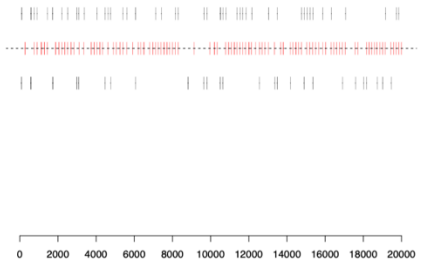

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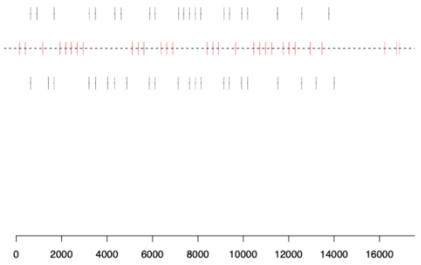

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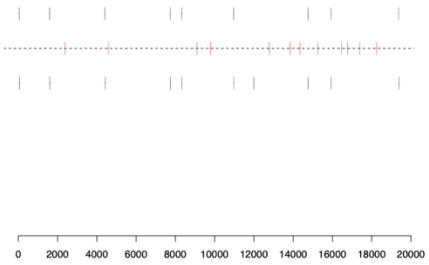

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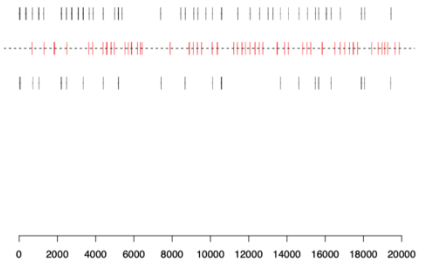

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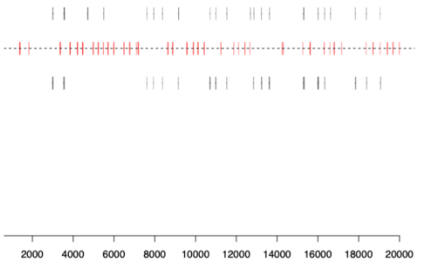

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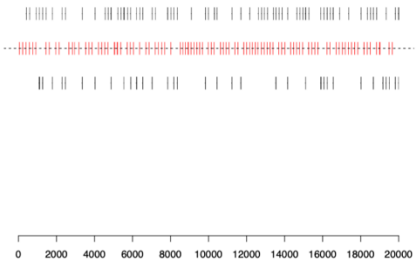

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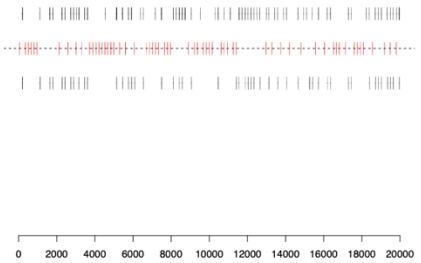

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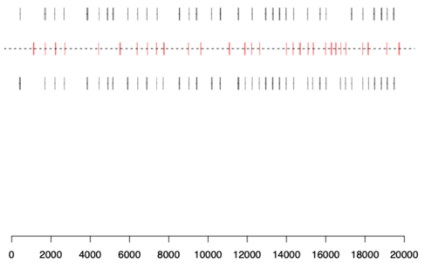

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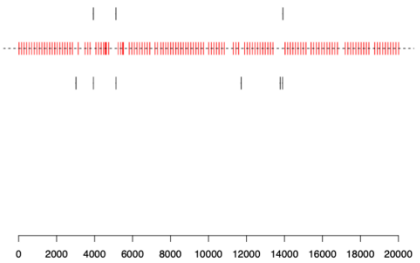

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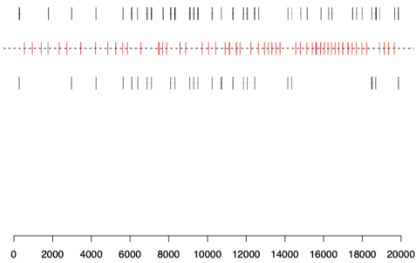

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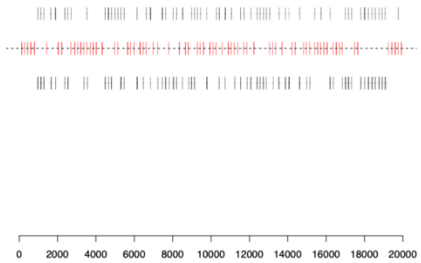

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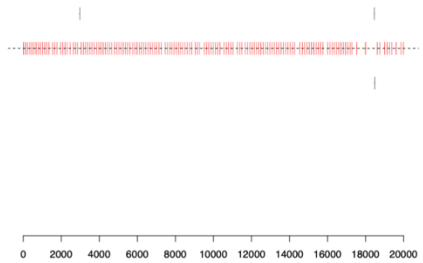

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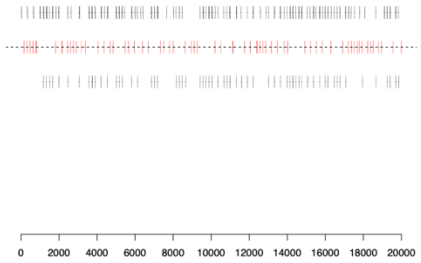

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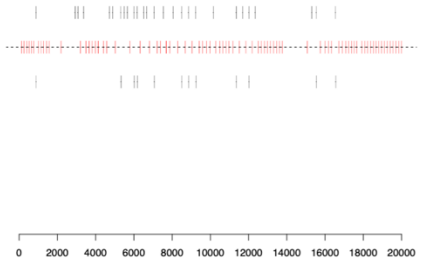

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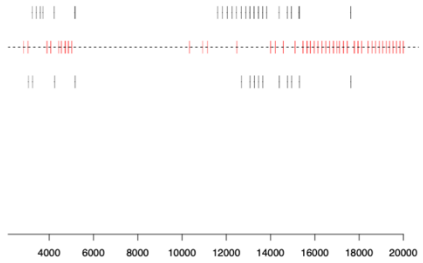
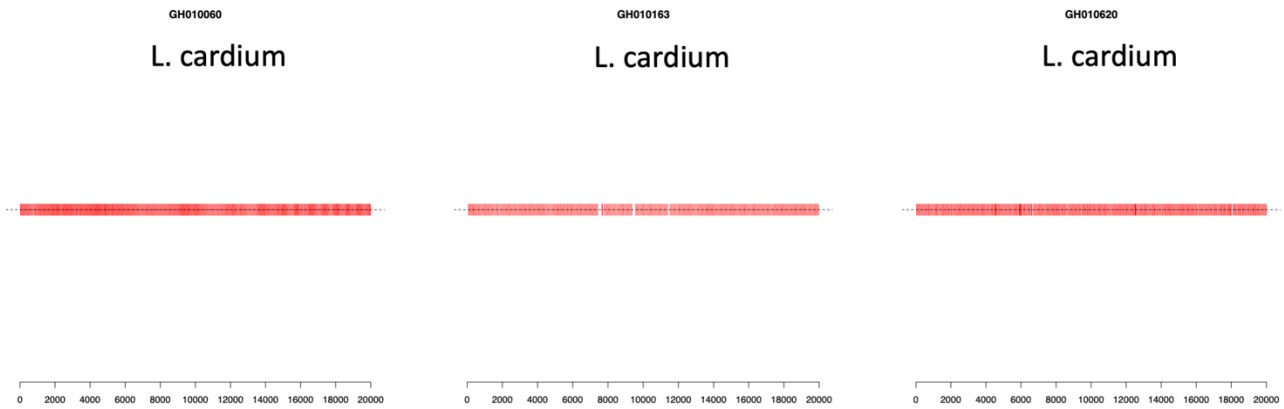
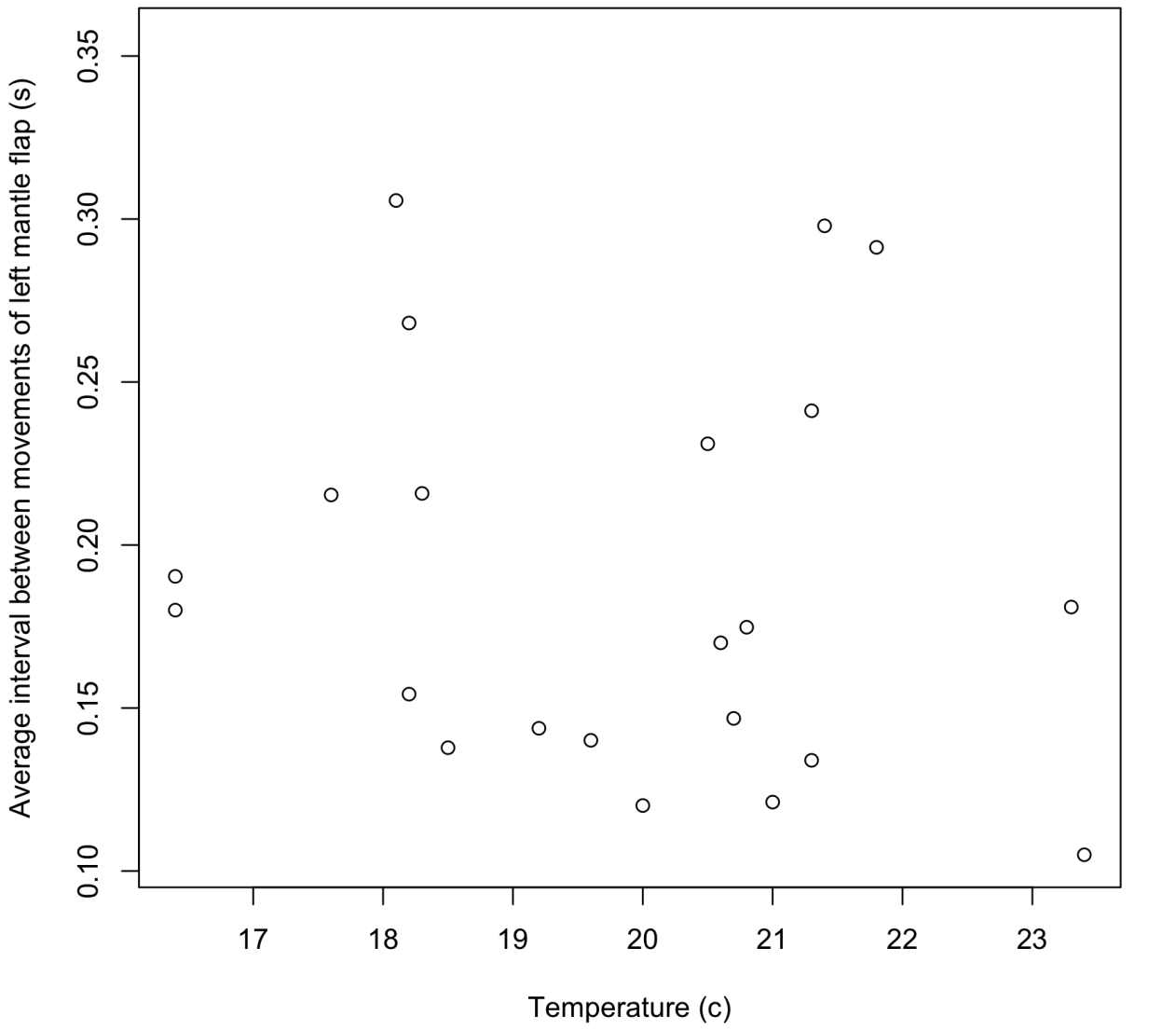




Supplementary Figure S3.4: Gait analyses for *Lampsilis fasciola* and *Lampsilis cardium* lure behavior. X axis denotes frame number. Videos were taken in Summer of 2018 from Sharon Mills (Fig. 2a) and Hudson Mills (Fig. 2b). Red lines on the center dotted line represent synchronized left/right movements, and black lines above the center line represent left side movements and below represent right side movements. Each graph is labelled as either a darter-like *L. fasciola*, leech-like *L. fasciola,* or *L. cardium.*



Supplementary Figure S3.5: Scatterplot showing the relationship between the average time interval between lure movements (left mantle flap) and temperature for *Lampsilis fasciola* and *Lampsilis cardium* lure displays videotaped in Summer 2018 and Sharon Mills (Fig. 2a) and Hudson Mills (Fig. 2b).