Evolution of diverse host infection mechanisms delineates an adaptive radiation of lampsiline freshwater mussels centered on their larval ecology

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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 centrarchids (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 (Psychobranchus 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, lampsilinid mussels may be legitimately viewed as an adaptive radiation.

**Subjects** Biodiversity, Evolutionary Studies, Genomics, Freshwater Biology

**Keywords** Phylogenomics, Unionidae, RADseq, Parasitism

**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, Ee & Pfeiffer (2020) found variation in tissue stoichiometry among unionid mussels that correlate with phylogeny. The consensus view (Coker et al., 1921; Bronmark & Malmqvist, 1982; Rashleigh & De Angelis, 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 the 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 & Foighil, 2019). Rashleigh & De Angelis (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 & De Angelis, 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 lampsilines 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 (Figs. 2B–2D) and brood lures (i.e., conglutinates and superconglutinates) containing larvae, that are released into the environment (Figs. 2E–2H).

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 lampshiline 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 lampshiline 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 lampshiline 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 & 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.
Figure 2  Panel depicting many of the common host infection strategies used by North American freshwater mussels. 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 *Psychobranchus*—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. Illustrations by John Megahan.

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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 Table S1.

**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. Ipypad 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,
##### Table 1  Summary table of samples used, life history traits, and summary data for genomic sequencing.  
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.

| Species name      | Infection strategy                  | Host information   | 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    | 414263         | 32713          | 1004             |
| *Cyrtonaias tampicoensis* | Broadcast | Gar          | UF                 | 438173   | 858098     | 339345         | 18540          | 208              |
| *Epioblasma triquetera* | 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 altillis* | Mantle lure; tethered, complex brood lure | Bass/Sunfish       | From Paul Johnson  | 306257   | 5387472    | 1266412        | 64930          | 1827             |
| *Hamiota australis* | Tethered, complex brood lure       | 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 higgoni* | 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 satrania* | Mantle lure and Simple brood lure  | Bass               | UF                 | 441167   | 4904722    | 916074         | 63718          | 2328             |
| *Lampsilis siliquoida* | 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 virens* | Mantle lure and Simple brood lure  | Bass               | Paul Johnson       | 306261   | 4169869    | 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              |
| *Lemiax rimosus* | Mantle lure                          | Darter/Sculpin     | NCS                | 47243    | 1911799    | 434117         | 38814          | 460              |
| *Leptoea fragilis* | Mantle lure                          | Drum               | INHS               | 79830    | 3519359    | 1143382        | 54580          | 484              |
| *Leptoea ochracea* | Mantle lure                          | 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   | 7915334    | 2253442        | 80037          | 660              |

(continued on next page)
### Table 1 (continued)

| Species name            | Infection strategy                        | Host information          | Tissue source | Museum ID | Raw reads | Total clusters | Consensus reads | Loci in assembly |
|-------------------------|-------------------------------------------|---------------------------|---------------|-----------|------------|----------------|-----------------|------------------|
| *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              |
| *Obbovaria choctawensis* | Mantle lure                               | Darter/Sculpin            | UF            | 441237    | 1470462    | 373459         | 32610           | 1052             |
| *Obbovaria 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              |
| *Psychobranchus fasciosaurus* | Complex brood lure           | Darter/Sculpin            | UF            | 438254    | 2517640    | 878247         | 37577           | 454              |
| *Psychobranchus foremanianus* | Complex brood lure                  | Darter/Sculpin            | Paul Johnson  | 306267    | 14377252   | 3961795        | 78567           | 659              |
| *Psychobranchus jonesi*  | Complex brood lure                      | Darter/Sculpin            | UF            | 441272    | 1455454    | 491977         | 29992           | 355              |
| *Quadrula quadrula*      | Mantle lure                             | Catfish                   | UF            | 438787    | 4999562    | 1569250        | 58525           | 148              |
| *Segittunio nasutus*     | Mantle lure and Simple brood lure        | Sunfish and Perch         | UF            | 438285    | 4608659    | 1458774        | 55120           | 1513             |
| *Segittunio 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 lug      | Darter/Sculpin            | INHS          | 87179     | 4434860    | 1022209        | 62605           | 1702             |
| *Venustaconcha trabalis* | Mantle lure and Simple brood lug        | Darter/Sculpin            | UF            | 438909    | 1660491    | 264191         | 36956           | 1469             |
| *Villosa amygdalá*       | Mantle lure and Simple brood lug         | unknown                   | UF            | 441054    | 2021257    | 400560         | 39674           | 1133             |
| *Villosa delumbis*       | Mantle lure and Simple brood lug         | Bass                      | UF            | 437984    | 4433617    | 1358338        | 61582           | 1544             |
| *Villosa vibex*          | Mantle lure and Simple brood lug         | Sunfish                   | UF            | 438545    | 1272879    | 370119         | 28877           | 941              |
| *Villosa villosa*        | Mantle lure and Simple brood lug         | Bass/Sunfish              | UF            | 441268    | 2756754    | 671290         | 48066           | 1340             |

**Notes.**

UF, University of Florida; INHS, Illinois Natural History Survey; NCS, North Carolina State University.

Newly sampled material Museum ID refers to their deposition in the UMMZ, University of Michigan Museum of Zoology.
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 (4,725 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 (Table S1). 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.

**Lampsilinae diversification rates**

Two different approaches were used to investigate the potential influence of host infection strategies on diversification rates in the Lampsilinae. 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 lampsilinae 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 Lampsilinae 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 5,000 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*) (Table 1, Table S2).

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 Fig. S1). 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 (*Toxoplasmata* (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...
Figure 3  **Phylogeny of lampsiline mussels.** 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 25% 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.

**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; Fig. S2). 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 *Ptychobranchus* (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 and 5).

**Lampsilide 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 (Table S3), 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 (Fig. S1), we found the BiSSE model was also the best-performing model (AICc) for broadcast release by a small margin (Table S3), 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,
Table 2  AIC and log likelihood values for SSE models performed for three different life history traits. 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.

| Model name   | Mantle lure | Brood lure | Broadcast strategy |
|--------------|-------------|------------|--------------------|
|              | 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         |

Figure 6  Diversification rate estimates for species with and without brood lures. 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.

DISCUSSION
Evolution of infection strategies in lampsilin 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 Hamiola 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, Wood & Foighil’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 & Murphy’s (2006) mt phylogeny-based inferences that lampsilines 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; Fig. S2). 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 altillis 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 & 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, Hove & Davis (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 and 5) to be provisional. For the taxa included here, our topology corroborates that of Smith, Pfeiffer & Johnson (2020) and our data support their decision to reclassify Leptodea ochracea to Atlanticconcha 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 (Figs. 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 lampsilin 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. 7I)—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 (Figs. 7Bii–7Biv) 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; Table S3) 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 lampsilines 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 lampsilines 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 lampsilines, 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 lampsilines 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 lampsilines (~50; Williams et al., 2017). Although new centrarchid 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 US 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 & De Angelis, 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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**ADDITIONAL INFORMATION AND DECLARATIONS**

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The authors declare there are no competing interests.

Author Contributions
• Trevor L. Hewitt conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
• Amanda E. Haponski performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
• Diarmaid Ó. Foighil conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

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The following information was supplied regarding the deposition of DNA sequences:
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Data Availability
The following information was supplied regarding data availability:
The ddRAD-seq data that was used in all analyses and the R code used to perform the analyses are available in the Supplemental Information.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.12287#supplemental-information.

REFERENCES

Araujo R, Cámara N, Ramos MA. 2002. Glochidium metamorphosis in the endangered freshwater mussel Margaritifera auricularia (Spengler, 1793): a histological and scanning electron microscopy study: Metamorphosis of M. auricularia Glochidia. Journal of Morphology 254:259–265 DOI 10.1002/jmor.10031.

Arbour JH, López-Fernández H. 2016. Continental cichlid radiations: functional diversity reveals the role of changing ecological opportunity in the Neotropics. Proceedings of the Royal Society B: Biological Sciences 283:20160556 DOI 10.1098/rspb.2016.0556.

Atkinson CL, Ee BC, Pfeiffer JM. 2020. Evolutionary history drives aspects of stoichiometric niche variation and functional effects within a guild. Ecology 101:ec03100 DOI 10.1002/ecy.3100.

Baker WH, Blanton RE, Johnston CE. 2013. Diversity within the Redeye Bass, Micropterus coosae (Perciformes: Centrarchidae) species group, with descriptions of four new species. Zootaxa 3635:379–401 DOI 10.11646/zootaxa.3635.4.3.
Barnhart MC, Haag WR, Roston WN. 2008. Adaptations to host infection and larval parasitism in Unionoida. *Journal of the North American Benthological Society* 27:370–394 DOI 10.1899/07-093.1.

Beaulieu JM, O’Meara BC. 2016. Detecting hidden diversification shifts in models of trait-dependent speciation and extinction. *Systematic Biology* 65:583–601 DOI 10.1093/sysbio/syw022.

Berg DJ, Haag WR, Guttman SI, Sickel JB. 1995. Mantle biopsy: a technique for nondestructive tissue-sampling of freshwater mussels. *Journal of the North American Benthological Society* 14:577–581 DOI 10.2307/1467542.

Bogan AE. 2007. Global diversity of freshwater mussels (Mollusca, Bivalvia) in freshwater. In: Balian EV, Lévêque C, Segers H, Martens K, eds. *Freshwater animal diversity assessment*. Dordrecht: Springer Netherlands, 139–147 DOI 10.1007/978-1-4020-8259-7_16.

Bronmark C, Malmqvist B. 1982. Resource partitioning between unionid mussels in a Swedish lake outlet. *Ecography* 5:389–395 DOI 10.1111/j.1600-0587.1982.tb01053.x.

Campbell DC, Serb JM, Buhay JE, Roe KJ, Minton RL, Lydeard C. 2005. Phylogeny of North American amblemines (Bivalvia, Unionoida): prodigious polyphyly proves pervasive across genera: Phylogeny of North American amblemines. *Invertebrate Biology* 124:131–164 DOI 10.1111/j.1744-7410.2005.00015.

Coker RE, Robert E, Clark HW, Howard AD, Arthur D, Shira AF. 1921. *Natural history and propagation of fresh-water mussels*. Washington, D.C.: Govt. Print. Off.

Corey CA, Dowling R, Strayer DL. 2006. Display behavior of ligumia (Bivalvia: Unionidae). *Northeastern Naturalist* 13:319–332 DOI 10.1656/1092-6194(2006)13[319:DBOLBU]2.0.CO;2.

Cummings KS, Watters GT. 2017. Freshwater mussel host database. The freshwater mussel host database, Illinois Natural History Survey & Ohio State University, 2017. Available at http://www.inhs.illinois.edu/collections/mollusk/data/freshwater-mussel-host-database (accessed on 12 June 2017).

Eaton DAR. 2014. PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics* 30:1844–1849 DOI 10.1093/bioinformatics/btu121.

Eaton DAR, Overcast I. 2020. ipyrad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics* 36:btz966 DOI 10.1093/bioinformatics/btz966.

Eaton DAR, Spriggs EL, Park B, Donoghue MJ. 2016. Misconceptions on missing data in RAD-seq phylogenetics with a deep-scale example from flowering plants. *Systematic Biology* 66:399–412 DOI 10.1093/sysbio/syw092.

Endler JA. 1988. Frequency-dependent predation, crypsis and aposematic coloration. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences* 319:505–523 DOI 10.1098/rstb.1988.0062.

FitzJohn RG. 2012. Diversitree: comparative phylogenetic analyses of diversification in R: Diversitree. *Methods in Ecology and Evolution* 3:1084–1092 DOI 10.1111/j.2041-210X.2012.00234.x.
Freeman BJ, Taylor A, Oswald KJ, Wares J, Freeman MC, Quattro JM, Leitner J. 2015. Shoal basses: a clade of cryptic identity. In: American fisheries society 143rd annual meeting. 449–466.

Garner J, McGregor S. 2001. Current status of freshwater mussels (Unionidae, Margaritiferidae) in the Muscle Shoals area of Tennessee River in Alabama (Muscle Shoals revisited again). American Malacological Bulletin 16:155–170.

Gascho Landis AM, Mosley TL, Haag WR, Stoeckel JA. 2012. Effects of temperature and photoperiod on lure display and glochidial release in a freshwater mussel. Freshwater Science 31:775–786 DOI 10.1899/11-082.1.

Gavrilets S, Losos JB. 2009. Adaptive radiation: contrasting theory with data. Science 323:732–737 DOI 10.1126/science.1157966.

Gittenberger A, Gittenberger E. 2011. Cryptic, adaptive radiation of endoparasitic snails: sibling species of Leptoconchus (Gastropoda: Coralliophilidae) in corals. Organisms Diversity & Evolution 11:21–41 DOI 10.1007/s13127-011-0039-1.

Givnish TJ, Spalink D, Ames M, Lyon SP, Hunter SJ, Zuluaga A, Iles WJ, Clements MA, Arroyo MT, Leebens-Mack J. 2015. Orchid phylogenomics and multiple drivers of their extraordinary diversification. Proceedings of the Royal Society B: Biological Sciences 282:20151553 DOI 10.1098/rspb.2015.1553.

Graf DL, Cummings KS. 2007. Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). Journal of Molluscan Studies 73:291–314 DOI 10.1093/mollus/eym029.

Graf DL, Ó Foighil D. 2000. The evolution of brooding characters among the freshwater pearly mussels (Bivalvia: Unionoidea) of North America. Journal of Molluscan Studies 66:157–170 DOI 10.1093/mollus/66.2.157.

Grant PR. 1999. Ecology and evolution of Darwin’s finches. Princeton: Princeton University Press.

Haag WR. 2012. North American freshwater mussels: natural history, ecology, and conservation. Cambridge: Cambridge University Press.

Haag WR. 2013. The role of fecundity and reproductive effort in defining life-history strategies of North American freshwater mussels: Fecundity and reproductive effort in mussels. Biological Reviews 88:745–766 DOI 10.1111/brv.12028.

Haag WR, Warren ML. 1998. Role of ecological factors and reproductive strategies in structuring freshwater mussel communities. Canadian Journal of Fisheries and Aquatic Sciences 55:297–306 DOI 10.1139/f97-210.

Haag WR, Warren ML. 1999. Mantle displays of freshwater mussels elicit attacks from fish: mussel and fish interactions. Freshwater Biology 42:35–40 DOI 10.1046/j.1365-2427.1999.00454.x.

Haag WR, Warren ML. 2000. Effects of light and presence of fish on lure display and larval release behaviours in two species of freshwater mussels. Animal Behaviour 60:879–886 DOI 10.1006/anbe.2000.1549.

Haag WR, Warren ML. 2003. Host fishes and infection strategies of freshwater mussels in large mobile basin streams, USA. Journal of the North American Bentholological Society 22:78–91 DOI 10.2307/1467979.
Hewitt TL, Wood CL, Foighil DÓ. 2019. Ecological correlates and phylogenetic signal of host use in North American unionid mussels. *International Journal for Parasitology* **49**:71–81 DOI 10.1016/j.ijpara.2018.09.006.

Hickley P, North R, Muchiri SM, Harper DM. 1994. The diet of largemouth bass, Micropterus salmoides, in Lake Naivasha, Kenya. *Journal of Fish Biology* **44**:607–619 DOI 10.1111/j.1095-8649.1994.tb01237.x.

Huang H, Knowles LL. 2016. Unforeseen consequences of excluding missing data from next-generation sequences: simulation study of RAD sequences. *Systematic Biology* **65**:357–365 DOI 10.1093/sysbio/syu046.

Jansen W, Bauer G, Zahner-Meike E. 2001. Glochidial mortality in freshwater mussels. In: Bauer G, Wächtler K, eds. *Ecology and evolution of the freshwater mussels Unionoida*. Berlin: Springer Berlin Heidelberg, 185–211 DOI 10.1007/978-3-642-56869-5_11.

Kim J, Sanderson MJ. 2008. Penalized likelihood phylogenetic inference: bridging the parsimony-likelihood gap. *Systematic Biology* **57**:665–674 DOI 10.1080/10635150802422274.

Kodandaramaiah U, Murali G. 2018. What affects power to estimate speciation rate shifts? *PeerJ* **6**:e5495 DOI 10.7717/peerj.5495.

Kraemer LR. 1970. The mantle flap in three species of Lampsilis (Pelecypoda: Unionidae). *Malacologia* **10**:225–282.

Lanave C, Preparata G, Sacone C, Serio G. 1984. A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution* **20**:86–93 DOI 10.1007/BF02101990.

Lefevre G, Curtis WC. 1912. *Studies on the reproduction and artificial propagation of freshwater mussels*. Washington, D.C.: Govt. print. off.

Losos JB. 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism: American Society of Naturalists E. O. Wilson Award Address. *The American Naturalist* **175**:623–639 DOI 10.1086/652433.

Lydeard C, Cowie RH, Ponder WF, Bogan AE, Bouchet P, Clark SA, Cummings KS, Frest TJ, Gargominy O, Herbert DG. 2004. The global decline of nonmarine mollusks. *BioScience* **54**:321–330 DOI 10.1641/0006-3568(2004)054[0321:TGDONM]2.0.CO;2.

Maddison WP, Knowles LL. 2006. Inferring phylogeny despite incomplete lineage sorting. *Systematic Biology* **55**:21–30 DOI 10.1080/10635150500354928.

Near TJ, Bossu CM, Bradburd GS, Carlson RL, Harrington RC, Hollingsworth Jr PR, Keck BP, Etnier DA. 2011. Phylogeny and temporal diversification of darters (Percidae: Etheostomatinae). *Systematic Biology* **60**:565–595 DOI 10.1093/sysbio/syr052.

Nichols SJ, Garling D. 2000. Food-web dynamics and trophic-level interactions in a multispecies community of freshwater unionids. *Canadian Journal of Zoology* **78**:871–882 DOI 10.1139/z99-256.

Nichols SJ, Silverman H, Dietz TH, Lynn JW, Garling DL. 2005. Pathways of food uptake in native (unionidae) and introduced (corbiculidae and dreissenidae)
freshwater bivalves. *Journal of Great Lakes Research* 31:87–96 DOI 10.1016/S0380-1330(05)70240-9.

Page LM, Burr BM. 2011. *Peterson field guide to freshwater fishes of North America north of Mexico*. Boston: Houghton Mifflin Harcourt.

Paradis E, Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35:526–528 DOI 10.1093/bioinformatics/bty633.

Parker RS, Hackney CT, Vidrine MF. 1984. Ecology and reproductive strategy of a South Louisiana Freshwater Mussel, *Glebula rotundata* (Lamarck) (Unionidae:Lampsilini). *Freshwater Invertebrate Biology* 3:53–58 DOI 10.2307/1467094.

Parsons KJ, Robinson BW. 2007. Foraging performance of diet-induced morphotypes in pumpkinseed sunfish (*Lepomis gibbosus*) favours resource polymorphism. *Journal of Evolutionary Biology* 20:673–684 DOI 10.1111/j.1420-9101.2006.01249.x.

Pfeiffer JM, Atkinson CL, Sharpe AE, Capps KA, Emery KF, Page LM. 2019. Phylogeny of Mesoamerican freshwater mussels and a revised tribe-level classification of the Ambileminae: XXXX. *Zoologica Scripta* 48:106–117 DOI 10.1111/zsc.12322.

Pfeiffer JM, Breinholt JW, Page LM. 2019. Unioverse: a phylogenomic resource for reconstructing the evolution of freshwater mussels (Bivalvia, Unionoida). *Molecular Phylogenetics and Evolution* 137:114–126 DOI 10.1016/j.ympev.2019.02.016.

Pillon Y, Hopkins HCF, Rigault F, Jaffré T, Stacy EA. 2014. Cryptic adaptive radiation in tropical forest trees in New Caledonia. *New Phytologist* 202:521–530 DOI 10.1111/nph.12677.

Rabosky DL. 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLOS ONE* 9:e89543 DOI 10.1371/journal.pone.0089543.

Rabosky DL, Goldberg EE. 2015. Model inadequacy and mistaken inferences of trait-dependent speciation. *Systematic Biology* 64:340–355 DOI 10.1093/sysbio/syu131.

Rabosky DL, Grundler M, Anderson C, Title P, Shi JJ, Brown JW, Huang H, Larson JG. 2014. BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods in Ecology and Evolution* 5:701–707 DOI 10.1111/2041-210X.12199.

Rabosky DL, Mitchell JS, Chang J. 2017. Is BAMM flawed? Theoretical and practical concerns in the analysis of multi-rate diversification models. *Systematic Biology* 66:477–498 DOI 10.1093/sysbio/syx037.

Rabosky DL, Santini F, Eastman J, Smith SA, Sidlauskas B, Chang J, Alfaro ME. 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nature Communications* 4:1958 DOI 10.1038/ncomms2958.

Raikow DF, Hamilton SK. 2001. Bivalve diets in a midwestern U.S. stream: a stable isotope enrichment study. *Limnology and Oceanography* 46:514–522 DOI 10.4319/lo.2001.46.3.0514.
Rashleigh B, De Angelis DL. 2007. Conditions for coexistence of freshwater mussel species via partitioning of fish host resources. *Ecological Modelling* **201**:171–178 DOI 10.1016/j.ecolmodel.2006.09.009.

R Core Team. 2018. *R: a language and environment for statistical computing*. Vienna, Austria: R foundation for statistical computing. Available at https://www.R-project.org/.

Revell LJ. 2012. *phytools: an R package for phylogenetic comparative biology (and other things): phytools: R package*. *Methods in Ecology and Evolution* **3**:217–223 DOI 10.1111/j.2041-210X.2011.00169.x.

Roe KJ, Harris PM, Mayden RL. 2002. Phylogenetic Relationships of the Genera of North American Sunfishes and Basses (Percoidei: Centrarchidae) as Evidenced by the Mitochondrial Cytochrome b Gene. *Copeia* **2002**:897–905 DOI 10.1643/0045-8511(2002)002[0897:PROTGO]2.0.CO;2.

Rubin BER, Ree RH, Moreau CS. 2012. Inferring phylogenies from RAD sequence data. *PLoS ONE* **7**:e33394 DOI 10.1371/journal.pone.0033394.

Sanderson MJ. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* **19**:101–109 DOI 10.1093/oxfordjournals.molbev.a003974.

Schluter D. 1996. Adaptive radiation along genetic lines of least resistance. *Evolution* **50**:1766–1774 DOI 10.1111/j.1558-5646.1996.tb03563.x.

Schluter D. 2000. *The ecology of adaptive radiation*. OUP Oxford.

Seehausen O. 2006. African cichlid fish: a model system in adaptive radiation research. *Proceedings of the Royal Society B: Biological Sciences* **273**:1987–1998 DOI 10.1098/rspb.2006.3539.

Sietman B, Hove M, Davis M. 2018. Host attraction, brooding phenology, and host specialization on freshwater drum by 4 freshwater mussel species. *Freshwater Science* **37**:000–000 DOI 10.1086/696382.

Smith CH, Pfeiffer JM, Johnson NA. 2020. Comparative phylogenomics reveal complex evolution of life history strategies in a clade of bivalves with parasitic larvae (Bivalvia: Unionoida: Ambleminae). *Cladistics* **36**:505–520 DOI 10.1111/cla.12423.

Smith SA, O’Meara BC. 2012. treePL: divergence time estimation using penalized likelihood for large phylogenies. *Bioinformatics* **28**:2689–2690 DOI 10.1093/bioinformatics/bts492.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**:1312–1313 DOI 10.1093/bioinformatics/btu033.

Strayer DL. 1981. Notes on the microhabitats of unionid mussels in some michigan streams. *American Midland Naturalist* **106**:411–415 DOI 10.2307/2425181.

Strayer DL, Ralley J. 1993. Microhabitat use by an assemblage of stream-dwelling unionaceans (Bivalvia), including two rare species of Alasmidonta. *Journal of the North American Benthological Society* **12**:247–258 DOI 10.2307/1467459.
Tran K, Ackerman JD. 2019. Mussels partition resources from natural waters under flowing conditions. *Science of The Total Environment* **696**:133870 DOI 10.1016/j.scitotenv.2019.133870.

Van Valen L. 1977. The red queen. *The American Naturalist* **111**:809–810 DOI 10.1086/283213.

Vaughn CC, Nichols SJ, Spooner DE. 2008. Community and foodweb ecology of freshwater mussels. *Journal of the North American Benthological Society* **27**:409–423 DOI 10.1899/07-058.1.

Watters GT. 2001. The evolution of the unionacea in North America, and its implications for the worldwide fauna. In: Bauer G, Wächtler K, eds. *Ecology and evolution of the freshwater mussels Unionoida*. Berlin, Heidelberg: Springer Berlin Heidelberg, 281–307 DOI 10.1007/978-3-642-56869-5_15.

Watters GT. 2018. A preliminary review of the nominal genus *Villosa* of freshwater mussels (Bivalvia, Unionidae) in North America.

Williams JD, Bogan AE, Butler RS, Cummings KS, Garner JT, Harris JL, Johnson NA, Watters GT. 2017. A revised list of the freshwater mussels (Mollusca: Bivalvia: Unionida) of the United States and Canada. *Freshwater Mollusk Biology and Conservation* **20**:33–58 DOI 10.31931/fmbc.v20i2.2017.33-58.

Yang Z, Kumar S, Nei M. 1995. A new method of inference of ancestral nucleotide and amino acid sequences. *Genetics* **141**:1641–1650 DOI 10.1093/genetics/141.4.1641.

Zanatta DT, Fraley SJ, Murphy RW. 2007. Population structure and mantle display polymorphisms in the wavy-rayed lampmussel, *Lampsilis fasciola* (Bivalvia: Unionidae). *Canadian Journal of Zoology* **85**:1169–1181 DOI 10.1139/Z07-089.

Zanatta DT, Murphy RW. 2006. Evolution of active host-attraction strategies in the freshwater mussel tribe Lampsilini (Bivalvia: Unionidae). *Molecular Phylogenetics and Evolution* **41**:195–208 DOI 10.1016/j.ympev.2006.05.030.