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Ecological correlates and phylogenetic signal of host use in North American unionid mussels

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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 was 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 specificity 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 the 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 the proportion of hosts shared, yet total variation as explained by the best model was low ($R^2 = 0.14$). There was evidence of a topological association between mussels and their hosts ($P = 0.001$) 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.

1. 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; Desdevises et al., 2002; 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 specificity (number of hosts used) of parasites is suspected to have strong effects on evolutionary rates and epidemiology

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(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 macroevolutionary 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 million years ago (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 comprises 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 (Fig. 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

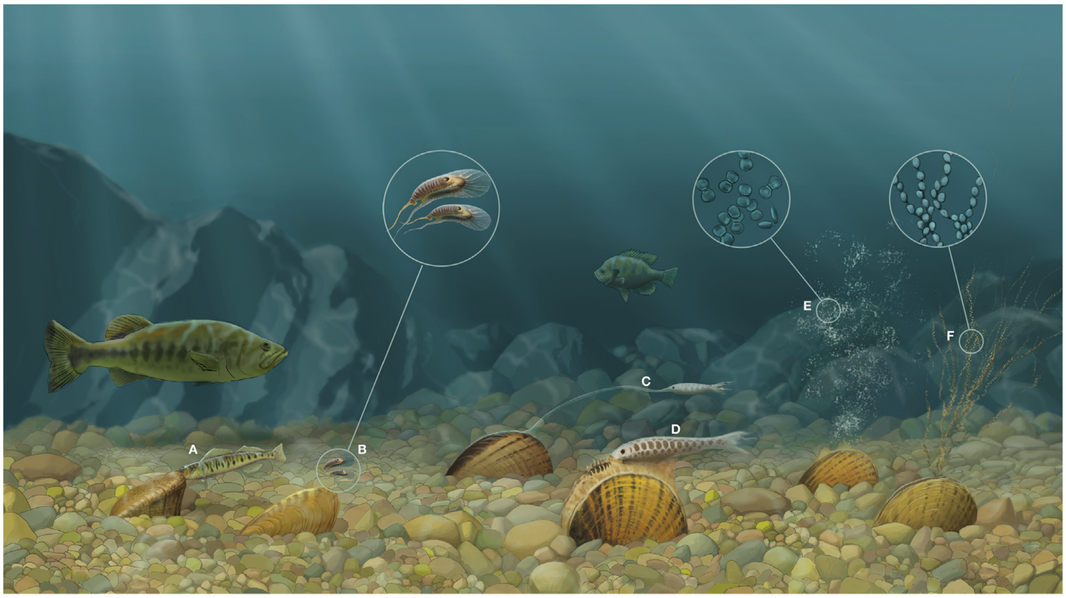

Fig. 1. A hypothetical benthic assemblage showing exemplars of North American freshwater mussel host infection strategies (Barnhart et al., 2008; Haag, 2012). (A) *Epioblasma triquetra*: physical entrapment of the host. (B) *Ptychobranchus subtentum*: releasing conglutinates containing glochidia that 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 and F represent "non-lure" broadcast strategies.

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 specificity is a conserved or labile trait in the Unionidae. We also do not understand whether the frequency distribution of unionid host specificity 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 specificity for diversification rates (Krasnov et al., 2005). Parasite taxa generally have a right-skewed distribution of host specificity, 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 specificity (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 factors are associated with host specificity in unionid mussels?

2. Materials and methods

2.1. 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) as are their fish hosts (Cummings and Watters, 2016). The phylogeny of Campbell et al. (2005) represented all 37 recognized genera of Amblemine (a subfamily that comprises approximately 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 (Illinois Natural History Survey Freshwater Mussel Host Database; https://wwx.inhs.illinois.edu/collections/mollusk/data/freshwater-mussel-host-database) 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 including natural infestation, natural metamorphosis observed, laboratory infestation, and laboratory metamorphosis observed. 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 host species reported for each mussel species), each mussel's binomial name was queried in Web of Science (Reuters, 2012; www.webofknowledge.com) 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; http://services.natureserve.org/index.jsp), 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 Global Biodiversity Information Facility (GBIF; https://www.gbif.org) occurrence point data, extracted using the 'dismo' package in the R (version 3.4.3) programing language (R foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org; Hijmans et al., 2015. dismo: species distribution modeling. R package version 1.0–12). 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., 2018. rgdal: bindings for the geospatial data abstraction library. R Package Version 1.2–18; Hijmans et al., 2012. Geosphere: spherical trigonometry. R Package Version 1–2). A phylogenetic tree for 7822 species of fish created using a mega-phylogeny approach (Smith et al., 2009) was obtained from Rabosky et al. (2013).

2.2. Frequency distribution of host specificity

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 specificity (number of hosts per mussel) across this subset of North American unionids. This frequency distribution was then compared with several ideal distributions (Poisson, negative binomial, log-normal, normal, exponential, geometric, and Weibull). Using the MASS package, implemented in R, the unionid host specificity 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).

2.3. 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 hosts 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 the 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 S1 (https://data.mendeley.com/datasets/d5cgjdgtpj/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.

2.4. 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., non-random 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 v 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.

2.5. Phylogenetic signal of host specificity

We used the Bayesian phylogenetic tree created for unionid mussels (see Section 2.4) and attached the number of hosts to each tip using the 'phylobase' package in R (Hackathon et al., 2013. phylobase: base package for phylogenetic structures and comparative data. R Package Version 06 5). 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 replications (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.

2.6. 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. We used phylogenetic least squared regression (PGLS) models instead of alternative models because the PGLS models have been shown to produce better estimates of phylogenetic parameters (Freckleton et al., 2011; Desdevises et al., 2015). 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 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 (see Section 2.4) 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.

3. Results

3.1. Database assembly

We searched the INHS mussel host database for the 69 species of unionid mussels 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).

3.2. Frequency distribution of host specificity is right-skewed

The frequency distribution of the number of hosts per mussel was right-skewed (Fig. 2), consistent with the frequency distribution of host specificity 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).

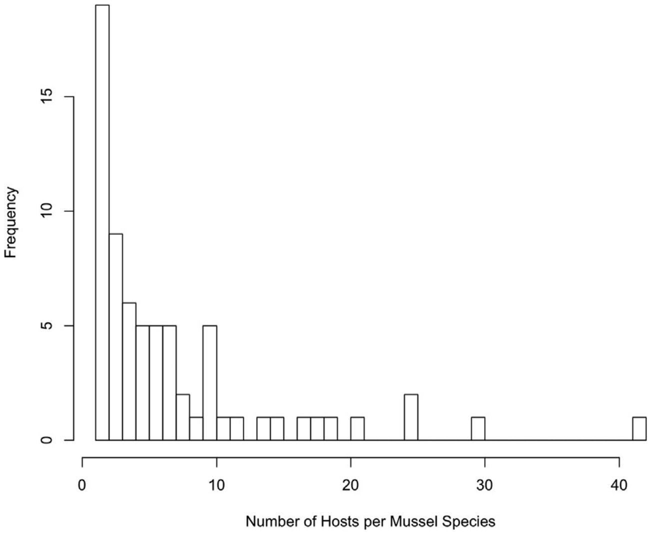

Fig. 2. A histogram displaying the frequency distribution of the number of hosts per mussel for 69 North American mussel species.

Table 1
The log-likelihood values for fitting the frequency distribution of the number of hosts per mussel to seven different frequency distributions.

Distribution Name	Log-likelihood Value
Log-normal	−198.8690
Weibull	−204.5227
Exponential	−204.9611
Negative Binomial	−207.2542
Geometric	−209.5612
Normal	−237.5575
Poisson	−325.0759

Table 2
Results from the multiple regression on distance matrices (MRM) model. Response variable is the number of shared hosts across all pairs of mussel species normalized by the total number of unique hosts per pair.

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

3.3. MRM: multiple variables show significant association with hosts shared

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

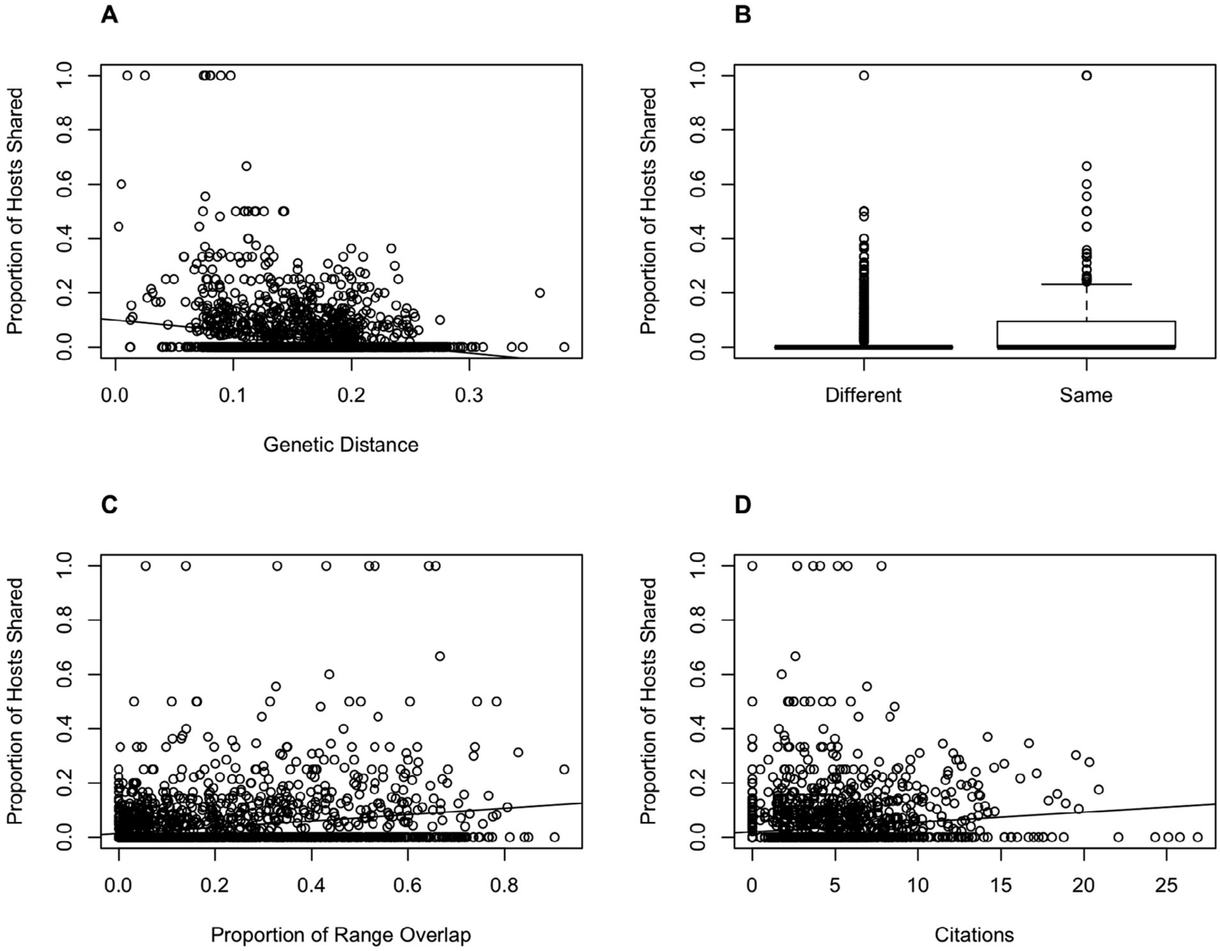

Fig. 3. Scatterplots and a boxplot displaying the proportion of hosts shared between pairs of North American unionid mussels over (A) genetic distance (K80; (Kimura, 1980)), (B) strategy similarity values, (C) proportion of range overlap, and (D) product of log number of citations for all pairs of unionid mussels in this analysis.

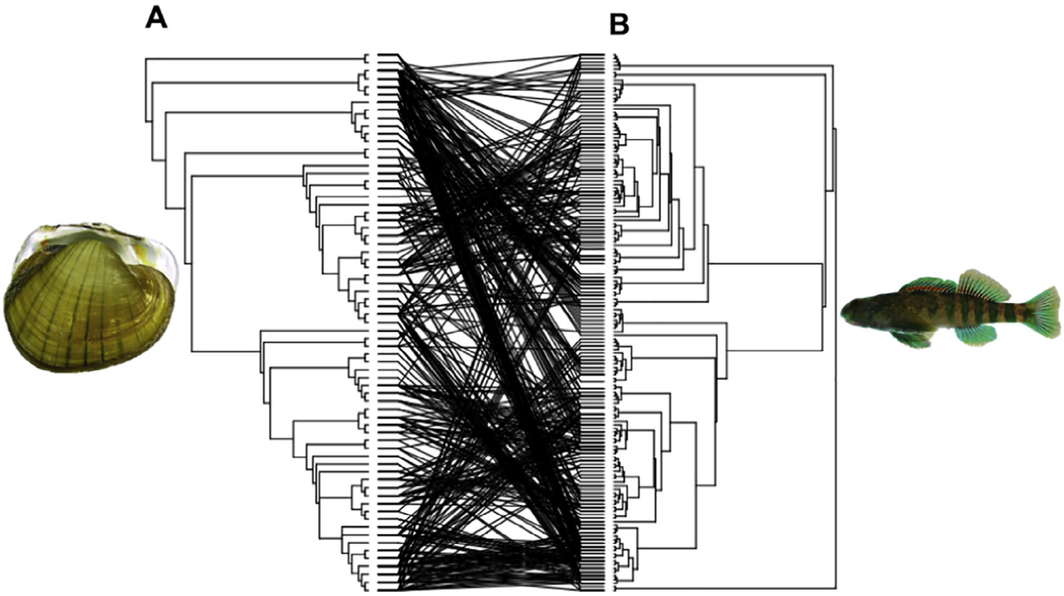

Fig. 4. A phylogeny of a subset of North American freshwater mussels (A) and a phylogeny of their host fish (B). Connecting lines represent all known parasitic interactions. The Parafit test of topological congruence suggests the interactions between mussels and fish are non-randomly distributed ($P = 0.001$).

single variable (range overlap, genetic distance, citations, and strategy similarity) were significant (Fig. 3). Range overlap, genetic distance, and citations were highly significant ($P < 0.001$), but the variation in the proportion of hosts shared as explained by any of the MRM models was very low. A large proportion of mussel pairs had zero hosts in common (1774 out of 2346 comparisons). This analysis was performed again on the subset of non-zero interactions. These models showed only a small increase in model performance, suggesting the abundance of zeros does not account for poor model performance.

3.4. ParaFit: significant 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 ($P = 0.001$). Fig. 4 displays the mussel and fish phylogenies used in this analysis, including all known parasitic interactions between the two groups.

3.5. Phylogenetic signal detected for host specificity in unionid mussels

Significant phylogenetic signal was detected for mussel host specificity 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 specificity) is displayed next to each tip on the unionid phylogeny and centered visualization (Fig. 5).

3.6. PGLS: habitat factors and phylogeny associated with host specificity

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 results are displayed in Table 3. 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 ($P = 0.020$). The variable glacial was positively associated with the 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 ($P = 0.102$; $\beta = -0.57$), low-gradient habitat ($P = 0.013$; $\beta = -0.75$), and riffle habitat ($P = 0.137$; $\beta = -0.45$). 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. The third, fourth and fifth models include combinations of the same variables found in the first and second-best models, except the fifth-best model which includes the creek variable together with glacial, low-gradient, and riffle habitats. The parameter estimates for all these models can be found in Table 3. See Fig. 6 for visual representations of all significant variables in the top five models (Glacier, Low-gradient, Riffle, and Number of Citations).

4. Discussion

This study identifies both habitat and phylogeny as important predictors of host specificity in a subset of North American unionid mussels. There is significant phylogenetic signal of host specificity in unionid mussels and, after controlling for phylogeny, certain habitat characteristics (low-gradient, riffle habitat, and post-glacial colonization) were associated with host specificity. Mussels and their host fish show evidence of topological congruence, which

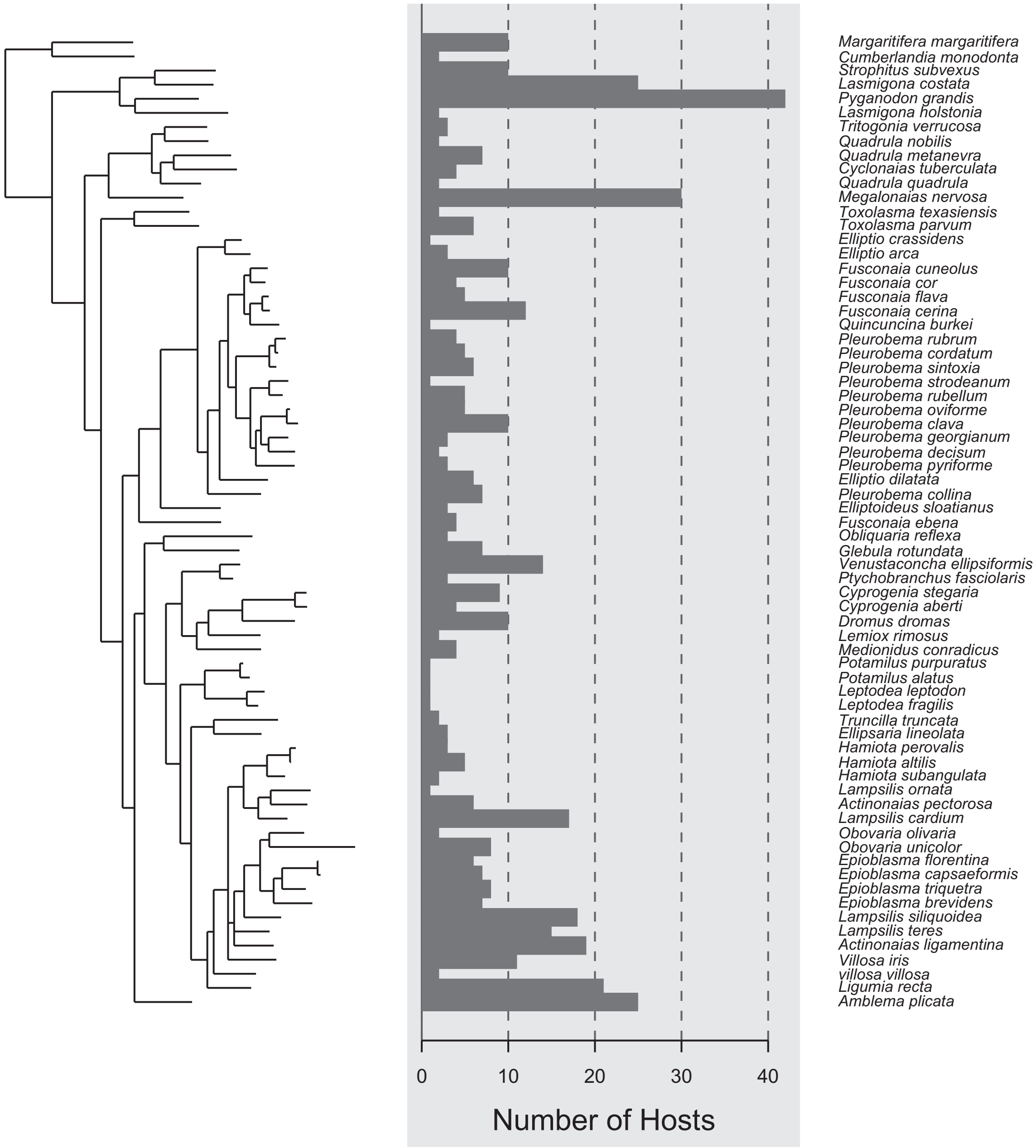

Fig. 5. The mussel phylogenetic tree alongside the trait value host specificity for each species of mussel. Phylogenetic signal was detected for host specificity in unionid mussels ($\lambda = 0.81$, $P = 0.003$).

Table 3

Results from the Phylogenetic Least Squares Regression models (PGLS) displaying Akaike Information Criterion (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 (http://services.natureserve.org/index.jsp). Glacial refers to a binary variable indicating species for which the 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 (www.webofknowledge.com). Parameter estimates for each model are included in parentheses and significant predictors are denoted with[a] ($\alpha = 0.05$).

Model	AIC	ΔAIC	Weight	λ
Glacial ($\beta^a = 0.54$) + Lowgrade ($\beta^a = -0.91$) + Riffle ($\beta^a = -0.71$)	224.5	0	0.015	0.88
Citations ($\beta^a = 0.42$) + Infection Strategy ($\beta = -0.57$) + Lowgrade ($\beta^a = -0.75$) + Riffle ($\beta = -0.45$)	224.6	0.1	0.014	0
Citations ($\beta^a = 0.47$) + Infection Strategy ($\beta = -0.53$) + Lowgrade ($\beta^a = -0.73$)	224.7	0.2	0.014	0
Citations ($\beta^* = 0.51$) + Lowgrade ($\beta^a = -0.61$)	224.8	0.3	0.013	0
Glacial ($\beta^a = 0.55$) + Creek ($\beta = 0.47$) + Lowgrade ($\beta^a = -0.98$) + Riffle ($\beta^a = -0.81$)	224.8	0.3	0.013	0.92

[a] Statistically significant ($P < 0.05$).

may be due to cospeciation or speciation via host shifts (de Vienne et al., 2013). Genetic distance, proportion of specificity overlap, number of citations, and strategy similarity were all significantly associated with the proportion of hosts shared between mussels.

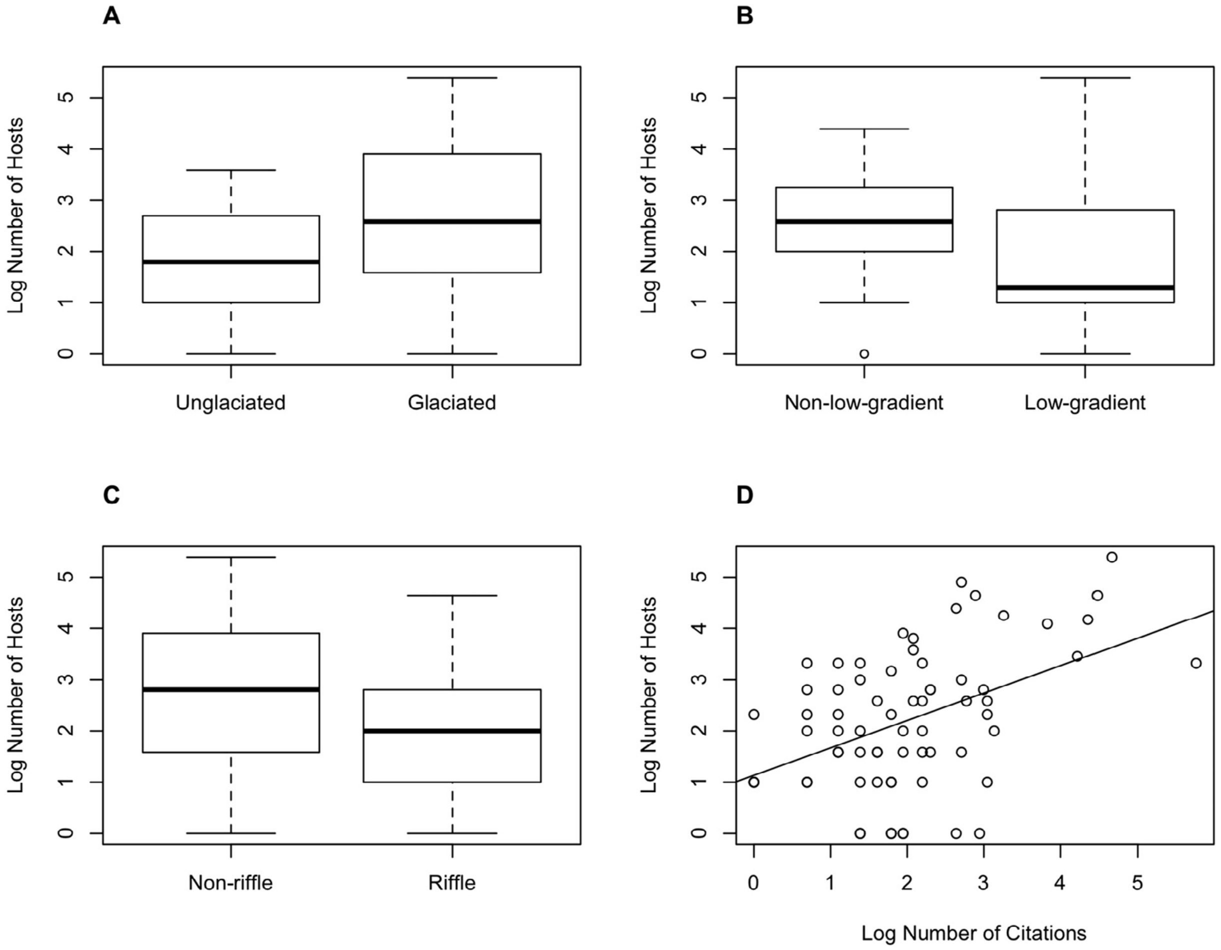

Fig. 6. Boxplots and a scatterplot displaying the log number of hosts over all significant variables found in the top five Phylogenetic Least Squares Regression models as indicated by Akaike Information Criterion including (A) post-glacial colonization, (B) low-gradient habitat, (C) riffle habitat, and (D) log number of Web of Science citations.

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). After removing all mussel pairs with zero hosts in common the R^2 increased to 0.20 for the model with all variables included. This small increase in model performance suggests that the poor fit of the model is not being driven by the large proportion of zeros in the dataset.

Phylogenetic signal of host specificity, 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 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 mussels can live for over 100 years, and the larval life stage usually lasts for only 3–4 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 specificity (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 (Moran's I and Abouheif's C_{mean}) were not significant. There is some evidence that statistical methods of estimating phylogenetic signal have low statistical power to detect phylogenetic signal relative to model-based approaches (Diniz-Filho et al., 2012). 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 specificity of unionid mussels is inherited, creating clades of predominately specialist and predominately generalist taxa. Given that host specificity in unionid mussels is inherited, and host specificity 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 an area that was covered by the Laurentide ice sheet during the last glacial maximum, a variable indicating a low-gradient habitat type, and a variable indicating a riffle type habitat. This model also had a very high estimated phylogenetic signal in the model residuals (Pagel's $\lambda = 0.88$), 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 are 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 in 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 specificity 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). The third best model also included the number of citations, infection strategy variable, and low-gradient variable, making it very similar to model #2, however it lacks the riffle variable. The fourth best model is also a subset of the variables found in model #2 and includes only the citations variable and the low-gradient variable. The fifth-best model included the glacial, creek, low-gradient, and riffle habitat variables. This model includes all the variables found in the best model, with the addition of the creek variable.

Parasitism provides a new axis in the bivalve niche that enables closely related species to diverge (Hutchinson, 1957. Population studies-animal ecology and demography-concluding remarks. Presented at the Cold Spring Harbor Symposia on Quantitative Biology, Plainview, NY, USA). 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 (Fig. 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 the transformation success rate between different host species and even different populations of the same host species (Riusech and Barnhart, 2000. Host suitability and utilization in *Venustaconcha ellipsiformis* and *Venustaconcha pleasii* (Bivalvia: Unionidae) from the Ozark Plateaus. Presented at the Freshwater mollusk symposia proceedings, Columbus, Ohio, USA; 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 specificity (number of hosts per mussel) was shown to have strong phylogenetic signal in this system. Given the theoretical importance of host specificity on speciation and extinction rates, diversification rates should be estimated for the Unionidae to determine whether these rates correlate with host specificity. The development of a large and comprehensive unionid phylogeny using high-throughput sequencing will increase the accuracy of these estimates.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2018.09.006.

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