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Author: Geheber, Aaron D.

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Contemporary and Historical Species Relationships Reveal Assembly Mechanism Intricacies among Co-occurring Darters (Percidae: Etheostomatinae)

Aaron D. Geheber[1]

Examination of contemporary (ecological) and historical (evolutionary) relationships within fish assemblages may provide understanding of the relative influences of competitive exclusion and habitat filtering assembly mechanisms. However, interpretation of relationship patterns (e.g., even or clustered) must consider niche conservatism, ecological convergence, abundance weighting, and spatial scale for proper understanding. The goals of this study were to identify influences of habitat filtering and competitive exclusion assembly mechanisms in darter (Percidae: Etheostomatinae) assemblages in the Duck River, Tennessee, and to build a conceptual framework describing the intricacies of assembly within this species-rich system. Phylogenetic relatedness, abundance weighting, habitat use similarity, functional morphological relatedness, and niche conservatism (for habitat use and morphology) were quantified for 15 darter assemblages along the stream gradient to elucidate magnitude and progression of assembly mechanisms. Habitat filtering was identified as a primary contributor to assembly based on findings of phylogenetic clustering and consistent co-occurrence of species that shared similar habitat requirements. Morever, lack of habitat use conservatism suggested that darters of two clades (both well represented in assemblages of the study) have converged on similar habitat needs in the system. Competitive interactions may have followed the initial habitat filter, as demonstrated by limiting morphological similarity within assemblages. This study demonstrates the importance of accounting for contemporary and historical factors to identify magnitude and progression of assembly mechanisms in fish assemblages. Furthermore, this study builds upon previous darter assembly work and provides a novel illustration of how habitat use convergence between phylogenetic clades can alter interpretations of assembly mechanisms in species-rich communities.

A fundamental goal in ecology is to identify mechanisms responsible for the distributions of organisms across time and space. Because organisms occur in complex communities where they engage in (and have historically engaged in) a multitude of interactions, much work has taken aim at understanding which interactions shape community composition (e.g., Grossman, 1982; Tilman, 2004; Maherali and Klironomos, 2007; Pearson et al., 2018). Although both biotic (competitive exclusion and predation) and abiotic (habitat filtering) mechanisms are thought to play important roles in community assembly (Kelt et al., 1995; Poff, 1997; Forsman et al., 2001; Vamosi et al., 2009), additional community features may influence interpretation of these mechanisms. For example, differences in scope of study (e.g., spatial, temporal, and trophic levels) have led to a variety of conclusions concerning the relative influences of competition and habitat filtering assembly mechanisms (Kraft and Ackerly, 2010; Fine and Kembel, 2011; Godoy et al., 2014; Geheber and Geheber, 2016; Pontarp and Petchey, 2016; Fitzgerald et al., 2017). For most systems, order and magnitude of assembly mechanisms are not well established due to the complex ecological and evolutionary relationships which may underlie species interactions. This is especially true for species-rich temperate and tropical stream fish assemblages, due to the numerous species interactions that are plausible at relatively fine spatial scales.

Multiple approaches towards quantifying assembly mechanisms have been developed, including quantification of contemporary habitat use and morphological similarity among community members (e.g., Weiher and Keddy, 1995; Kraft et al., 2008; Ingram and Shurin, 2009; Fitzgerald et al., 2017), species occurrence patterns based on historical biogeographic reconstructions (e.g., Leibold et al., 2010; Weeks et al., 2016), and patterns of evolutionary relatedness between co-occurring species (e.g., Vamosi et al., 2009). In general, these approaches suggest competitive exclusion will result in patterns of evenness (i.e., limiting similarity) among co-occurring species (Webb, 2000; Vamosi et al., 2009), and habitat filtering will result in ecologically similar species “clustering” together due to their assumed overlap in habitat requirements (Weiher and Keddy, 1995; Poff, 1997; Webb et al., 2002).

Although community assembly mechanisms (e.g., habitat filtering and competitive exclusion) are likely dependent on both contemporary ecological relationships and historical evolutionary factors, numerous studies have approached assembly dynamics in an “either/or” context. This is problematic, because interpreting phylogenetic relatedness patterns as proxies for contemporary ecological functionality can be misleading (Barnagaud et al., 2014; Gerhold et al., 2015). On the other hand, examining assembly mechanisms based on ecologically relevant trait distributions without acknowledging the underlying evolutionary relationships among community members may be equally concerning. In addition, past studies of community assembly have often ignored species’ abundances (or biomass), which likely have some bearing on interpretation of assembly dynamics (Anderson et al., 2004; Hardy, 2008; Vamosi et al., 2009). For example, if the most abundant species in a community is also the most “distinct” (i.e., ecologically and/or phylogenetically) compared to all other community members, competitive exclusion may well be a driving assembly mechanism. However, under this scenario, if all other co-occurring species are rare yet similar (i.e., ecologically and/or phylogenetically clustered), this signal for competitive exclusion might be overlooked without inclusion of abundance weighting.

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[1] University of Central Missouri, School of Natural Sciences, W.C. Morris 111, Warrensburg, Missouri 64093; Email: geheber@ucmo.edu.
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In stream fishes, it is often assumed that close phylogenetic relatives have retained ecologically informative ancestral traits (i.e., niche conservatism); however, these relationships are not always cut-and-dried, and understanding how ecologies have evolved is crucial for untangling relative influences of assembly mechanisms. Specifically, phylogenetic niche conservatism is met when ancestral ecological factors are more similar among clade members than what would be expected under a Brownian motion model of evolution (Losos, 2008). If this assumption is not met, recognizing the possibility of ecological convergence among co-occurring species, or between clades of species, may be of great importance when inferring habitat filtering and competitive exclusion assembly mechanisms. In general, a conceptual framework that integrates ecological and evolutionary data appropriately may provide necessary tools for teasing apart and illuminating the relative influences of assembly mechanisms (Kraft et al., 2007; Graham et al., 2012; Barnagaud et al., 2014).

Multiple scenarios may arise when attempting to understand how ecological and evolutionary relationships explain habitat filtering and/or competitive exclusion assembly mechanisms (Fig. 1). As previously stated, it is typically reasoned that patterns of clustering are resultant of habitat filtering and patterns of evenness are resultant of competitive exclusion mechanisms; however, these patterns are only expected when niche conservatism has been met (Fig. 1A). In cases where underlying habitat use convergence exists among co-occurring darters, species with similar habitat affinities may be evenly distributed across the phylogeny tips as a result of habitat filtering, rather than clustered. Furthermore, competitive exclusion mechanisms may lead to phylogenetic clustering despite habitat use disparity among co-occurring darters (Fig. 1B). Assembly scenario can become more complex when clade level habitat use convergence within darter assemblages exists, because of the possibility that niche conservatism is maintained within individual clades, but not across the entirety of the regional phylogeny. Under such a scenario, clustering patterns are indicative of habitat filtering, although habitat use convergence (at the clade level) can lead to multiple species clusters (Fig. 1C). Patterns of evenness under this scenario (Fig. 1C) are resultant of a competitive exclusion mechanism limiting co-occurrence of close relatives that share habitat use requirements. This resultant pattern is like that expected from competitive exclusion under niche conservatism despite the loss of correlation between lineage and habitat use among darters. These scenarios shed light on constraints and possible interpretations of assembly mechanisms in species-rich assemblages of stream fishes where phylogenetic, morphological, and habitat use features are not always in “agreement.”

The southeastern United States harbors the most diverse temperate freshwater fish fauna in the world (Burr and Mayden, 1992; Warren et al., 1997; Lundberg et al., 2000). Large components of the ichthyofauna within these systems are darters (Percidae: Etheostomatinae), a group that is endemic to North America and contains an estimated 250 species (Scharpf, 2008; Near et al., 2011). Darters are small-bodied, benthic stream fishes that often occur in shallow areas with high flows known as riffles (Matthews et al., 1982; Harding et al., 1998). Darters rely on body shape characteristics to maintain position on the streambed and to navigate the substrate without being displaced by high flows. Within the Duck River system (Lower Tennessee River drainage), ~30 species of darters are known to occur, and multiple species often co-exist in local-scale assemblages. Because these assemblages harbor exceptional species diversity at small spatial scales (i.e., as compared to other vertebrate assemblages), interactions between species within assemblages are plausible (Vamosi et al., 2009). Moreover, high levels of darter co-occurrence in stream assemblages provide a suitable testing ground for examining the relative influences (and complexities) of habitat filtering and competitive exclusion mechanisms.

Phylogenetic relationships, habitat use, and functional morphology among darter assemblage members were integrated to evaluate the importance of competitive exclusion and habitat filtering assembly mechanisms in a temperate stream system. The objectives were i) to test for significant phylogenetic clustering or evenness among co-occurring darters, using both species presence/absence and weighted abundance data, ii) to test levels of habitat use and morphological clustering or evenness within darter assemblages, and iii) to test for phylogenetic niche conservatism among Duck River darter species by incorporating habitat use and functional morphology as proxies for species' niches. Here, niche conservatism is met when close phylogenetic relatives have retained ancestral habitat usage and morphological features. The overarching goals of this study were to identify the relative influences of competitive interactions and habitat filtering mechanisms responsible for present day structure in Duck River, Tennessee, darter assemblages, and build a conceptual framework describing the intricacies of darter assembly within this species-rich system.

MATERIALS AND METHODS

Data collection.—Fishes and abiotic data were collected at 15 stream sites across a ~340 km stretch of the Duck River in Tennessee, United States (Fig. 2). Collections were made during June 2012 (19th–27th), sites were representative of summer low flow conditions in the system, and weather conditions remained stable throughout the sampling period. Each site was chosen *a priori* based on the presence of riffle habitat (mean stream surface area for sites ~0.045 ha). Within each stream site, darters and abiotic parameters were collected from 18 quadrats (1.5 m x 1.5 m) evenly distributed across three transects. Transects were established at the lower, intermediate, and upper portion of each site ($n = 270$ quadrats total in the study). Transects spanned the width of the stream channel perpendicular to stream flow, and six quadrats were evenly spaced across each transect to ensure unbiased sampling of available habitat. At each quadrat, fishes were collected via kick seining using a weighted 1.8 m seine net (3 mm mesh). Species collected from the 18 quadrats within each stream site were pooled prior to phylogenetic community analyses, so that assembly at the site scale could be examined (see Geheber and Geheber, 2016 for comparisons of quadrats, sites, and drainages). Specimens were preserved in 10% formalin and later cataloged at the Sam Noble Oklahoma Museum of Natural History (SNOMNH), University of Oklahoma.

Abiotic data collected from each quadrat included water depth (cm), flow velocity (m/s), and substrate size. Three flow velocity measures were taken from each quadrat using a FLO-MATE flowmeter (model 2000; Marsh-McBirney, Frederick, MD), and stream depth was measured at the same three points. Five random substrate grabs per quadrat were measured using a substrate sizer (AL-SCI Field Sieve, West

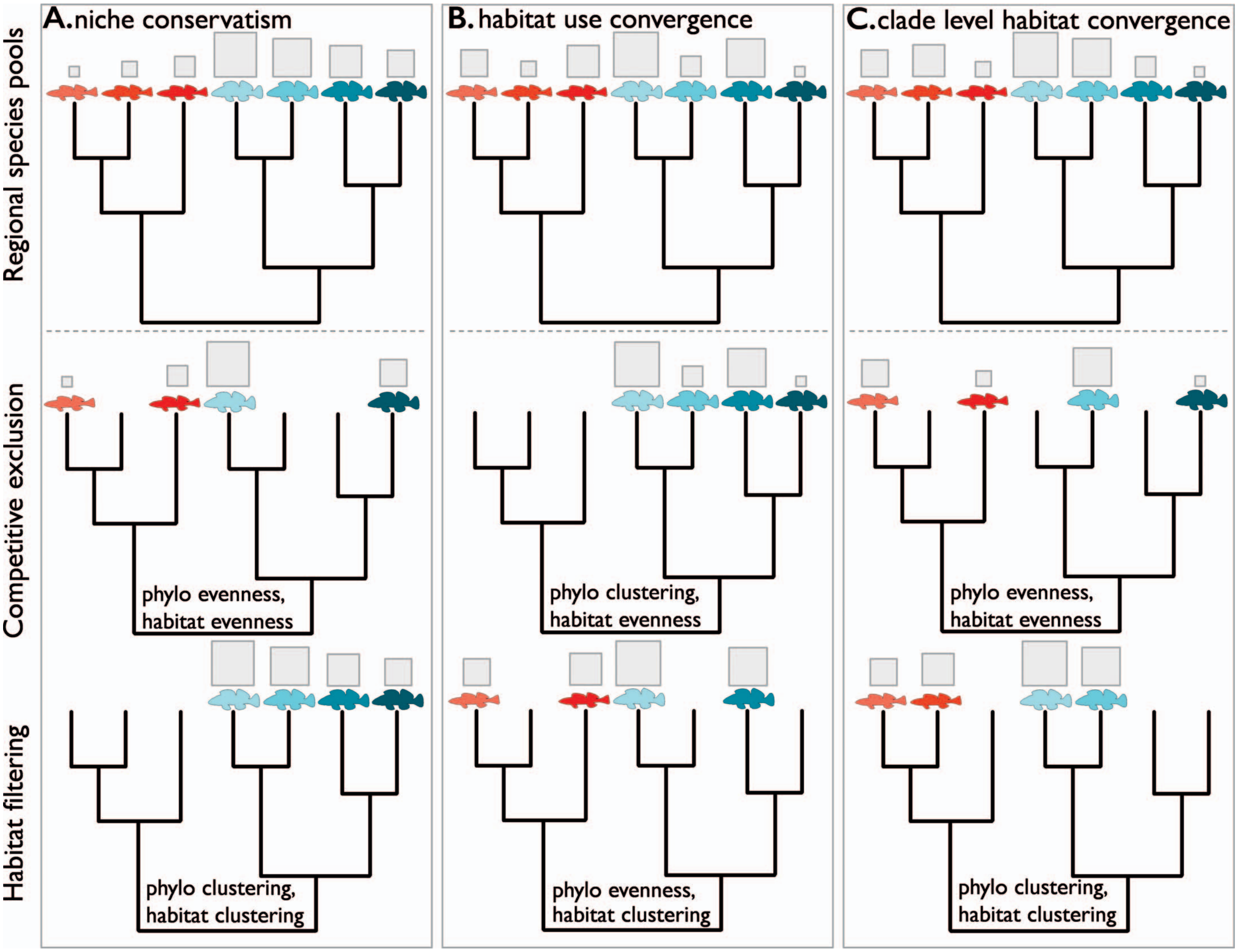

Fig. 1. Schematic diagram depicting influences of habitat filtering and/or competitive exclusion and the hypothetical data patterns generated by these two mechanisms across three assembly scenarios. Interpretation of data patterns for each scenario is influenced by the presence of niche conservatism (i.e., whether closely related species have retained ecologically important ancestral traits) or by the presence of convergence in habitat use among co-occurring species. Regional species pool phylogenies and the predicted scenario outcomes are encompassed by boxes (A, B, and C). Darter morphology is shown primarily by the darter silhouettes on the tree tips, and secondarily by darter color shade. Therefore, darter morphology is depicted as conserved in all three scenarios. Habitat use for each "species" is indicated by the size of the boxes located above each darter depiction in each scenario (e.g., darters with similar sized boxes have similar habitat use). Hypothetical assemblages containing four species each are depicted below each regional species phylogeny, and active assembly mechanisms related to each assemblage outcome (habitat filtering or competitive exclusion) are indicated to the left. Associated phylogenetic (phylo) and habitat use (habitat) patterns are indicated by the terms "clustering" or "evenness" within each possible outcome.

Trenton, NJ), and substrate was categorized as gravel (2–16 mm), pebble (16–64 mm), cobble (64–256 mm), boulder (>256 mm), or bedrock.

***Phylogeny*.**—The regional species pool was defined as species within the family Percidae occurring within the Duck River drainage, which was determined based on species distributions from Etnier and Starnes (1993) and all available museum records (accessed through the Fishnet2 Portal, http://www.fishnet2.net). Twenty-eight species were included in the regional pool. A maximum likelihood molecular phylogeny for the regional species pool was generated using multiple nuclear and mitochondrial genes (cytochrome *b* [*Cyt b*], s7 intron 1 [*S7*], cytochrome oxidase 1 [*CO1*], NADH dehydrogenase 2 [*ND2*], recombination activating gene 1 [*RAG1*]). Sequences were downloaded from the National Center for Biotechnology Information's GenBank and aligned using the MUSCLE algorithm (Edgar, 2004). Randomized Axelerated Maximum Likelihood (RAxML) was run on the unpartitioned data set using BlackBox (Stamatakis, 2006; Stamatakis et al., 2008). The general time-reversible model of nucleotide evolution (GTRCAT) was selected for the analysis. The RAxML tree was transformed to ultrametric using the *chronopl* function in the R package *ape* (Sanderson, 2002; Paradis et al., 2004) prior to all subsequent assemblage structure analyses. This is commonly done and necessary prior to phylogenetic community structure analyses (e.g., Gomez et al., 2010). Additionally, the phylogenetic hypothesis of Near et al. (2011) was also used in the following analyses (in addition to the phylogenetic hypothesis generated for the present study). Because assemblage structure results were nearly identical when using either phylogeny, results from the phylogenetic hypothesis generated for the present study are presented.

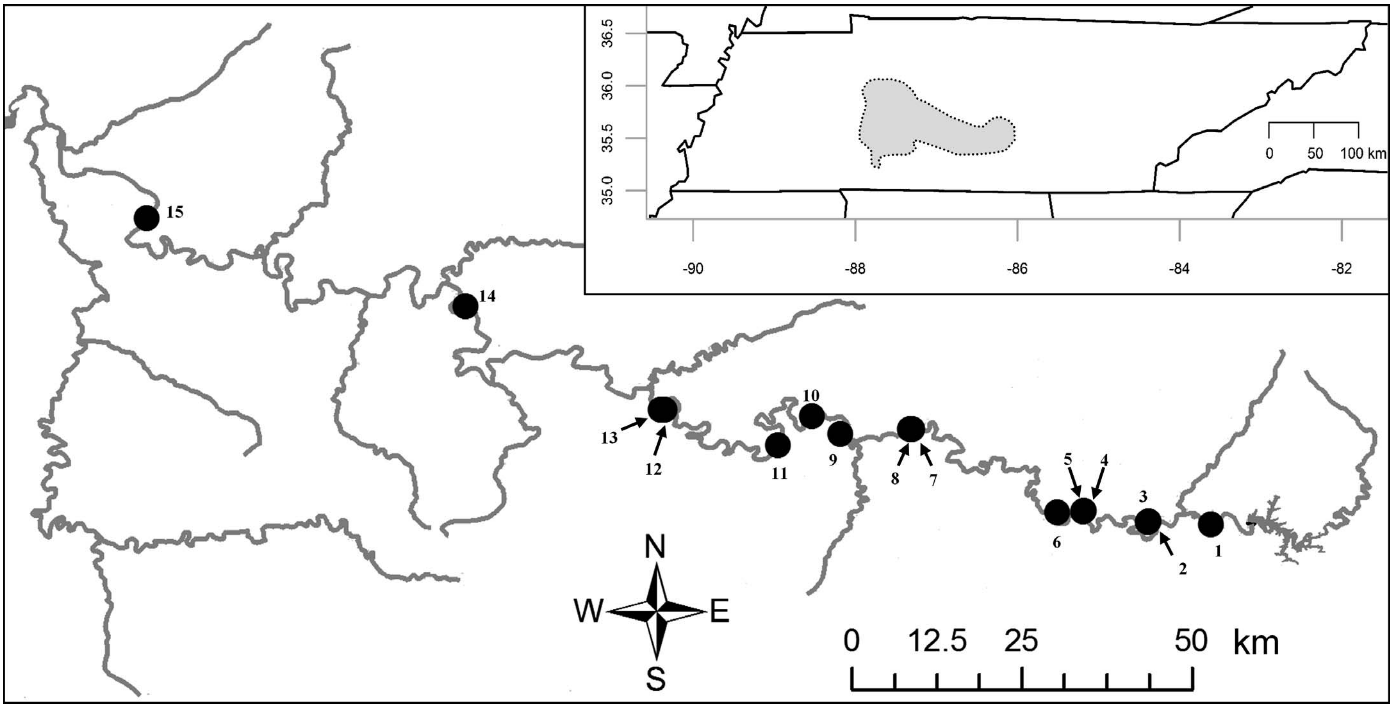

Fig. 2. Map of Duck River drainage showing the 15 study sites. Inserted panel depicts an overview of the location of the Duck River drainage in Tennessee, USA.

Phylogenetic assemblage structure.—To address the first study objective, metrics of phylogenetic relatedness were calculated using Phylocom version 4.2 (Webb et al., 2008, 2011). The *comstruct* function was used to calculate Mean Pairwise Distance (MPD) and Mean Nearest Phylogenetic Taxon Distance (MNTD) within each assemblage. Observed MPD and MNTD values were compared to those of 999 randomly generated assemblages in order to determine significance ($P \leq 0.05$). Null assemblages maintained the species richness of the observed assemblage, where species were randomly drawn without replacement from the regional pool. This model was chosen because of the assumption that species included in the regional phylogeny pool were not dispersal limited due to basic stream connectivity across sites; therefore, it was plausible that each species had an opportunity to occur in every site. To explore potential influence of this assumption, an additional null model which was limited to species present in samples was also run (i.e., randomly drew from species collected during the study rather than from the entire regional species pool). The two models yielded nearly identical results; therefore, results from the first model are presented. Using this null model, two metrics of standard effect size were calculated for each assemblage; the Net Relatedness Index (NRI) and the Nearest Taxon Index (NTI; Equations 1 and 2; from Webb, 2000).

$$\text{Equation 1} \qquad NRI = -1 \times \frac{MPD_{sample} - MPD_{rndsample}}{sd(MPD_{rndsample})}$$

$$\text{Equation 2} \qquad NTI = -1 \times \frac{MNTD_{sample} - MNTD_{rndsample}}{sd(MNTD_{rndsample})}$$

Net relatedness index (NRI) was calculated from MPD and measured the standard effect size of mean phylogenetic distance across the phylogeny between all species pairs co-occurring in an assemblage. This is a measure of how far apart species are on the phylogeny. Nearest taxon index (NTI) was calculated from the MNTD and measured the standard effect size of phylogenetic distance separating each species from its closest relative within an assemblage (i.e., nearest neighbor measure). Therefore, NRI is sensitive to phylogeny-wide patterns of clustering or evenness based on overall mean phylogenetic distances among co-occurring species, and NTI is sensitive to clustering or evenness patterns across the phylogeny tips because it measures distances between the closest phylogenetic relatives rather than distances among all co-occurring species (Webb, 2000). It should be noted that both NRI and NTI aim to measure the same two patterns of phylogenetic structure (i.e., clustering and evenness); however, each uses a slightly different approach. Both metrics were included due to the different strengths and weaknesses provided when identifying patterns of phylogenetic structure (Kraft et al., 2007). Positive values of NRI or NTI indicate phylogenetic clustering, whereas negative values suggest phylogenetic evenness (Webb et al., 2002). All *comstruct* procedures were initially run using presence/absence data for each of the 15 assemblages, and additional runs incorporating species abundance weighting followed. Abundances within each site were depicted across the tips of the phylogeny using the R package *pez* (Pearse et al., 2015). Generally speaking, clustering patterns support habitat filtering-based assembly and evenness patterns support competitive exclusion-based assembly in the previously described analyses.

Phylomorphospace.—Body shape is an ecologically relevant feature in fishes that can be indicative of swimming ability, position holding, trophic niche, and predator–prey interactions (Webb, 1984; Matthews, 1985; Guill et al., 2003). Geometric morphometric techniques were used to quantify body shape for all darter species collected during the study. Twelve homologous landmarks were assigned/digitized to the left side of each specimen using TPSDig version 2.12 (Rohlf, 2008). All specimens were then aligned using Procrustes fit, and Principal Component Analysis (PCA) was performed on aligned landmark coordinates using MorphoJ version 1.01b (Klingenberg, 2008). To illustrate phylogeny–morphology relationships, the phylomorphospace approach of Sidlauskas (2008) and the R package *geomorph* (Adams and Otarola-Castillo, 2013) was employed. Principal component scores for the first three PCs were deemed interpretable based on the broken stick model (Jackson, 1993), and all three were retained for subsequent tests of phylogenetic niche conservatism.

Habitat use and morphological structure.—To address the second study objective, habitat use and morphological variance, range, and mean nearest trait distance (MNTD) among species within assemblages were examined. Habitat use factors were created for each species using the mean flow velocity, depth, and proportions of gravel, pebble, cobble, boulder, and bedrock from quadrats where each species occurred (for species-specific habitat use descriptions, see Geheber and Frenette, 2016). Morphology was assessed using PC scores (1–3) for each species (i.e., PC scores from previous geometric morphometric analyses). For these analyses, the species pool contained all species collected during the study period. Patterns of clustering and evenness were measured using standard effect size (SES) of variance, range, and MNTD among species for each habitat use and morphological factor within each assemblage (i.e., based on 999 randomized runs). These SES metrics were selected due to the known ability of variance and range measures to identify clustering patterns and the ability of MNTD to identify evenness patterns (Aiba et al., 2013). The SES equation used was a modified version of the *comtrait* function from Phylocom (Equation 3 modified from Webb et al., 2011). Standard Effect Size metrics were multiplied by –1 to maintain positive and negative value interpretations that were consistent with the interpretations of phylogenetic assemblage structure metrics (NRI and NTI). A similar equation modification has been used in previous work (see Geheber and Geheber, 2016). Therefore, SES values greater than zero indicated that co-occurring darter species were more similar than expected based on random distribution (clustered), and values less than zero indicated dissimilarity among co-occurring species (evenness) as compared to a random distribution.

Equation 3 $$SES_{metric} = -1 \times \frac{metric_{obs} - metric_{rnd}}{sd(metric_{rnd})}$$

Phylogenetic niche conservatism.—To examine the third study objective, niche conservatism for each morphological axis (PC1–3) and habitat use factor was tested among darter species collected during the study. Here, phylogenetic niche conservatism is a measure of whether ancestral morphology or habitat use factors are retained among closely related species (more similar than expected under a Brownian motion model of evolution; Losos, 2008). Using the *phylosignal* function in the R package *picante*, the *K* statistic for each morphological and habitat factor was calculated to quantify conservatism across the phylogeny in relation to rate of evolution under Brownian motion (Blomberg et al., 2003). The *K* statistic represents the strength of phylogenetic signal, where values of one indicate morphological or habitat use differences are proportional to tree branch lengths, and values greater than one indicate morphology or habitat use factors are conserved across the regional pool phylogeny. Therefore, phylogenetic niche conservatism has been met when species within a clade are more similar to one another than would be expected under Brownian motion evolution. Although *phylosignal* also outputs randomization tests for phylogenetic signal using independent contrasts, these values were not reported due to the low power of this analysis when examining trees with $<$20 species (Blomberg et al., 2003). It should be noted that the *K* statistic is a standardized measure and is not influenced by sample size. It is therefore an interpretable descriptor of phylogenetic niche conservatism regardless of the number of species included in the analysis (Blomberg et al., 2003).

RESULTS

Phylogenetic assemblage structure.—Most assemblages were found to have positive SES metric values with or without the incorporation of species' abundances. *Comstruct* results based on presence/absence were positive for 10 of the 15 assemblages (NRI) and showed significant phylogenetic clustering within three assemblages (Fig. 3). Moreover, nearest taxon index (NTI) values were positive for 12 of the 15 assemblages, and significant clustering was recovered within six assemblages (Fig. 3). Similarly, positive values were recovered from the *comstruct* procedure that included weighted abundances (Fig. 3). The regional phylogeny is presented alongside species abundance data (for each site) and illustrates the two main clades to which clustered species belong (Fig. 4). In addition, greater clustering was recovered upstream as compared to downstream assemblages (Fig. 3).

Phylomorphospace.—Relationships between phylogeny and body shape among Duck River darter species are depicted in Figure 5. The first three principal components (PCs) explained 80.46% of variance in body shape. Principal component 1 (48.25% of variation) described variation in head size where positive values indicated larger heads in proportion to body length (Fig. 5). Principal component 2 (18.85%) described overall variation in body length proportions (i.e., the length from the first dorsal-fin insertion and pelvic-fin insertion to the second dorsal-fin insertion and anal-fin insertion; Fig. 5). Furthermore, PC3 (13.37%) represented variation in head shape and mouth position (not depicted in Fig. 5).

Habitat use and morphological structure.—Co-occurring species showed similar (i.e., clustered) habitat use for all measured factors, as indicated by positive SES metric values, apart from "boulder" (Table 1). Negative values for "boulder," however, are most likely due to the rarity of boulders in the system, as null assemblages would often have a variance and range of zero for boulder affinity due to most species never occurring with boulders. Descriptions of individual species' habitat use, and differences in habitat use among Duck River darters, are explicitly discussed in Geheber and Frenette (2016).

Negative SES variance and range values for darter morphology were recovered for PC1 and PC2 (i.e., even) within assemblages, whereas PC3 values were positive within assemblages (i.e., clustered; Table 1). Darter morphology within assemblages had greater variation and range than expected in random assemblages based on head size (PC1) and body length (PC2). However, the MNTD measure resulted in PC1 and PC2 values close to zero indicating no

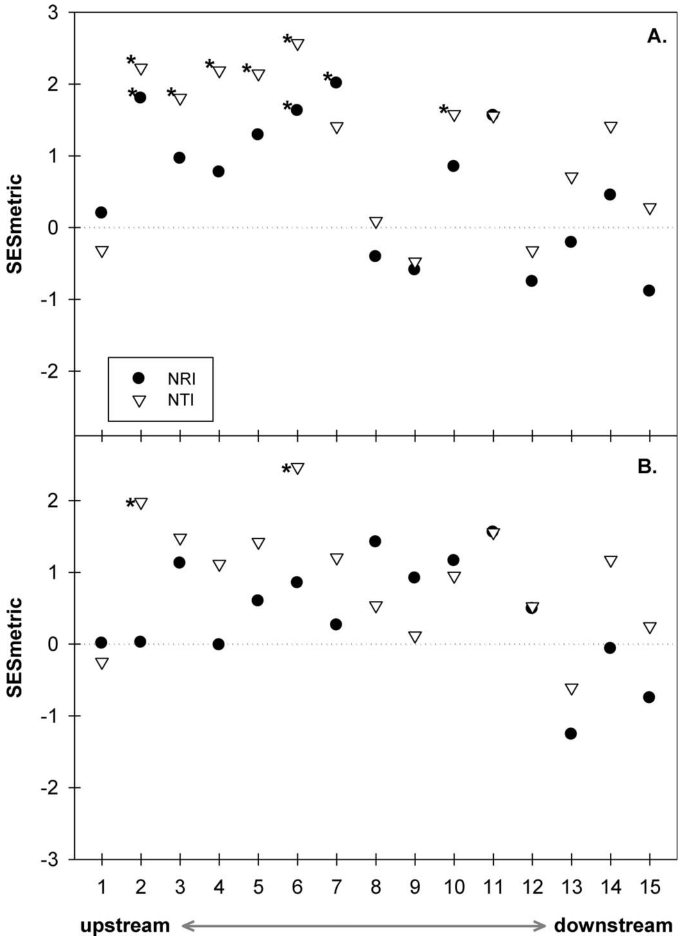

Fig. 3. Scatterplots depicting relationships between assemblages and degree of clustering or evenness based on the Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) standard effect size metrics (SESmetrics). Positive values indicate phylogenetic clustering and negative values indicate phylogenetic evenness. NRI values are black circles and NTI values are open triangles (*indicate significant phylogenetic structure in assemblages [$P \leq 0.05$]). (A) Depicts analyses based on species presence/absence within each assemblage, and (B) depicts analyses including weighted abundances, and therefore indicates relationships among individuals within assemblages.

clear clustering or evenness signal within assemblages (Table 1). All three SES metrics resulted in positive values for PC3 among species within assemblages (Table 1).

Phylogenetic niche conservatism.—Principal component 1 was conserved across the phylogeny and yielded a Blomberg's *K* > 1 (Table 1). This PC was the only factor to show phylogenetic conservatism within Duck River darters, although several other habitat use and morphological factors yielded *K* values approaching one (Table 1). Habitat use factors resulting in *K* values less than one may be indicative of habitat use convergence among more distantly related species.

DISCUSSION

An overarching goal of this study was to address the influences and progression of habitat filtering and competitive exclusion mechanisms during darter community assembly. Results suggest that habitat filtering was the primary, initial, assembly mechanism based on findings of consistent phylogenetic clustering and strong co-occurrence among species sharing similar habitat preference. The clustering patterns that were recovered indicated ecologically similar species were filtered into stream sites from the regional species pool, and this mechanism was identified despite clade level convergence in darter habitat use (see Fig. 1C). Secondary competitive interactions may have followed the initial habitat filter, resulting in the present-day assemblage structure observed. Influence of secondary competitive pressures was determined by the presence of morphological evenness among species within riffle sites (i.e., morphologically similar species showed low co-occurrence). Interpretation of the relative influences and sequential nature of these mechanisms was dependent on understanding both contemporary (ecological) and historical (evolutionary) relationships within assemblages (e.g., inter-clade convergence on habitat use requirements). Moreover, the conceptual framework presented (Fig. 1) aids in describing the intricacies of darter assembly within this species-rich system and has application towards understanding assembly in other systems.

Historical influences on assemblage structure.—A tendency towards phylogenetic clustering among darters in stream sites, with and without abundance weighting, indicated that locally abundant species did not increase phylogenetic "repulsion" of close relatives. Analyses including species abundances were important due to the possibility that competition among close relatives could have been masked using presence/absence data alone but apparent when incorporating species' abundances (Anderson et al., 2004; Vamosi et al., 2009). This, however, was not the case, and clustering patterns that were recovered likely represented a habitat filtering mechanism.

A greater frequency of significant clustering was found using NTI, as compared to NRI, suggesting the patterns recovered were representative of clustering among nearest neighbors at the tips of the phylogeny (Webb, 2000; Kraft et al., 2007; Cardillo, 2011). Visual inspection of phylogenetic co-occurrence indicated prevalent clustering of assemblage members in two main clades (Fig. 4). Although intra-clade clustering indicated importance of habitat filtering mechanisms, the phylogenetic distance between these two recurring clades may suggest inter-clade evenness (i.e., possible competition at lower phylogenetic resolution) between the two lineages. Cumulative influences of competition and habitat filtering have been found in sunfish assemblages at the lake scale (Helmus et al., 2007); however, other fish assembly studies have suggested a greater importance of habitat filtering at relatively large spatial scales (Peres-Neto, 2004; Geheber and Geheber, 2016). In the present study, I propose that the high darter richness within stream sites persists through sequential assembly mechanisms (i.e., habitat filtering followed by competitive pressures). As previously stated, most individuals collected during the study represented two clades that were evenly spaced (distantly related) across the regional phylogeny (Fig. 4). A scenario where habitat filters remove all species except those of the two representative clades from riffle sites, followed by competitive segregation among morphologically similar species in riffle sites, is plausible (see Fig. 1C: "habitat filtering" and visualize subsequent partitioning based on the morphological differences between the two well-represented clades).

Additionally, phylogenetic structure analyses revealed an interesting trend moving from upstream to downstream assemblages. In all cases (i.e., using presence/absence,

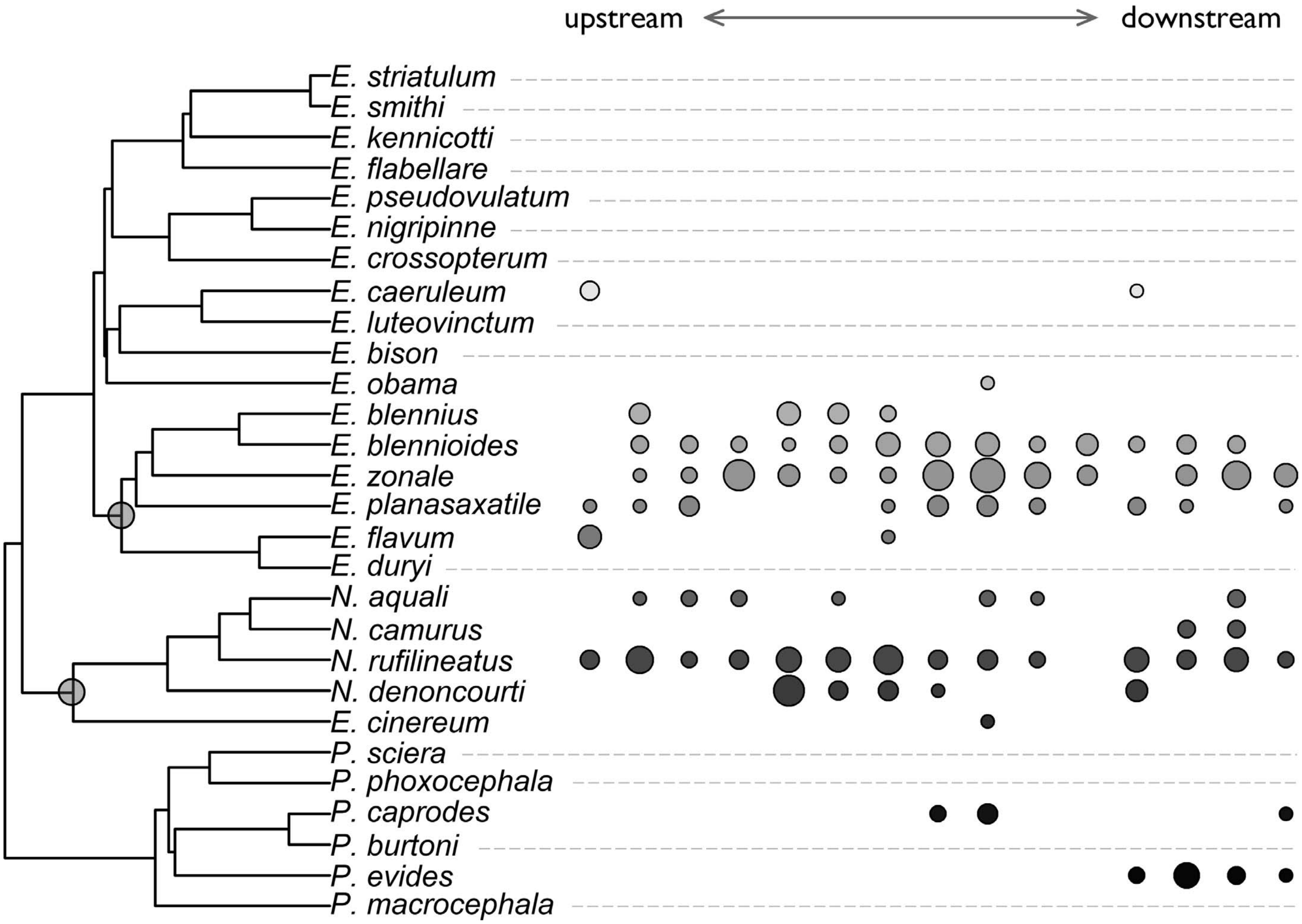

Fig. 4. Ultrametric tree including all darter species known to occur in the Duck River drainage (regional species pool). Species present at each site are illustrated as circles, and abundances are represented by size of circles (i.e., circle sizes are sqrt transformed abundances). For reference, the greatest abundance of a species collected from a single site during the study was *E. zonale* (site nine; $n = 43$). Different circle shades represent the different species. Sites one through 15 are depicted from upstream to downstream (left to right). Encircled nodes indicate the two main clades in which species were phylogenetically clustered. See Data Accessibility for tree file.

abundance weighted, NRI, and NTI), a stronger signal for phylogenetic clustering was observed in upstream sites as compared to further downstream sites. This pattern seemed to be driven by the addition of *Percina* spp. in the more downstream assemblages, which is likely related to greater availability of deeper riffle areas in these sites (Geheber and Frenette, 2016). This pattern is quite intriguing considering the predicted shift towards a habitat filtering mechanism (clustering patterns) at greater spatial scales due to a competitive release related to increased habitat heterogeneity (Cavender-Bares et al., 2006; Geheber and Geheber, 2016). Here the opposite was found, where the addition of *Percina* spp. in downstream sites (i.e., greater volume and surface area) resulted in a more even distribution of occupied clades across the phylogeny (Fig. 4). Moreover, the shift towards patterns of clade evenness in several downstream sites may aid in explaining why Geheber and Geheber (2016) found an increase in phylogenetic clustering with increased spatial scale in darter assemblages of three surrounding drainages, but not at the full drainage scale in the more species-rich Duck River.

Habitat use assemblage structure.—Species showing similar habitat use (i.e., substrate, depth, and flow) were more likely to co-occur in stream site assemblages. This suggests habitat filtering is an important assembly mechanism in darter assemblages, and moreover, habitat use clustering corroborated patterns of phylogenetic clustering. It should be noted that habitat use data for each species was generated from quadrats where individuals occurred during the study period; however, this does not necessarily mean species with very similar habitat usage are co-occurring in quadrats. Here, competitive exclusions may still occur in cases where species that have similar habitat requirements avoid competitive interactions by persisting in neighboring quadrats (with similar habitat characteristics) within stream sites. Although habitat use and phylogenetic clustering patterns were observed in stream site assemblages, phylogenetic niche conservatism was not recovered for habitat use factors among species present during the study. Rather, it seems habitat use convergence was observed among species collected during the study, and this was based on habitat use similarity among several distant phylogenetic relatives (i.e., habitat use convergence among species from the two main clades that "made it through" the initial habitat filter). For example, *E. blennius* has statistically similar habitat use to the main representatives of the *Nothonotus* clade (*N. rufilineatus* and *N. denoncourti*), and *N. aquali* has statistically similar habitat use

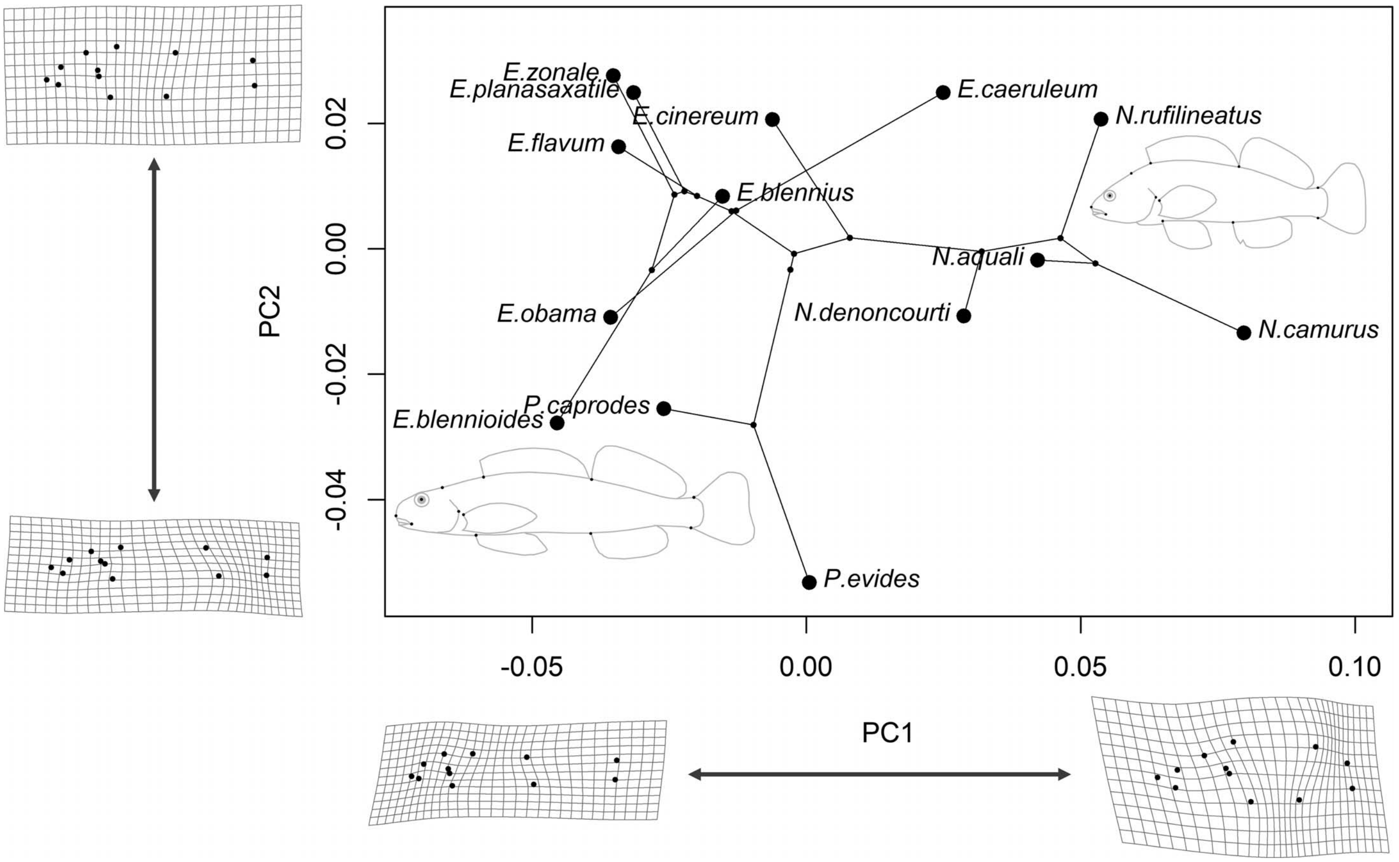

Fig. 5. Body shape relationships among darter species (PC1 vs. PC2) based on 12 homologous landmarks used in geometric morphometric analyses. The molecular phylogeny is overlaid to illustrate phylogenetic–morphological relationships among species. Gray wireframe warped grids show body shape extremes along PC1 (48.25% of variation) and PC2 (18.85% of variation). All warped grids are magnified 2.5 times in order to better illustrate changes in variation across each axis. Darter line drawings depict *E. blennioides* (lower left in morphospace) and *N. rufilineatus* (upper right in morphospace), and each shows the locations of the 12 homologous landmarks included. Phylogenetic niche conservatism of PC1 (determined by Blomberg's *K* value) can be visualized in the figure.

to *E. blennioides*, *E. zonale*, and *E. planasaxatile* (Geheber and Frenette, 2016). A schematic depiction of such a scenario is outlined and visualized in the "habitat filtering" outcome of Figure 1C, where clustering (i.e., habitat filtering) is shown across the phylogeny tips, and habitat usage has converged between members of the two commonly represented darter lineages. This scenario reflects the outcome of the present study, and the two well-represented clades can be viewed in Figure 4. It should be noted that patterns of ecological convergence were described based on darter distributions during the summer. Inclusion of seasonal sampling would be of great interest in future work, considering not much is

Table 1. Blomberg's *K* values for all habitat use and morphological traits. Values of $K > 1$ indicate that the habitat use factor or morphological trait shows conservatism (niche conservatism) among the collected species. Niche conservatism implies that closely related species have retained the ancestral trait/habitat usage. Standard effect size (SES) of variance, range, and mean nearest trait distance (MNTD) for each habitat use and morphological trait across all stream site assemblages are reported (i.e., means are presented). For these three SES metrics, positive values indicated greater similarity than expected in random assemblages (clustering), and negative values indicated less similarity than expected at random (evenness).

	Blomberg's *K*	SES (variance)	SES (range)	SES (MNTD)
Habitat use				
Gravel	0.851	0.691	0.598	0.347
Pebble	0.646	0.706	0.736	0.429
Cobble	0.591	0.367	0.405	0.065
Boulder	0.584	−1.330	−0.938	0.127
Bedrock	0.875	0.524	0.451	0.161
Velocity	0.785	0.739	0.404	0.293
Depth	0.839	0.503	0.721	−0.092
Morphology				
PC1	1.498	−0.489	−0.478	0.050
PC2	0.871	−0.455	−0.350	−0.032
PC3	0.494	0.497	0.331	0.221

known about changes in mechanistic assembly influences across temporal scales and life history stages in stream fishes (but see work by Fitzgerald et al. [2017] on seasonal assembly dynamics in tropical fishes, and work by Cavender-Bares and Reich [2012] and Norden et al. [2012] addressing successional assembly in plant communities).

Morphological influence on assembly.—Morphologies of co-occurring species were evenly distributed within assemblages based on body shape variation and range (described by PC1 and PC2), and furthermore, body shape showed phylogenetic conservatism among species collected. Multiple scenarios could explain such patterns of morphological evenness in the light of phylogenetic and habitat use clustering. One possible explanation for body shape evenness within assemblages is that multiple morphologies may be capable of similar ecological functions in Duck River riffles. If this were true, distant phylogenetic relatives may have converged upon near identical habitat use within assemblages but remained morphologically distinct due to lack of environmental selection pressures "pushing" towards a specific body shape. However, I suggest this morphological evenness may have resulted from competitive exclusion following the initial habitat filter. Here, distant phylogenetic relatives with similar habitat usage are able to co-occur due to small-scale functional (morphological) differences which have allowed species to avoid competitive exclusion following the initial habitat filter. In short, a habitat filter effectively removes all species from the regional pool except those that are members of the two well-represented clades (this is true with some exceptions, see Fig. 4), and competitive exclusion then limits the number of species present from each of these clades within each riffle. Because darters generally share a similar external morphology, as compared to other non-darter stream fish lineages, morphological differences within darter assemblages may represent quite small-scale differences in darter functionality. It should be noted that the MNTD metric did not recover strong signal for morphological evenness or clustering within assemblages, which may suggest minimal effects of competitive exclusion based on morphology altogether. However, MNTD values based on PC1 and PC2 were quite low relative to all other habitat use and morphological factor values recovered using the MNTD metric.

Past work has suggested darter maximum body size is impacted by the presence of close relatives in Tennessee River drainage assemblages (i.e., individuals were larger in the absence of competitors), which infers some level of competition between similar species (Page and Schemske, 1978). Because habitat homogeneity is expected to increase the strength of competitive interactions among similar species at fine spatial scales (Cavender-Bares et al., 2006, 2009; Emerson and Gillespie, 2008; Geheber and Geheber, 2016), it is plausible that competition becomes a more influential mechanism at even smaller scales among darters in the Duck River system (Geheber and Geheber, 2016). Segregation among species due to competitive exclusion at fine scales could result from darter micro-habitat use specificity and/or specific life history characteristics reliant on fine-scale habitat characteristics (e.g., use of specific substrates and micro-flows; Matthews, 1985; Kessler and Thorp, 1993). Multiple studies have shown patterns of differential use, or habitat partitioning, among co-occurring darter species (e.g., Matthews et al., 1982; Schlosser and Toth, 1984; Stauffer et al., 1996; Pratt and Lauer, 2013; Geheber and Frenette, 2016). Moreover, previous work indicates that darter species segregate habitat use when co-occurring at relatively fine scales (e.g., within a riffle), and these segregation patterns have been equated to competitive interactions based on niche expansion of one species in the absence of the other (Greenberg, 1988). Specifically, Greenberg (1988) illustrates how a primary habitat filtering mechanism may be followed by secondary competitive interactions which "refine" a species' habitat use.

Conclusions.—Habitat filtering and competitive exclusion mechanisms act sequentially during assembly in local-scale darter assemblages. Patterns of phylogenetic and habitat use clustering indicated habitat filtering acted as a primary assembly mechanism in darter assemblages, and patterns of morphological evenness suggested secondary influences of competitive exclusion likely acting within the strongly represented clades of darters. The integrated framework (i.e., incorporation of phylogeny, habitat use, and morphology) used in this study provides new insights into understanding the influence of ecological convergence on the interpretation of assemblage patterns. This study illustrates how views of phylogenetic community structure (e.g., interclade evenness vs. intra-clade clustering) can alter mechanistic interpretations. Overall, this study reveals the importance of accounting for contemporary and historical factors to identify magnitude, progression, and complexities of acting assembly mechanisms in species-rich stream fish assemblages.

DATA ACCESSIBILITY

Supplemental material is available at https://www.copeiajournal.org/ci-18-155.

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