

Widespread convergence in stream fishes

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Convergent evolution, the evolution of similar phenotypes among distantly related lineages, is often attributed to adaptation in response to similar selective pressures. Here, we assess the prevalence and degree of convergence in functional traits of stream fishes at the microhabitat scale in five zoogeographical regions across the world. We categorized species by microhabitat, water velocity and preference for substrate complexity and calculated the prevalence of convergence, degree of convergence and functional diversity for each category. Among species occupying similar microhabitats of small, low-gradient streams, 34% had combinations of convergent traits. Convergence occurred at higher rates than expected by chance alone, implying that adaptation to similar environmental conditions often resulted in similar evolutionary patterns along multiple niche dimensions. Two of the microhabitat groupings had significantly convergent species represented in all zoogeographical regions. Fishes occupying microhabitats with high water velocity and low structural complexity generally occupied a restricted morphospace and exhibited greater prevalence and higher degrees of convergence. This suggests that water velocity and habitat structural complexity interact, selecting a restricted distribution of trait distributions and higher degrees of convergence in stream fish assemblages. Furthermore, these results suggest that microhabitat features in streams select for fish trait distributions in a fairly predictable and deterministic manner worldwide.

ADDITIONAL KEYWORDS: convergent evolution – environmental filtering – fish – functional trait – intercontinental – microhabitat.

INTRODUCTION

Convergent evolution is the independent evolution of similar phenotypes, a phenomenon that can arise via equivalent or different developmental pathways (Arendt & Reznick, 2008; Wake *et al.*, 2011; Agrawal, 2017). Some remarkable examples of convergent evolution occur when similar functional trait patterns are observed among species from different regions with distinct evolutionary histories, such as animals adapted to subterranean habitats (Trontelj *et al.*, 2012), desert lizards (Melville *et al.*, 2006), island lizards (Mahler *et al.*, 2013) and freshwater fishes (Winemiller, 1991; Winemiller *et al.*, 1995). These and other examples of convergence among assemblages occupying habitats with similar environmental conditions suggest that convergent evolution arises from similar responses to selective challenges rather

than expression of constraints or random processes (Melville *et al.*, 2006; Conway Morris, 2010; Morinaga & Bergmann, 2017). Nonetheless, few studies have tested rigorously for convergence over large geographical and evolutionary scales (e.g. Wiens *et al.*, 2006; Moen *et al.*, 2016). Instead, studies often restrict comparisons to two or three regions (e.g. Melville *et al.*, 2006) while focusing on only a single genus or family (e.g. Serb *et al.*, 2017; Zelditch *et al.*, 2017). Furthermore, early studies of convergence among species assemblages were constrained by a paucity of large phylogenies (e.g. Winemiller, 1991), but the data and methods to create large phylogenies are now available to support analyses of convergence (e.g. Rabosky *et al.*, 2018). Here, we test for convergence of teleost fishes from similar microhabitats of streams within five major zoogeographical regions around the world.

Evolutionary convergence is thought to be common, if not ubiquitous, throughout the tree of life, occurring at all biological levels from DNA sequences to communities (Conway Morris, 2003;

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Losos, 2011; McGhee, 2011; Winemiller *et al.*, 2015). Nonetheless, the means to assess convergence are not always straightforward, and inferences about mechanisms that generate convergence are often vague. Convergent taxa are rarely perfect replicas; instead, they have varying degrees of phenotypic similarity involving functional traits inferred to be associated with various niche dimensions. Convergent evolution is often taken as evidence of adaptation to similar selective pressures, implying that certain aspects of evolution are deterministic and potentially repeatable (Losos *et al.*, 1998; Conway Morris, 2010; Mahler *et al.*, 2013). However, convergence can also result from developmental or functional constraints that limit phenotypic variation, inevitably leading to the appearance or reappearance of similar phenotypes among multiple lineages (Losos, 2011; Frédérick *et al.*, 2013; Agrawal, 2017). Adaptation and constraints are not mutually exclusive, and together can produce convergent forms (Frédérick *et al.*, 2013; Agrawal, 2017; Morinaga & Bergmann, 2017). Alternatively, convergence might result simply from random acquisition of similar phenotypes in distantly related lineages (Gould, 1990; Stayton, 2008). Efforts to reconstruct the evolutionary sequence that resulted in convergence are even more complicated for species-rich regions with complex biogeographical histories, because traits adaptive for a given set of conditions might persist long after the lineage has evolved in response to novel conditions in a different time or place.

The interaction between a trait and function can be complex, further complicating interpretations of convergence. For example, more than one phenotype or trait might perform a given ecological function, a many-to-one relationship (Hulsey & Wainwright, 2002; Wainwright *et al.*, 2005; Collar *et al.*, 2014). This could result in the independent evolution of species with divergent traits that have similar functions for a given niche dimension (Zelditch *et al.*, 2017). Alternatively, a single trait might have multiple functions, allowing the organism to exploit multiple resources (Zelditch *et al.*, 2017). The multidimensionality of ecology and functions might lead to a many-to-many relationship between traits and functions, and multidimensionality itself might reduce the probability of convergence (Stayton *et al.*, 2008).

A long-standing evolutionary concept is the idea of adaptive landscapes, whereby a species' fitness increases during evolution toward an adaptive peak defined by a multivariate phenotypic surface. The breadth and height of the adaptive peak can be determined by the strength of constraints, whereby strong ecological and functional constraints would correspond to narrow tall peaks. In contrast, weak or

no selective pressure would result in a broad peak or several close shallow peaks, reflecting weak selection for a single phenotype or suite of phenotypes. This raises the question: in habitats with stronger environmental constraints, would the degree of convergent evolution be greater? If this were the case, it would suggest that convergent evolution is deterministic and potentially predictable (Trontelj *et al.*, 2012).

Like any organism, stream fishes are subject to multiple selective pressures. Stream hydrology plays a powerful role in shaping fish ecology and evolution (Townsend & Hildrew, 1994; Poff & Allan, 1995; Mims & Olden, 2012; Bower *et al.*, 2019). At local scales, water velocity can influence stream fish ecology and evolution (Lamouroux *et al.*, 2002; Bower & Pillar, 2015; Haas *et al.*, 2015; Lujan & Conway, 2015) and therefore function as a strong environmental filter (Willis *et al.*, 2005; Bower & Winemiller, 2019). For example, deep-bodied fish generally do not perform well in microhabitats with rapid water velocity, owing to the high energetic cost of maintaining position against strong drag (Webb, 1988; Bower & Piller, 2015). Stream substrates also influence species ecology and evolution (Kovalenko *et al.*, 2012). Habitats with structurally complex substrates can provide refuge from predators and adverse environmental conditions (Bartholomew *et al.*, 2000; Tokeshi & Arakaki, 2012), in addition to providing greater diversity of resources and microhabitats than non-structured substrates (Willis *et al.*, 2005; Kovalenko *et al.*, 2012), potentially increasing the number of species and functional diversity (Richardson *et al.*, 2017; Leitão *et al.*, 2018). Conversely, habitats with uniform, simple substrates tend to be associated with less functional diversity (Willis *et al.*, 2005). Here, we hypothesize that natural selection in response to abiotic features of stream microhabitats has resulted in the evolution of similar suites of functional traits among distantly related lineages.

This study assesses the prevalence of convergent evolution in teleost fishes from similar microhabitats within small, low-gradient streams across five zoogeographical regions. Specifically, we test the following predictions: (1) fishes occupying similar microhabitats will have convergent phenotypes across all zoogeographical regions; (2) species from microhabitats with high water velocity and/or low structural complexity will have a greater degree of convergence and lower phenotypic richness; and (3) species from microhabitats with low water velocity and structurally complex substrates will have relatively weak convergence and higher functional richness.

MATERIAL AND METHODS

DATA ACQUISITION AND PREPARATION

Fishes were surveyed in low-gradient streams of similar size and environmental conditions (for details, see [Bower & Winemiller, 2019](#)). Five distinct zoogeographical regions were chosen to provide an opportunity to test for convergent evolution within microhabitat types across different regional assemblages: Afrotropical (Benin), Neotropical (Brazil), Mesoamerican (Belize), Nearctic (USA: New Jersey, South Carolina and Texas) and Indo-Malayan (Cambodia). Four to seven streams were sampled in each region during low-water periods when the streams were wadeable, when densities of fish per unit area were highest and fish capture was most efficient, allowing us to capture nearly all of the fish diversity within these streams.

At each stream sampling site, we collected fishes from several types of microhabitats encountered within a 200–500 m reach surveyed while sampling in an upstream direction to obtain reliable samples of fish diversity within these streams (modified from [Barbour *et al.*, 1999](#); [Bower & Piller, 2015](#)). Microhabitats were designated based on the consistency of current velocity, substrate composition, position in the water column, and in-channel cover. Once any fish specimens were captured from a given microhabitat, a labelled flag was placed at the site of capture. After being euthanized, specimens collected from a microhabitat were placed in labelled Whirlpaks matching the flag. The water velocity, substrate composition and depth were recorded at each flagged capture point, allowing us to associate these habitat variables with every specimen collected. Measurements of velocity were taken in the middle of the water column within each microhabitat using a Marsh–McBirney Flo-Mate Portable Flow Meter. Microhabitats were sampled only if the substrate composition could be categorized as sand (> 90% cover), woody structure (> 80% cover), aquatic macrophytes (> 80% cover), leaf packs (> 90% cover), root banks (banks with dense root structures, > 90%) or gravel (6–25 cm diameter, > 80% cover). Given the challenge of sampling fish from diverse microhabitats, various methods, including dipnetting and backpack electrofishing, were used depending on which suited the stream conditions best. For example, dipnets were effective for extracting fish from undercut banks, seining was effective in areas lacking submerged structure, and electrofishing was effective around submerged structures. At each study site, habitat variables of water temperature (in degrees Celsius), dissolved oxygen (DO), pH, specific conductivity (in microsiemens per centimetre) and salinity (in parts per thousand) were measured. Specimens were euthanized via anaesthetic (MS222) overdose and

preserved in 10% formalin, following the animal care protocol (IACUC 2014-0173 and 2017-0233).

We measured 25 morphometric features that reflect body shape and other functional traits that affect how fishes feed and use habitats ([Gatz, 1979](#); [Winemiller, 1991](#); [Table 1](#)). Traits of five specimens of each species (for rare species, $N = 1–4$; sample sizes are provided in [Supporting Information, Table S1](#)) were measured to the nearest 0.1 mm using callipers. In addition, we grouped species into life history categories based on information from the literature ([Supporting Information, Table S1](#)). To reduce intraspecific morphological variation associated with ontogeny, only adult size classes were analysed. We standardized traits using proportions of linear measurement to eliminate the influence of body size on shape components ([Winemiller, 1991](#); [Casatti *et al.*, 2006](#); [Table 1](#)). Species averages were computed for each morphological trait, and two datasets were compiled. An ‘all-traits dataset’ included traits inferred to influence performance for habitat use, trophic and life history dimensions, and a ‘habitat-trait dataset’ included only traits inferred to influence swimming performance and microhabitat use ([Gatz, 1979](#); [Winemiller, 1991](#)).

DATA DIMENSION REDUCTION

We performed principal coordinates analyses (PCOAs) to reduce data dimensions and ordinate species within trait space using the ‘all-traits’ dataset and Gowers distance, because this dataset included both categorical and continuous traits. In addition, a principal components analysis (PCA; based on an eigenanalysis of the correlation matrix) was used for the ‘habitat’ dataset, which includes only continuous traits. Data were scaled and centred to a mean = 0 and variance = 1 before performing the PCOA. Following the Kaiser-Guttman (KG) rule for PCA, we selected all principal component (PC) axes with eigenvalues greater than one for use in further analyses ([Guttman, 1954](#); [Kaiser, 1960](#)). Anguilliform species (eel-like body shape) were removed owing to their extreme morphology; their inclusion produced strongly skewed gradients and assemblage ordinations that separated anguilliform fish from all other species, with the latter being tightly clustered within morphospace.

MICROHABITAT CLUSTERS

To test the first hypothesis, we divided species into seven microhabitat clusters: top-water species in microhabitats with low water velocities and varying amounts of instream structure, mid-water species in microhabitats with high water velocities and low instream structure, mid-water species in microhabitats with low water velocity and low instream structure,

Table 1. All measured traits, trait standardization, trait codes and trait definitions

Trait	Standardization	Trait definition
Average standard length*	SL	Maximum standard length from the populations in this study
Head length*	HEAD_L/SL	Distance from the tip of the jaw to the posterior edge of the operculum
Head depth*	HEAD_D/BOD_D	Vertical distance from dorsum to ventrum passing through the pupil
Oral gape	GAPE/BOD_D	Vertical distance measured inside of fully open mouth at tallest point
Mouth position*	MOUTH_P	The angle between an imaginary line connecting the tips of the open jaws and an imaginary line running between the centre of the pupil and the posterior-most vertebra (e.g. 90 representing a terminal mouth)
Eye position*	EYE_POS/ HEAD_D	Vertical distance from the ventral pigmented region to the ventrum
Eye diameter*	EYE_D/HEAD_D	Vertical distance from eye margin to eye margin
Snout length*	SNT_L/HEAD_L	Distance from the posterior pigmented region of the eye to the tip of the upper jaw with mouth shut
Snout protrusion	SNT_PR/HEAD_L	Additional distance from the posterior pigmented region to the tip of the upper jaw with mouth fully open and extended
Body depth*	BOD_D/SL	Maximum vertical distance from dorsum to ventrum
Body width*	BOD_W/SL	Maximum horizontal distance from side to side
Caudal peduncle length*	PED_L/SL	Distance from the posterior proximal margin of the anal fin to the caudal margin of the ultimate vertebra
Caudal peduncle depth*	PED_D/BOD_D	Minimum vertical distance from dorsum to ventrum of caudal peduncle
Caudal peduncle width*	PED_W/BOD_W	Horizontal width of the caudal peduncle at mid-length
Dorsal fin length*	DORS_L/SL	Distance from the anterior proximal margin to the posterior proximal margin of the dorsal fin
Dorsal fin height*	DORS_HT/SL	Maximum vertical distance from the proximal to distal margin of the dorsal fin (excluding filaments)
Anal fin length*	ANAL_L/SL	Distance from the anterior proximal margin to the posterior proximal margin of the anal fin
Anal fin height*	ANAL_HT/SL	Maximum vertical distance from proximal to distal margin of the anal fin
Caudal fin depth*	CAUD_D/SL	Maximum vertical distance across the fully spread caudal fin
Caudal fin length*	CAUD_L/SL	Maximum distance from proximal to distal margin of the caudal fin (excluding filaments)
Pectoral fin length*	PEC_L/SL	Maximum distance from proximal to distal margin of pectoral fin
Pelvic fin length*	PELV_L/SL	Maximum distance from the proximal to distal margin of the pelvic fin
Gut length	GUT_L/SL	Length of gut from the beginning of the oesophagus to the anus (extended without stretching)
Gill raker	RAKER	Coded as: 0 for absent; 1 for short, blunt or toothlike; 2 for intermediate or long and sparse; and 3 for long and comb-like
Tooth shape	TOO_S	Coded as: 0 for absent; 1 for unicuspid (rasping); 2 for multicuspid (crushing); 3 for short conical (grasping); 4 for long conical (piercing); and 5 for triangular serrated (shearing)

*Trait used in the habitat-trait dataset.

mid-water species in microhabitats with low water velocity and high instream structure, benthic species in microhabitats with high water velocity and low instream structure, benthic species in microhabitats with high water velocity and high instream structure, and benthic species in microhabitats with low water velocity and varying amounts of instream structure (Supporting Information, Table S1). These

clusters were created based on species–microhabitat associations, while being sufficiently broad to include species from multiple regions. To create the microhabitat clusters, we used a three-table ordination (RLQ analysis, RLQ stands for the R [site x environment], L [site x species], and Q [species x trait] matrices and is, therefore, called RLQ analysis) to determine the microhabitat preference for each

species (for details, see [Bower & Winemiller, 2019](#)). The first two dimensions of R site scores from the RQL analysis (microhabitat preference) and positions in the water column (benthic, mid-water or surface) were used in a hierarchical cluster analysis (Ward's method) to classify species into microhabitat clusters.

SUBSTRATE COMPLEXITY AND WATER VELOCITY CATEGORIES

To address hypotheses 2 and 3, we categorized species by habitat gradients that independently evaluated water velocity and structural complexity preferences. Substrates were separated into non-structurally complex and structurally complex microhabitats (e.g. sand vs. wood). Hereafter, we use the term 'complexity' in reference to this substrate grouping. For each species, we calculated the mean water velocity weighted by abundance. Structural complexity preference was calculated by dividing the number of individuals captured from structurally complex microhabitats by the total number of individuals for a given species. We grouped species into low (0–0.1 m/s), medium (0.1–0.2 m/s) and high (> 0.2 m/s) water velocity categories. Species were also classified by substrate complexity preference: low association with structured substrates (proportion < 0.33), medium association with structured substrates (proportion 0.33–0.67) and high association with structured substrates (proportion > 0.67). These water velocity and substrate complexity preference categories were analysed separately from the microhabitat clusters.

FUNCTIONAL DIVERSITY ANALYSES

We calculated the average functional diversity using Rao's quadratic entropy (RaoQ) for each microhabitat, water velocity and substrate complexity category. Rao's quadratic entropy is the sum of species pairwise distances weighted by their relative abundance ([Botta-Dukát, 2005](#)). To account for differences in the number of species in each category ([Table 2](#)), we randomly subset ten species from within each water velocity and substrate category to generate RaoQ values. This was done 999 times for each water velocity and substrate category, and the differences among these generated values were tested using an analysis of variance (ANOVA) and Tukey's honestly significant different test. The 'FD' package in R was used to find RaoQ ([Laliberté et al., 2014](#); R Core Team, 2020).

CONVERGENCE ANALYSES

To examine trait convergence within each microhabitat, water velocity and substrate complexity category, tanglegrams were created to visualize the morphological similarity of groupings relative to phylogenetic relationships, following [Zelditch et al. \(2017\)](#). A tanglegram pairs two branching diagrams; in this case, a phylogenetic tree and a phenogram created from hierarchical clustering analyses of the PCA or PCOA axes using Ward's method ([Zelditch et al., 2017](#)). A line was drawn from the position of each species in the phylogeny to its position in the dendrogram that was based on trait similarity to illustrate convergent species pairs. Convergent evolution is defined as the

Table 2. Mean C_1 value, standard error of C_1 values, proportion of significantly convergent species within each category, total number of species, and number of convergent species for habitat clusters, water velocity groupings and substrate complexity groupings

Categories	Mean C_1	SE C_1	Proportion	Total species	Convergent species
TOP-LVVS	0.595	0.057	0.379	29	11
MID-LVHS	0.391	0.012	0.672	58	39
BEN-HVLS	0.662	0.051	0.250	12	3
BEN-HVHS	0.554	0.029	0.311	45	14
BEN-LVVS	0.521	NA	0.333	6	2
Low velocity	0.399	0.011	0.417	108	45
Medium velocity	NA	NA	NA	60	NA
High velocity	0.566	0.026	0.370	27	10
High complexity	0.401	0.010	0.411	124	51
Medium complexity	0.671	0.057	0.225	40	9
Low complexity	0.681	0.040	0.167	30	5

Microhabitat clusters are labelled as follows: benthic species in microhabitats with high water velocity and high instream structure (BEN-HVHS), benthic species in microhabitats with high water velocity and low instream structure (BEN-HVLS), benthic species in microhabitats with low water velocity and varying amounts of instream structure (BEN-LVVS), mid-water species in microhabitats with low water velocity and high instream structure (MID-LVHS), and top-water species in microhabitats with low water velocities and varying amounts of instream structure (TOP-LVVS). Abbreviations: NA, not assessed; SE, standard error.

independent evolution of similar phenotypes (Agrawal, 2017). Therefore, convergence is demonstrated by high phenotypic similarity but a distant phylogenetic relationship of species occupying the same microhabitat type. Convergent species will be in close proximity on the dendrogram but widely separated on the phylogeny and in the same microhabitat category. These convergent taxa were then analysed with the 'C₁ values' (see next paragraph). A recently published time-calibrated tree was used in this study (Rabosky *et al.*, 2018; for details, see Bower & Winemiller, 2019). Tanglegrams were created using the 'cophylo' function in the phytools package (Revell, 2012).

We calculated the degree and significance of convergence for each microhabitat, water velocity and substrate complexity category using a recently developed convergence metric (Stayton, 2015). This method requires the identification of potentially convergent species a priori, which was done using the tanglegram, following Zelditch *et al.* (2017). After identifying possibly convergent species, we tested the degree of convergence using Stayton's C metrics (Stayton, 2015). For our study, we selected the C₁ metric, which measures the similarity between two extant taxa relative to their shared ancestor, giving the proportion of morphological distance reduced in convergent taxa by evolution. For details of the method, see Stayton (2015). The C₁ metric characterizes the inverse of the proportional morphological distance between pairs of extant taxa (D_{tip}) and the maximum morphological distance between their shared ancestors (D_{max}), giving the reduction in morphological divergence ($C_1 = 1 - D_{\text{tip}}/D_{\text{max}}$; Stayton, 2015). Values of one indicate complete convergence and morphological similarity, and zero would indicate no convergence at all. It is important to note that the C₁ values are sensitive to the species included in the dataset. For example, the inclusion of related but morphologically divergent species in the clades of interest will greatly inflate the resulting C₁ value because of the large increase in D_{max} . In this case, the D_{tip} would not be reduced by convergence; rather, including the related but morphologically divergent species would increase the C₁ value purely by increasing D_{max} . The degree of convergence was not tested below the genus level. We also estimated the frequency of convergent evolution using C₅. This metric calculates the number of lineages that enter a morphospace of interest and also provides the proportion of simulated convergences greater than the observed convergences as a *P*-value (for details, see Stayton, 2015).

To test whether these metrics were significantly different from random, the observed morphological data were compared with datasets that simulated trait distributions within the phylogeny using the Brownian motion evolutionary model (Stayton,

2015). We used a false discovery rate correction for multiple comparisons. We acknowledge that additional evolutionary models, such as Ornstein–Uhlenbeck models, are also used to test for convergence. However, recent studies show that when analysing datasets with high trait dimensionality or deep phylogenetic scales, such as the dataset used in our study, these evolutionary models can lead to increased error rates, overfitting and misclassification of convergence (Cooper *et al.*, 2016; Khabbazian *et al.*, 2016; Adams & Collyer, 2018).

RESULTS

We analysed a total of 197 species representing 16 different orders: 57 species from the USA, 52 from Brazil, 41 from Cambodia, 25 from Benin and 21 from Belize. Brazil had the most families represented (19), followed by Benin (17), Cambodia (13), USA (10) and Belize (9) (Supporting Information, Table S1). The PCOA accounted for 78.6% total trait variation in the first 12 axes (Supporting Information, Fig. S11; Table S2), and 76.4% of variation in traits relevant for microhabitat function was captured in seven PCA axes (Supporting Information, Fig. S10; Table S3). The 12 PCOA axes and seven PCA axes were used to build tanglegrams for determining convergence.

In support of the hypothesis that fishes occupying similar microhabitats will be convergent, the tanglegram and C₁ values based on the 'habitat-trait dataset' showed many cases of convergence within microhabitat clusters (Fig. 1; Supporting Information, Fig. S1), with 67 species (34% of all species) being significantly convergent with at least one other species when based only on traits associated with habitat use. Significantly convergent species from the cluster of top-water species in microhabitats with low water velocities and varying amounts of instream structure were found in all regions (Supporting Information, Fig. S1; Tables S1 and S4). Within this cluster, three groups of convergent species were identified (Table 2; Supporting Information, Table S4): group 1 ($\bar{C}_1 = 0.672$; $C_5 = 3$, $P = 0.021$; three species from Brazil and USA), group 2 ($\bar{C}_1 = 0.660$; $C_5 = 4$, $P = 0.022$; four species from Belize, Benin and Brazil) and group 3 ($\bar{C}_1 = 0.491$; $C_5 = 4 = 0.018$; four species from Brazil and Cambodia). None of the 19 species associated with the mid-water species in microhabitats with high water velocities and low instream structure or mid-water species in microhabitats with low water velocity and low instream structure exhibited strong convergence (Supporting Information, Fig. S1; Table S4).

Two major subsets of convergent species associated with the mid-water microhabitats with low water velocity and high instream structure cluster were

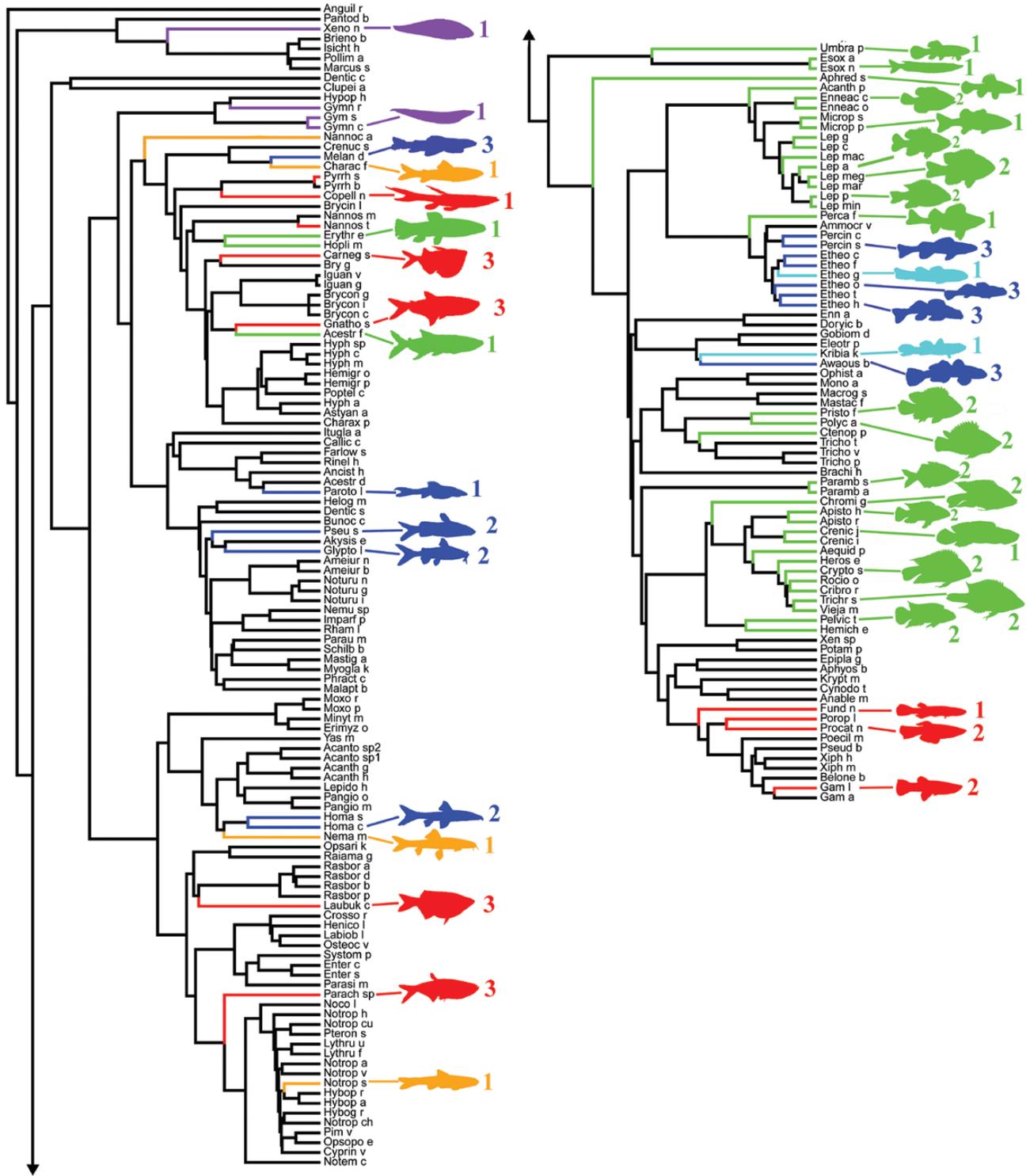


Figure 1. Phylogeny showing all species included in this study, split into two clades for ease of interpretation. Species that were significantly convergent with at least one other species within their microhabitat cluster are coloured. Significant convergence was based on C_1 values using the ‘habitat-traits dataset’ and the tanglegram in the [Supporting Information \(Fig. S1\)](#). Convergent species within microhabitat clusters are labelled as follows: benthic species in microhabitats with high water velocity and high instream structure (BEN-HVHS, blue), benthic species in microhabitats with high water velocity and low instream structure (BEN-HVLS, light blue), benthic species in microhabitats with low water velocity and varying

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identified (Fig. 1; Supporting Information, Tables S2 and S4): group 1 ($\bar{C}_1 = 0.358$; $C_5 = 3$, $P = 0.012$; 13 species from Benin, Brazil, and USA) and group 2 ($\bar{C}_1 = 0.399$; $C_5 = 5$, $P = 0.027$; 26 species from all regions). At least one significantly convergent species within this cluster was found in every region (Supporting Information, Fig. S1; Tables S1 and S4).

Within the benthic species cluster in microhabitats with high water velocity and low instream structure, three species from Brazil, Cambodia and USA were significantly convergent ($\bar{C}_1 = 0.662$; Fig. 1; Table 2; Supporting Information, Table S4). The cluster of benthic species in microhabitats with high water velocity and high instream structure contained three different groups (Fig. 1; Table 2; Supporting Information, Table S4): group 1 ($\bar{C}_1 = 0.643$; $C_5 = 3$, $P = 0.017$; three species from Brazil, Cambodia and USA), group 2 ($\bar{C}_1 = 0.568$; $C_5 = 3$, $P = 0.449$; three species from Cambodia) and group 3 ($\bar{C}_1 = 0.563$; $C_5 = 5$, $P = 0.001$; nine species from Belize, Brazil, and USA). Within this cluster, no significantly convergent species were found in Benin (Supporting Information, Fig. S1; Tables S1 and S4). In the cluster of benthic species in microhabitats with low water velocity and varying amounts of instream structure, two species from Benin and USA were convergent ($\bar{C}_1 = 0.521$).

Analysis of the 'all-traits dataset' also supported hypothesis 1. The tanglegram and C_1 values based on the 'all-traits dataset' identified 60 significantly convergent species within the microhabitat clusters (Supporting Information, Fig. S2; Table S5). Two convergent groups were found within the mid-water species cluster in microhabitats with low water velocity and high instream structure: group 1 ($\bar{C}_1 = 0.279$; $C_5 = 5$, $P = 0.001$; 24 species from all regions) and group 2 ($\bar{C}_1 = 0.389$; $C_5 = 8$, $P = 0.001$; 11 species from Benin, Brazil and USA). Three species from Brazil, Cambodia and USA were convergent in the cluster of benthic species in microhabitats with high water velocity and low instream structure ($\bar{C}_1 = 0.513$; $C_5 = 3$, $P = 0.028$). Two distinct groups of convergent species were identified within the cluster of top-water species in microhabitats with low water velocities and varying amounts of instream structure. The first group included nine significantly convergent species from Benin, Brazil and Africa ($\bar{C}_1 = 0.482$; $C_5 = 6$, $P = 0.001$). The second group, consisting of four species, had no significant pairwise comparisons (Supporting Information, Table S5). The benthic species in microhabitats with high water velocity and

high instream structure cluster had three distinct groups: group 1 ($\bar{C}_1 = 0.268$, two species from Brazil and Cambodia), group 2 ($\bar{C}_1 = 0.277$; $C_5 = 3$, $P = 0.492$; three species from Cambodia) and group 3 ($\bar{C}_1 = 0.395$; $C_5 = 5$, $P = 0.001$; eight species from Belize, Brazil and USA).

We used two tanglegrams (Supporting Information, Figs S3, S4) and C_1 values (Table 2) based on the 'habitat-trait dataset' to address hypotheses 2 and 3. These tanglegrams and C_1 values revealed significant convergent species within the water velocity and substrate complexity categories (Table 2; Supporting Information, Figs S3, S4; Table S4). Supporting hypotheses 2 and 3, the high water velocity category had the highest average C_1 value and smallest measures of functional diversity (Table 2; Fig. 2). No species from the medium water velocity category were significantly convergent. Also supporting hypotheses 2 and 3, low structural complexity had the greatest degree of convergence and smallest measures of functional diversity (Table 2; Fig. 2). We found significant different RaoQ values among all high values of substrate complexity habitats when accounting for the number of species by subsetting species from within each substrate category ($P < 0.05$). The highest RaoQ values were found in high substrate complexity habitats and the lowest in low substrate complexity habitats (Fig. 2). Species in the high water velocity category had significantly lower RaoQ values compared with the low and medium water velocity categories ($P < 0.05$; Fig. 2). However, the RaoQ values of high water velocity category did not significantly differ from the medium water velocity category ($P > 0.05$).

DISCUSSION

We found a high prevalence of significant convergence among stream fishes occupying similar microhabitats in streams from five zoogeographical regions. Congruent with our first prediction, species occupying similar microhabitats (with the exception of mid-water species in microhabitats with high water velocity and low instream structure and mid-water species in microhabitats with low water velocity and low instream structure) had convergent traits associated with swimming performance and habitat use in addition to traits associated with feeding behaviour, habitat use and life history strategies. In addition,

amounts of instream structure (BEN-LVVS, orange), mid-water species in microhabitats with low water velocity and high instream structure (MID-LVHS, green), and top-water species in microhabitats with low water velocity and varying amounts of instream structure (TOP-LVVS, red). Individual groupings found within each cluster are indicated by the numbers next to example fish images.

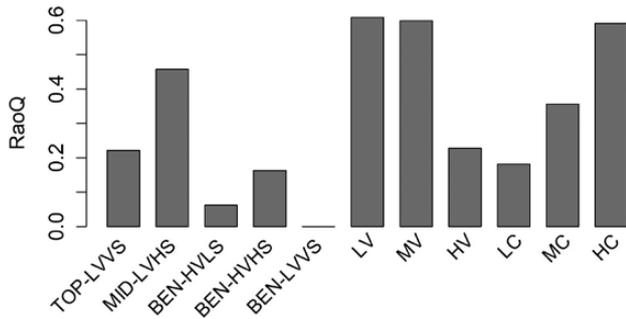


Figure 2. Functional diversity values for microhabitat, water velocity and substrate complexity clusters. Clusters are labelled as follows: benthic species in microhabitats with high water velocity and high instream structure (BEN-HVHS), benthic species in microhabitats with high water velocity and low instream structure (BEN-HVLS), benthic species in microhabitats with low water velocity and varying amounts of instream structure (BEN-LVVS), mid-water species in microhabitats with low water velocity and high instream structure (MID-HVLS), mid-water species in microhabitats with high water velocity and low instream structure (MID-LVHS), top-water species in microhabitats with low water velocity and varying amounts of instream structure (TOP-LVVS), low water velocity (LV), median water velocity (MV), high water velocity (HV), low substrate complexity (LC), median substrate complexity (MC) and high substrate complexity (HC).

convergence was higher than expected by random chance based on the C_5 values. Taken together, this suggests that adaptation to environmental conditions resulted in repeated patterns of evolution along multiple niche dimensions. Supporting our second and third predictions, fishes in the high water velocity category and those inhabiting microhabitats with little or no structural complexity generally occupied a reduced morphological space and had higher convergence values (Fig. 2; Table 2). Thus, it appears that high water velocity and low structural complexity act as an environmental filter, influencing the trait distributions of stream fish assemblages.

ECOMORPHOLOGICAL CONVERGENCE

Supporting hypothesis 1, many examples of convergence in similar microhabitats were identified in this study. For example, convergence among top-water species in microhabitats with low water velocity and varying instream structure was found based on analyses of the ‘all-trait dataset’ and the ‘habitat-trait dataset’. All these species have superior-oriented mouths, a trait often associated with surface feeding (Keast & Webb, 1966). These surface feeders often have elongate bodies with unpaired medial fins positioned posteriorly. This body shape and fin arrangement are well suited for

burst swimming, but not for prolonged movement in fast water, which might explain their prevalence in microhabitats with low water velocity (Keast & Webb, 1966; Webb, 1984, 1988). This species group is widespread, with representative species collected in all regions except Cambodia. Similar species, such as species within Aplocheilidae, exist in Cambodia, but were not collected in our study. Another group of convergent top-water species was characterized by a relatively deep body, superior mouth orientation and large pectoral fins. The large, wing-like pectoral fins of these fishes are used to propel the fish upwards through the water column, which facilitates a rapid burst to the surface to capture floating food items and to escape from predators by leaping into the air (Eaton *et al.*, 1977; Saidel *et al.*, 2004). This group of fishes was fairly rare, being collected only in Brazil and Cambodia, with no known counterparts in North America.

Other examples of convergence were seen for two groups of species from the cluster of mid-water species in microhabitats with low water velocity and high instream structure. The first group consisted of deep-bodied, largely invertivorous species that tend to have a terminal mouth and relatively large anal, dorsal and pectoral fins. A laterally compressed, deep body allows for efficient lateral movement, with a narrow turning radius (Videler, 2012), but increases drag on the body surface, which reduces the velocity of burst swimming and efficiency of sustained unidirectional swimming (Webb, 1984, 1988). Large dorsal and pectoral fins enhance deceleration and lateral manoeuvres, such as yawing (Lauder & Drucker, 2004). Fishes with these traits might be well suited for living in highly structured habitats with low water velocity, wherein manoeuvrability is important for foraging and escape. Other studies also have reported remarkable convergence in deep-bodied fishes, such as between Centrarchidae and Cichlidae (Montaña & Winemiller, 2013). In our study, representatives of this group were found in every region. In contrast, a second group within the cluster of mid-water species in microhabitats with low water velocity and high instream structure consisted of predatory fishes that tend to have torpedo-shaped bodies and posteriorly positioned unpaired median fins that enhance swimming in rapid bursts. However, these traits compromise the ability to make precise lateral movements in a small radius (Webb, 1988). No representatives of this group were collected in Cambodia or Belize. However, similar species can be found in these regions, such as Channidae species (Cambodia).

The cluster of convergent benthic species in microhabitats with high water velocity and high instream structure shared a number of morphological features, such as a dorsoventrally compressed body,

inferior mouth position and relatively large pectoral fins. A dorsoventrally compressed body is strongly associated with benthic fishes occupying microhabitats with fast-flowing water. This body shape reduces drag and lessens the energetic costs of maintaining position in fast water (Webb, 1984, 1988). Benthic fishes, such as darters (North American Percidae), can use their large pectoral fins to create negative lift, forcing them against the substrate to prevent slippage downstream in fast-flowing water (Page & Swofford, 1984; Lujan & Conway, 2015). This is a common ecomorphotype, with species representatives found in all regions except Benin. However, if more species from the *Nannocharax* genus were collected, we might also have found significant convergence in Benin. One odd case of convergence was between *Etheostoma gracile* (North American percid with riffle-dwelling ancestors) and *Kribia kribensis* (African eleotrid that had estuarine dwelling ancestors). Both species have characteristics common in benthic fishes that occupy microhabitats with high water velocity, yet they were captured from areas with low water velocity. This convergent pair is of particular interest because behaviour might be influencing habitat use more than morphological specialization. For example, behaviour might be more important in determining prey exploitation than morphology for an intertidal fish assemblage (Grossman, 1986). Likewise, *E. gracile* and *K. kribensis* might select habitats with low water velocity even though they retain morphological traits adapted for holding position in fast water. It is important to note that these species were convergent only based on analysis of the ‘habitat-trait dataset’ and were not convergent when all traits were analysed, apparently owing to differences in life histories.

No convergent species were identified within either clusters of mid-water species in microhabitats with high water velocity and low instream structure or mid-water species in microhabitats with low water velocity and low instream structure, probably because most of the species in these clusters belonged to two ecologically and morphological similar families, Cyprinidae and Leuciscidae (Cypriniformes). Convergent evolution can be limited by various types of genetic, physiological and mechanical constraints that can facilitate phylogenetic niche conservatism, whereby lineages retain ancestral niches and phenotypes (Prinzing *et al.*, 2001; Brändle *et al.*, 2002; Entling *et al.*, 2007; Wiens *et al.*, 2010; Losos, 2011; Ernst *et al.*, 2012; Moen *et al.*, 2013). Genetic and developmental pathways can become increasingly canalized over time, which would also constrain the potential for convergent evolution. This might be particularly true for lineages that have evolved specialized niches (Schoener, 2009; Wiens *et al.*, 2010). Conversely, a sufficiently long period of evolution might allow distantly related lineages to

overcome phylogenetic and developmental constraints and converge in response to similar environmental conditions (Cody & Mooney, 1978; Melville *et al.*, 2006; Losos, 2011). Evolutionary constraints leading to niche conservatism and convergence resulting from adaptation to similar environments can occur to varying degrees, and often both can be recognized when comparing assemblage trait distributions (Cooper *et al.*, 2011; Moen *et al.*, 2013).

Multiple convergent groups were observed within certain microhabitat clusters. This suggests that more than one viable niche optimum (adaptive peak) exists within these microhabitats and is also consistent with the idea of many-to-one mapping of form and function (Wainwright *et al.*, 2005). In addition to the extensive convergence seen in this study, evolutionary constraint was also evident from the strong phylogenetic signal in traits and the morphological clustering of related species (see Bower & Winemiller, 2019), even within convergent groups.

Convergence across zoogeographical regions was common, but relatively little convergence was observed within assemblages of the same region (Table 3; Supporting Information, Figs S5–S9). This finding was not unexpected. Within zoogeographical regions, convergence between sympatric taxa should be rare, because most niches are likely to be occupied by species already possessing adaptive traits preventing species with similar niches from establishing in these habitats during community assembly. In contrast, evolutionarily independent lineages in different regions might evolve similar traits in response to similar selective environments over long time periods, thereby contributing to the diversity of species pools in their respective regions. Not all microhabitat clusters had representatives in every region that were significantly convergent. This might be attributable to incomplete sampling of the regions or to certain species types not evolving within a region.

Table 3. Number of convergent species across regions and number of convergent species within a single region

Categories	Proportion	Total species	Convergent species
All regions	0.340	197	67
Belize	0.048	21	1
Benin	NA	25	0
Brazil	0.038	52	2
Cambodia	0.122	41	5
USA	0.053	57	3

Abbreviation: NA, not assessed.

HABITAT GRADIENT CATEGORIES

To address our second and third hypotheses, we assessed convergence along habitat gradients, focusing independently on categories of water velocity and structural complexity. Species occupying microhabitats with high water velocity revealed greater convergence (C_1 values) and a smaller functional trait space when compared with species associated with slow-flowing water. This suggests that water velocity is a strong environmental filter that restricts the performance of species with certain traits. Drag on the body and the high energetic costs of maintaining position in habitats with fast-flowing water appear to exclude most deep-bodied fishes from occupying these habitats (Webb, 1984, 1988). In an adaptive landscape defined by a multivariate phenotypic surface, these high velocity and non-structured habitats with strong environmental filters would be defined by narrow, tall peaks. Consequently, the narrow peaks would limit the morphological variation and increase the probability that individuals occupying this peak would be strongly convergent. In microhabitats with low water velocities, this source of selection is relaxed, which permits coexistence of species with diverse morphologies and a larger assemblage morphospace, resulting in lower likelihood of convergence.

Fishes from structurally complex microhabitats also displayed lower degrees of convergence (lower average C_1 values) and greater functional richness, a finding similar to other studies (Willis *et al.*, 2005; Montaña *et al.*, 2014). Structurally complex habitats generally support more species and greater functional diversity (MacArthur & MacArthur, 1961; Gorman & Karr, 1978; Willis *et al.*, 2005), presumably because these habitats tend to provide more niche space and refuge from predators or harsh environmental conditions (Kovalenko *et al.*, 2012). Higher species richness in structurally complex habitats could also be associated with predation-induced increase in diversity, also known as aspect diversity, another dimension of functional diversity (Rand, 1967; Ricklefs, 2009). Although low C_1 values were found, on average, for species pairs in structurally complex and low water velocity categories, a few of the convergent species pairs in these habitats had high C_1 values. Structurally complex microhabitats with low water velocity should have greater availability of niches and weaker environmental filtering, which should support species packing, niche diversification and the evolution of specialized niches (Poff & Allan, 1995). Ecological specialists have provided some of the most impressive examples of convergence (Harmon *et al.*, 2005; Mahler *et al.*, 2013; Moen *et al.*, 2013; Blom *et al.*, 2016).

Although the results supported our second and third hypotheses, inferences might have been weakened by

an interaction between water velocity and substrate. For example, a fish can avoid the force of flowing water by occupying hydraulic refuges within the laminar boundary layer near the substrate surface or behind logs and other solid structures (Carlson & Lauder, 2011). However, the negative relationship of water velocity and substrate complexity with functional diversity and the positive relationships with convergence values still held when finer microhabitat categories were compared (e.g. benthic species in microhabitats with high water velocity and high instream structure vs. benthic species in microhabitats with high water velocity and low instream structure). Many microhabitat clusters with low water velocity and high substrate complexity tended to have larger functional diversity and small C_1 values, further supporting hypotheses 2 and 3.

CONCLUSIONS

Studies of evolutionary convergence have the potential to improve predictions about how species and assemblages will respond to a changing biosphere. The significant convergence found throughout the zoogeographical regions in the present study suggests that deterministic processes and some universal constraints strongly influence fish evolution and local community assembly in streams. Further exploration of convergence could facilitate development of models capable of forecasting changes in assemblage functional composition in response to anthropogenic habitat alterations. For example, our findings suggest that a reduction of instream structure, a likely consequence of deforestation of riparian habitats, would reduce the functional diversity of fish assemblages. Research is needed to determine whether convergence is common and predictable among fishes in other habitats and regions, not to mention other taxa. In addition, further research in molecular ecology and evolutionary developmental biology could prove particularly fruitful for understanding genetic mechanisms behind the widespread convergence observed in freshwater fishes. Convergent traits could evolve owing to mutations in similar developmental pathways, such as the armour plate patterning of threespine sticklebacks (Colosimo *et al.*, 2005; Stern, 2013), or have genetically disparate origins, such as the neofunctionalization of gene duplications resulting in the antifreeze glycoproteins of Antarctic notothenioid fish and Arctic cod (Chen *et al.*, 1997; Roelants *et al.*, 2010). Studies using molecular and evo-devo approaches could elucidate mechanisms that produce convergent traits, whereas ecological investigations, such as the one presented here, are needed to gain a better understanding of the role of environmental factors in species and assemblage convergence.

The present study revealed extensive convergence among fishes from similar microhabitats in low-gradient streams. Environmental factors at the microhabitat scale, especially hydraulics, appear to influence functional diversity of local assemblages at the habitat scale and result in convergence at the inter-continental scale. Following an adaptive landscape framework, consistent selective pressures should yield tall, narrow peaks, limiting the morphologies that can occupy this peak and increasing the likelihood of convergence. In contrast, weak selective pressure would produce broad, low peaks with greater functional richness and a lower likelihood of convergence. Our results match these expectations with regard to the apparent influence of water velocity and substrate complexity on convergence and functional diversity of stream fishes. The prevalence of convergence among stream fishes implies that predictable deterministic mechanisms play a strong role not only in evolution, but also during local community assembly.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Tanglegram of all species, depicting possible convergent taxa of the microhabitat clusters. The phylogeny is on the left side, and the phenogram from a cluster analysis using the 'habitat-trait dataset' (includes only habitat-associated traits) is on the right. The lines connect the position of each species on the phylogeny and dendrogram based on species trait similarity phenogram, showing only possible convergent taxa. Microhabitat clusters are labelled as follows: benthic species in microhabitats with high water velocity and high instream structure (BEN-HVHS, blue), benthic species in microhabitats with high water velocity and low instream structure (BEN-HVLS, black), benthic species in microhabitats with low water velocity and varying amounts of instream structure (BEN-LVVS, orange), mid-water species in microhabitats with low water velocity and high instream structure (MID-LVHS, green), and top-water species in microhabitats with low water velocities and varying amounts of instream structure (TOP-LVVS, red). Key to species names can be found in the [Supporting Information \(Table S1\)](#).

Figure S2. Tanglegram of all species, depicting possible convergent taxa of the microhabitat clusters. The phylogeny is on the left side, and the phenogram from a cluster analysis of the 'all-traits dataset' is on the right. The lines connect the position of each species on the phylogeny and dendrogram based on species trait similarity, showing only possible convergent taxa. Microhabitat clusters are labelled as follows: benthic species in microhabitats with high water velocity and high instream structure (BEN-HVHS, blue), benthic species in microhabitats with high water velocity and low instream structure (BEN-HVLS, black), mid-water species in microhabitats with low water velocity and high instream structure (MID-LVHS, green), and top-water species in microhabitats with low water velocities and varying amounts of instream structure (TOP-LVVS, red). Key to species names can be found in the [Supporting Information \(Table S1\)](#).

Figure S3. Tanglegram of all species, depicting possible convergent taxa from the water velocity categories. The phylogeny is on the left side, and the phenogram from a cluster analysis of habitat-associated traits is on the right. The lines connect the position of each species on the phylogeny and dendrogram based on species trait similarity, showing only possible convergent taxa. Water velocity categories are labelled as follows: high water velocity (blue lines) and low water velocity (green lines). Key to species names can be found in the [Supporting Information \(Table S1\)](#).

Figure S4. Tanglegram of all species, depicting possible convergent taxa from the substrate complexity categories. The phylogeny is on the left side, and the phenogram from a cluster analysis of habitat-associated traits is on the right. The lines connect the position of each species on the phylogeny and dendrogram based on species trait similarity, showing only possible convergent taxa. Substrate complexity categories are labelled as follows: high substrate complexity (blue lines), medium substrate complexity (red lines) and low substrate complexity (green lines). Key to species names can be found in the [Supporting Information \(Table S1\)](#).

Figure S5. Tanglegram of species from Benin, depicting possible convergent taxa of the microhabitat clusters. The phylogeny is on the left side, and the phenogram from a cluster analysis of habitat-associated traits is on the right. The lines connect the position of each species on the phylogeny and dendrogram based on species trait similarity, showing only possible convergent taxa. Microhabitat clusters are labelled as follows: benthic species in microhabitats with high water velocity and high instream structure (BEN-HVHS, blue), benthic species in microhabitats with high water velocity and low instream structure (BEN-HVLS, black), benthic species in microhabitats with low water velocity and varying amounts of instream structure (BEN-LVVS, orange), mid-water species in microhabitats with low water velocity and high instream structure (MID-LVAS, green), mid-water species in microhabitats with high water velocity and low instream structure (MID-HVLS, pink), and top-water species in microhabitats with low water velocities and varying amounts of instream structure (TOP-LVVS, red). Key to species names can be found in the [Supporting Information \(Table S1\)](#).

Figure S6. Tanglegram of species from Belize, depicting possible convergent taxa of the microhabitat clusters. The phylogeny is on the left side, and the phenogram from a cluster analysis of habitat-associated traits is on the right. The lines connect the position of each species on the phylogeny and dendrogram based on species trait similarity, showing only possible convergent taxa. Microhabitat clusters are labelled as follows: benthic species in microhabitats with high water velocity and high instream structure (BEN-HVHS, blue), benthic species in microhabitats with high water velocity and low instream structure (BEN-HVLS, black), benthic species in microhabitats with low water velocity and varying amounts of instream structure (BEN-LVVS, orange), mid-water species in microhabitats with low water velocity and high instream structure (MID-LVHS, green), mid-water species in microhabitats with high water velocity and low instream structure (MID-HVLS, pink), mid-water species in microhabitats with low water velocity and low instream structure (MID-LVLS, purple), and top-water species in microhabitats with low water velocities and varying amounts of instream structure (TOP-LVVS, red). Key to species names can be found in the [Supporting Information \(Table S1\)](#).

Figure S7. Tanglegram of species from Brazil, depicting only possible convergent taxa of the microhabitat clusters. The phylogeny is on the left side, and the phenogram from a cluster analysis of habitat-associated traits is on the right. The lines connect the position of each species on the phylogeny and dendrogram based on species trait similarity, showing only possible convergent taxa. Microhabitat clusters are labelled as follows: benthic species in microhabitats with high water velocity and high instream structure (BEN-HVHS, blue), benthic species in microhabitats with high water velocity and low instream structure (BEN-HVLS, black), benthic species in microhabitats with low water velocity and varying amounts of instream structure (BEN-LVVS, orange), mid-water species in microhabitats with low water velocity and high instream structure (MID-LVHS, green), mid-water species in microhabitats with high water velocity and low instream structure (MID-HVLS, pink), mid-water species in microhabitats with low water velocity and low instream structure (MID-LVLS, purple), and top-water species in microhabitats with low water velocities and varying amounts of instream structure (TOP-LVVS, red). Key to species names can be found in the [Supporting Information \(Table S1\)](#).

Figure S8. A tanglegram of species from Cambodia, depicting only possible convergent taxa of the microhabitat clusters. The phylogeny is on the left side, and the phenogram from a cluster analysis of habitat-associated traits is on the right. The lines connect the position of each species on the phylogeny and dendrogram based on species trait similarity, showing only possible convergent taxa. Microhabitat clusters are labelled as follows: benthic species in microhabitats with high water velocity and high instream structure (BEN-HVHS, blue), benthic species in microhabitats with high water velocity and low instream structure (BEN-HVLS, black), benthic species in microhabitats with low water velocity and varying amounts of instream structure (BEN-LVVS, orange), mid-water species in microhabitats with low water velocity and high instream structure (MID-LVHS, green), mid-water species in microhabitats with high water velocity and low instream structure (MID-HVLS, pink), mid-water species in microhabitats with low water velocity and low instream structure (MID-LVLS, purple), and top-water species in microhabitats with low water velocities and varying amounts of instream structure (TOP-LVVS, red). Key to species names can be found in the [Supporting Information \(Table S1\)](#).

Figure S9. A tanglegram of species from the USA, depicting only possible convergent taxa of the microhabitat clusters. The phylogeny is on the left side, and the phenogram from a cluster analysis of habitat-associated traits is on the right. The lines connect the position of each species on the phylogeny and dendrogram based on species trait similarity, showing only possible convergent taxa. Microhabitat clusters are labelled as follows: benthic species in microhabitats with high water velocity and high instream structure (BEN-HVHS, blue), benthic species in microhabitats with high water velocity and low instream structure (BEN-HVLS, black), benthic species in microhabitats with low water velocity and varying amounts of instream structure (BEN-LVVS, orange), mid-water species in microhabitats with low water velocity and high instream structure (MID-LVHS, green), mid-water species in microhabitats with high water velocity and low instream structure (MID-HVLS, pink), mid-water species in microhabitats with low water velocity and low instream structure (MID-LVLS, purple), and top-water species in microhabitats with low water velocities and varying amounts of instream structure (TOP-LVVS, red). Key to species names can be found in the [Supporting Information \(Table S1\)](#).

Figure S10. Principal components analysis (PCA) ordination plot of the habitat-traits dataset for each zoogeographical region. Zoogeographical regions are labelled as follows: Benin (BEN), Belize (BEL), Brazil (BRA), Cambodia (CAM) and USA (USA). Left plot shows the species coloured by microhabitat preference: benthic species in microhabitats with high water velocity and high instream structure (BEN-HVHS, blue), benthic species in microhabitats with high water velocity and low instream structure (BEN-HVLS, black), benthic species in microhabitats with low water velocity and varying amounts of instream structure (BEN-LVVS, orange), mid-water species in microhabitats with low water velocity and high instream structure (MID-LVHS, green), mid-water species in microhabitats with high water velocity and low instream structure (MID-HVLS, pink), mid-water species in microhabitats with low water velocity and low instream structure (MID-LVLS, purple), and top-water species in microhabitats with low water velocities and varying amounts of instream structure (TOP-LVVS, red).

species in microhabitats with low water velocity and low instream structure (MID-LVLS, purple), and top-water species in microhabitats with low water velocities and varying amounts of instream structure (TOP-LVVS, red). Right plot shows the trait scores.

FigureS11. Principal coordinates analysis (PCOA) ordination plot of the all-traits dataset for each zoogeographical region. Zoogeographical regions are labelled as follows: Benin (BEN), Belize (BEL), Brazil (BRA), Cambodia (CAM) and USA (USA). Left plot shows the species coloured by microhabitat preference: benthic species in microhabitats with high water velocity and high instream structure (BEN-HVHS, blue), benthic species in microhabitats with high water velocity and low instream structure (BEN-HVLS, black), benthic species in microhabitats with low water velocity and varying amounts of instream structure (BEN-LVVS, orange), mid-water species in microhabitats with low water velocity and high instream structure (MID-LVHS, green), mid-water species in microhabitats with high water velocity and low instream structure (MID-HVLS, pink), mid-water species in microhabitats with low water velocity and low instream structure (MID-LVLS, purple), and top-water species in microhabitats with low water velocities and varying amounts of instream structure (TOP-LVVS, red). Right plot shows the trait scores.

TableS1. List of species, their acronym, common name, family, order, collection location, habitat cluster, life history grouping and number of individuals collected. Asterisks denote significant convergence with at least one other species. Habitat groupings: top-water species in microhabitats with low water velocities and varying amounts of instream structure (TOP-LVVS), mid-water species in microhabitats with high water velocities and low instream structure (MID-HVLS), mid-water species in microhabitats with low water velocity and low instream structure (MID-LVLS), mid-water species in microhabitats with low water velocity and high instream structure (MID-LVHS), benthic species in microhabitats with high water velocity and low instream structure (BEN-HVLS), benthic species in microhabitats with high water velocity and high instream structure (BEN-HVHS), and benthic species in microhabitats with low water velocity and varying amounts of instream structure (BEN-LVVS).

Table S2. The principal component (PC) scores, eigenvalues, proportion of variance explained and cumulative variance explained from the principal components analysis of the habitat-traits dataset.

TableS3. The principal component (PC) scores, eigenvalues, proportion of variance explained and cumulative variance explained from the principal coordinates analyses (PCOA) of the all-traits dataset.

Table S4. The results of the Stayton's C_1 tests using the habitat-traits dataset. A list of each species' pairwise comparisons with their C_1 values, uncorrected P -values, microhabitat cluster and grouping within microhabitat cluster.

Table S5. Results of the Stayton's C_1 tests using the all-traits dataset. A list of each species' pairwise comparisons with their C_1 values and uncorrected P -values.

SHARED DATA

The data underlying the study are available from the Dryad Digital Repository (Bower & Winemiller, 2020).