Ancient and contingent body shape diversification in a hyperdiverse continental fish radiation

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The characiform fishes of the Neotropics and Africa radiated remarkably in ecomorphology, but the macroevolutionary processes responsible for their biodiversity remain unexplored, and the degree to which their continental diversification parallels classic adaptive radiations remains untested. We reconstruct their diversification using a new fossil-calibrated molecular phylogeny, dietary information, and geometric morphometrics. Though body shape diversified early in a manner consistent with an ancient continental adaptive radiation, trophic shifts did not always coincide with shape changes. With the notable exception of piscivores, lineages that converged in diet did not converge closely in body shape. Shifts in habitat or other variables likely influenced body shape evolution in addition to changes in diet, and the clade's history departs from many classic adaptive radiations in lakes or on islands, in which trophic convergence drives morphological convergence. The contrast between the Neotropical radiation's exhaustive exploration of morphospace and the more restrained diversification in Africa suggests a major role for contingency in characiform evolution, with the presence of cypriniform competitors in the Old World, but not the New, providing one possible explanation. Our results depict the clearest ecomorphological reconstruction to date for Characiformes and set the stage for studies further elucidating the processes underlying its diversification.

KEY WORDS: Adaptive radiation, characiformes, convergence, macroevolution, morphological evolution, phylogenetics.

Much of life's rich diversity evolved during adaptive radiations, in which clades speciate rapidly while generating spectacular ecomorphological variation (Simpson 1953; Schluter 2000; Glor 2010). The best understood radiations occurred in geographically restricted ecosystems (Schluter 2000), in which increases in ecological opportunity catalyzed early bursts of linked morphological and lineage diversification (Schluter 2000; Givnish 2015). Physical invasion of a new environment with unoccupied niches can provide that ecological opportunity, as occurred in Darwin's famous finches (Lack 1947; Grant and Grant 2006), honeycreepers, silverswords, and *Drosophila* on the Hawaiian archipelago (Carson et al. 1970; Givnish et al. 2009; Lerner et al. 2011), Caribbean *Anolis* lizards (Losos et al. 1998; Harmon et al. 2005), and the cichlids of Africa's crater (Machado-Schiaffino et al. 2015) and rift lakes (Kocher et al. 1993; Muschick et al. 2012;

Machado-Schiaffino et al. 2015). Functional innovations can also promote diversification, as in the fusion of the cichlid pharyngeal jaw, which increased ecological opportunity by allowing novel prey consumption (Liem 1973; Hulsey et al. 2006; Hulsey 2006). Regardless of the process opening ecological opportunity, adaptive radiations tend to follow a predictable pattern that modern phylogenetic comparative methods can detect (Schluter 2000; Gavrilets and Losos 2009; Glor 2010).

Insular and lacustrine radiations tend to exhibit rapid initial bursts of phenotypic innovation (Simpson 1944), a link between divergent ecologies and divergent morphologies (Schluter 2000), and convergence across replicated radiations (Losos and Ricklefs 2009). In the most famous ichthyological example, the cichlid fishes of Africa's rift lakes radiated in parallel, with each lake's fishes diversifying across habitat and depth early in cladogenesis,

then convergently adapting their heads and bodies to diverse diets (Rüber and Adams 2001; Hulsey 2006; Hulsey et al. 2013). Sticklebacks and mountain whitefishes replicated their ecomorphological radiations following multiples invasions of postglacial lakes (Schluter 1995; Rundle et al. 2000; Vonlanthen et al. 2009). In nonfish systems, Antillean anoles (Mahler et al. 2010), Hawaiian spiders (Gillespie 2004), and French Polynesian land snails all exhibit substantial morphological convergence during adaptation to varied habitats and diets, indicating substantial determinism when adaptively radiating in insular systems.

Fewer studies have attempted to reconstruct patterns of geographically widespread radiations. Continental radiations of ratsnakes, cichlids, and mainland anoles (Pinto et al. 2008; Burbrink and Pyron 2010; López-Fernández et al. 2013) exhibit some signatures of classical adaptive radiations including early bursts and high rates of phenotypic change. Cenozoic and Mesozoic mammalian radiations produced many convergent taxa (Luo 2007), much like the replicated island radiations discussed above. However, the relationships among ecological opportunity, species formation, and adaptive divergence appear much more varied at larger scales (Maestri et al. 2017). For example, rates of speciation and morphological diversification were decoupled in New World ratsnakes, leading to clades with high species diversity, but limited ecological variability (Burbrink et al. 2012). Patterns within Neotropical cichlids vary regionally, with South American riverine lineages exhibiting an initial spike in disparity that quickly declined, consistent with patterns in the lacustrine adaptive radiations, while Central American riverine lineages diversified their morphology throughout cladogenesis (Arbour and López-Fernández 2016). Despite that temporal difference, both continental cichlid radiations overlap in morphospace and produced lineages that converge in diet and body shape (Winemiller et al. 1995; López-Fernández et al. 2013; Arbour and López-Fernández 2016). These studies suggest that the tempo and mode of continental-scale radiations may be less predictable and more susceptible to historical contingency than adaptive radiations in lake and island systems, and that morphological convergence may remain a hallmark of replicated radiations at any scale.

Scientists have long debated the degree to which adaptation and diversification are deterministic or dependent on historical contingency (Gould 1989, 2002; Conway Morris 2003; Losos 2017). If adaptive evolution is highly deterministic then repeated invasion of the same ecological niche will produce convergent morphologies, as seen in the numerous *Anolis* lizard radiations (Losos 1992; Losos et al. 1998; Harmon et al. 2005; Mahler et al. 2013), sticklebacks (Schluter 1995; Rundle et al. 2000), and lake whitefishes (Vonlanthen et al. 2009). Yet, clades that evolve under similar selective pressures may not always converge because the contingencies of history can change the trajectory

of diversification in lineages under the same functional demands (Gould 1989). These contingencies can include underlying genetic or behavioral differences (Donoghue 2005), clade-specific morphological innovation (Losos 2011), and variation in ecological opportunity caused by the presence or absence of competitors in disparate ecosystems (Hansen 1997; Langerhans and DeWitt 2004).

Because clades respond to the evolutionary dynamics of other organisms that share their environments, one might expect more contingency than determinism in the evolutionary history of geographically expansive radiations (Foote 1997; Ricklefs 2010). Evolutionary determinism would be less likely in such cases because different portions of the radiation evolve in the presence of different species communities (Gould 1989; Losos 2011). Prior occupancy by other species in a community potentially restricts a clade's ability to diversify by reducing niche space, and once morphospace has saturated, diversification in some clades must be balanced by shrinking in others (Ricklefs 2010). In other words, diversifying clades fill niche space vacated by extinction or force out other lineages through competitive exclusion. This heightened contingency due to variation in community composition may help explain why patterns of morphological diversification appear less predictable in continental radiations than insular and lacustrine radiations. However, the field requires many more studies of widespread radiations to understand how and why diversification differs at small versus expansive scales.

The fish order Characiformes provides an excellent opportunity to infer the evolutionary model underlying a major continental ecomorphological radiation. Comprising more than 2000 species distributed primarily in South America and Africa, characiforms vary dramatically in body, jaw, and tooth morphology (Géry 1977). They include elongate piscivores like the famous African tigerfish or the Neotropical pike-characins, compressiform tetras, fusiform headstanders, deep-bodied herbivorous pacus, cylindrical darter-like insectivores, and many other diverse forms. Most morphological studies have focused on the diversification of subclades (Buckup 1993a; Sidlauskas 2008) or revised systematics (Fink 1996; Buckup 1998; Malabarba and Weitzman 2003; Mirande 2009; Mirande 2010), rather than studying morphological disparity across this order.

Guisande et al. (2012) represents the most comprehensive study to date of characiform ecomorphological diversification. They found shorter dorsal-fin bases in species inhabiting high flow habitats, which may reduce drag in fast water (Guisande et al. 2012). That study also inferred a link between tooth shape and diet, with carnivorous species possessing caniniform teeth, omnivores possessing multicuspidate dentition, and herbivores tending toward molariform teeth. They postulated that an ancient adaptive radiation along habitat and trophic axes shaped characiform morphological diversity, but without tests of adaptive

evolution or comparison of alternative modes of diversification, the exact evolutionary mechanism and extent that ecology influences morphology in Characiformes remain unknown.

Because fishes adapt their bodies simultaneously to the ecological demands of habitat and diet, variation in body shape can help reconstruct the history of an ichthyological radiation. Most obviously, trunk shape and fin position can adapt a fish to a specific water column position or flow regime (Webb 1982; Webb and Weihs 1986; McLaughlin et al. 1999; Proulx and Magnan 2002) and gape size, eye placement and mouth position covary strongly with diet (Wainwright and Richard 1995). Postcranial body shape also adapts fishes trophically (Rüber and Adams 2001), for example by increasing maneuverability in compressiform fishes that hunt zooplankton (Keast and Webb 1966), or by optimizing the fast-start lunges that sagittiform fishes (pikes, gars, needlefishes, and many others) use to ambush prey (Domenici and Blake 1997). Studies of body shape variation in fishes have a long tradition (Thompson 1942; Bookstein et al. 1985; Claverie and Wainwright 2014), due to the ease of locating homologous landmarks and measurements, even across large phylogenetic scales.

Our study examines the evolution of characiform body shape in the context of shifts among different trophic ecologies and against the backdrop of independent evolution on two continents to reconstruct the history of the radiation and infer the relative influence of determinism versus contingency. Did characiform evolution on Africa mirror their evolution in South and Central America? Does the ecomorphological history of this major continental radiation parallel classic insular adaptive radiations? To answer these general questions, we infer a large, robust molecular phylogeny by synthesizing data from previous studies and link it to a novel body shape dataset. Phylogenetic comparative methods then address five specific questions about body shape evolution in the order: (1) Does a simple Brownian model best explain body shape diversification, or did characiforms evolve under a more complicated model characterized by distinct adaptive optima? (2) Did body shape diversify continuously, or primarily early in the clade's history? (3) Did dietary shifts coincide frequently with major shifts in body shape? (4) Did distinct lineages switching to the same trophic ecology converge morphologically, or do multiple morphological solutions correspond to each trophic niche? (5) Did body shape diversify primarily contingently or deterministically, as would be indicated by low versus high levels of convergence, and mostly dissimilar or similar evolutionary patterns in Africa and the Neotropics?

Materials and Methods
PHYLOGENETIC ANALYSIS
We synthesized data for the four most commonly sequenced loci in characiform systematics from nine previous publications and added new data from 27 taxa to assemble a dataset spanning 129 taxa in 21 characiform families. Taxon sampling averaged 30% of species within each family. See Supporting Information S1 for details on data acquisition and alignment. Macroevolutionary analyses employed an ultrametric tree estimated under a Bayesian relaxed-clock model in BEAST 2.0 (Bouckaert et al. 2014) through the CIPRES web server (Miller et al. 2010) using seven fossil calibrations. Parameters and priors for the BEAST analysis were set up using BEAUTi 2.0 (Bouckaert et al. 2014; see Supporting Information S3 and Table S2).

We performed four independent MCMC runs for 250 million generations, sampling every 25,000. The first 10% of the posterior was disregarded as burn-in after visualizing the posterior distribution in Tracer 1.5 (Rambaut and Drummond 2007) using effective sample size (ESS), prior convergence, and likelihood ($-\ln L$) of the priors and posterior estimates. An ESS >200 generally indicates that a MCMC has searched the landscape sufficiently (Drummond et al. 2006). The remaining trees were used to construct a maximum clade credibility tree.

We also conducted Bayesian and Maximum-Likelihood reconstructions in Mr. Bayes and RAxML to ensure that these methods did not yield a substantively different topology from the BEAST analysis (see Supporting Information S2 for details). Because the interfamilial relationships inferred from the synthetic dataset using any of these methods differed slightly from a recent molecular phylogeny inferred from similar data (Oliveira et al. 2011), we also used BEAST 2.0 to infer an ultrametric tree constrained to that topology (see Supporting Information S3 and Fig. S1 for details) and repeated all macroevolutionary analyses using that constrained phylogeny. We did not repeat analysis with a phylogeny constrained to the very recent reconstruction of Betancur-R et al. (2018), because that paper appeared while our contribution was in the final stages of revision after peer review.

MORPHOLOGICAL DATA ACQUISITION
Seven museum collections provided specimens for morphometric analysis (detailed in the Supporting Information). One to ten specimens (average = 7.1 ± 2.3) from each of 329 species were included, totaling 2210 specimens from 21 families. To avoid the confounding effects of known ontogenetic allometry (Fink and Zelditch 1995; Sidlauskas et al. 2011) only putative adult specimens (>50% of the maximum reported length for the species) were used. Morphospaces were constructed using all 329 species to capture the major axes of morphological variation in the order with the maximum rigor possible. However, only the 118 species that corresponded to tips on the synthetic phylogeny and had matching ecological data were used in comparative phylogenetic analysis.

Geometric morphometric data were collected from radiographs (Fig. 1; Supporting Information S4). Because the
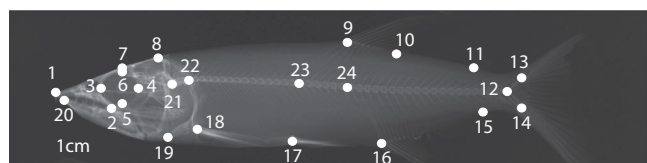
Figure 1. Radiograph of *Acestrorynchus minimus* USNM 311177 50.0 mm SL, showing position of 24 landmarks used in geometric morphometric analysis. (1) anterior limit of premaxilla; (2) posterior limit of premaxilla; (3) anterior limit of orbit; (4) posterior limit of orbit; (5) ventral limit of orbit; (6) dorsal limit of orbit; (7) dorsal margin of neurocranium at vertical through center of orbit; (8) posterodorsal tip of supraoccipital; (9) dorsal-most portion of the base of the first dorsal-fin ray pterygiophore; (10) dorsal-most portion of the base of the last dorsal-fin ray pterygiophore; (11) base of the body dorsal of the fourth most posterior; (12) compound vertebral centrum at posterior of verebral column; (13) posterodorsal limit of hypurals; (14) ventrodorsal limit of hypurals; (15) dorsal-most portion of the base of the of last anal-fin ray pterygiophore; (16) dorsal-most portion of the base of the first anal-fin ray pterygiophore; (17) pelvic-fin origin; (18) pectoral-fin origin; (19) ventral limit of joint between contralateral cleithra; (20) anterior tip of dentary; (21) joint between basioccipital and first vertebra of Weberian apparatus; (22) anterior limit of fifth vertebra (first vertebra not incorporated into Weberian apparatus and first bearing full-sized pleural ribs); (23) vertebral centroid posterior to first dorsal-fin ray pterygiophore; (24) vertebral centroid posterior to first anal-fin ray pterygiophore.

phylogenetic comparative methods required representation of each species by a single data point, we calculated the centroid position for each species in morphospace, and used those coordinates in subsequent analysis.

Morphological disparities for families and continental assemblages were calculated as Procrustes variance using the morphol.disparity function in geomorph (Adams and Otarola-Castillo 2013), and as Euclidean variances by applying the disparity function in geiger 2.0 (Pennell et al. 2014) to the morphospace distributions. Procrustes variance is the mean squared Procrustes distance of each specimen in a group from the mean shape of that group (Zelditch et al. 2012).

ECOLOGICAL CLASSIFICATION

Each species was assigned to a generalized trophic guild through a primary literature review of diet in characiform fishes in Academic Search Premier, Web of Science, and Google Scholar. We also examined species descriptions, field guides, and checklists, and searched FishBase (Froese and Pauly 2000) for dietary data. Supporting Information S5 contains further details.

Ancestral dietary shifts were determined using Bayesian stochastic character mapping (1000 simulations; Huelsenbeck et al. (2003)) implemented in the simmap function in the phy-

tools package (Revell 2012). We used the "all rate different" (ARD) model and allowed different forward and backward rates, estimated the prior distribution of the states at the root of the tree and used the MCMC option to set the parameters of the Q matrix.

EVOLUTIONARY MODEL SELECTION

We performed model selection in mvMORPH (Clavel et al. 2015) using small sample-size corrected AICc to determine whether a simple stochastic model of evolution could reasonably explain the history of diversification in the order, or whether evidence exists for a more complicated scenario. We included two basic stochastic scenarios, a univariate Brownian model and a mixed rate Brownian model in which clades may differ in their underlying rate of morphological change. We also tested the modified Brownian "early burst" model of Harmon et al. (2010), in which morphology evolves more quickly early in cladogenesis, and multiple a priori Ornstein-Uhlenbeck (OU) models designed to distinguish alternative hypotheses of body shape evolution toward distinct optima in morphospace (Fig. 2). The morphological optima in these OU models are most frequently interpreted as adaptive peaks, and indeed, in an adaptive radiation scenario one expects either the early burst model or a multiple-peak OU model to fit best. However, we acknowledge that the Brownian model may fit certain types of adaptive evolution well (particularly a scenario in which the positions of the adaptive peaks shifts frequently) and that a single-peak OU model may also describe evolution within a constrained or bounded morphospace. All models fitted were multivariate, because analyzing principal components individually can artefactually privilege the early burst model (Uyeda et al. 2015).

Because preliminary analyses revealed that Old World and New World assemblages differed in morphospace occupancy, one model (OUcontinent) hypothesized that characiform lineages diversifying on each continent evolved toward separate optima. Another model (OUtrophic) hypothesized a distinct optimum for each trophic category, directly testing whether species with different diets evolved toward different optima, and whether lineages converging in diet also converged in morphospace. OUfamily hypothesized a distinct optimum for each family, as expected if body shape coincided with lineage formation early in evolutionary history. OU family + piscivore was similar to OUfamily, but also hypothesized that all piscivorous species converged on a single adaptive optimum regardless of familial assignment. This model was included because preliminary analyses and inspections of morphospace suggested that piscivorous lineages, and no others, converged.

To determine if our a priori models were appropriately comprehensive, we also included the best a posteriori model selected by an unconstrained SURFACE analysis (Ingram and Mahler 2013), and a constrained SURFACE model in which piscivorous
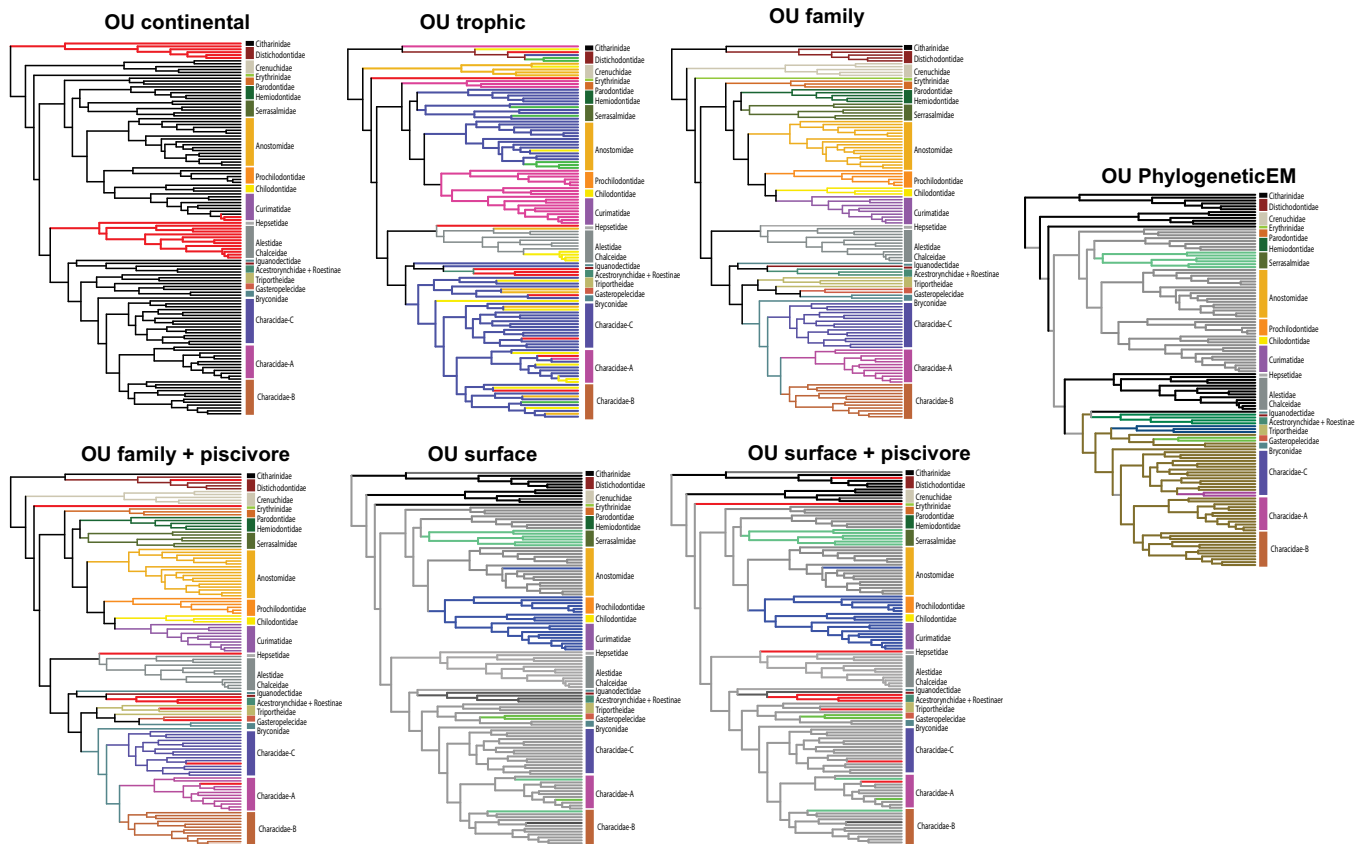
Figure 2. Alternative multivariate OU hypotheses for the evolution of body shape in characiform fishes. The trees show the different OU regimes hypotheses used in model selection analyses performed in mvMORPH. The first model (OU continental) hypothesizes that species on the same continent share an adaptive peak. The second model (OU trophic) hypothesizes that species that share a trophic ecology also share an adaptive peak. The third model (OU family) is a phylogenetic hypothesis where species share an adaptive peak with other members of their family. The fourth model (OU family + piscivore) is phylogenetic hypothesis where species share an adaptive peak with other members of their family except that all piscivorous fishes share an adaptive optimum irrespective of their familial affiliation. The fifth model (OU surface) is the best-fitting model returned by the SURFACE analysis. The sixth model (OU surface + piscivore) is the best-fitting model returned by the SURFACE analysis, but with all piscivorous fishes sharing an adaptive optimum. We also fitted two different Brownian motion models, an early burst model, and an alternative multipeak model in PhylogeneticEM.

lineages converged. SURFACE was applied to the MCC phylogeny identified in BEAST using the first four PC axes for the entire dataset. We implemented a delta AICc threshold of -3, rather than the default zero because SURFACE can overfit models and identify biologically unreasonable regime shifts or convergences (Ingram and Mahler 2013; Pennell et al. 2014) without such correction. The function MEDUSA in geiger (Pennell et al. 2014) estimated that AICc threshold.

Even with the ΔAICc correction, SURFACE may still overfit the number of optima, and it has attracted criticism for its unreasonable assumption of no evolutionary correlation among PC axes (Adams and Collyer 2017). In light of the potential issues with the SURFACE analysis, we also used PhylogeneticEM (Bastide et al. 2018) to identify optima on the MCC phylogeny a posteriori, and included that model in the mvMORPH analysis. This newer likelihood method uses an ex-

pectation maximization algorithm (multivariate OU model, or scOU) to estimate the shift positions without assuming uncorrelated axes (Bastide et al. 2018). We compare the optima identified in the SURFACE and PhylogeneticEM analyses in the results.

Accurate ranking of complex models such as those we implemented in mvMORPH requires substantial statistical power (Boettiger et al. 2012), and insufficient power can lead to complex OU models being incorrectly favored (Ho and Ané 2014; Cooper et al. 2016). To determine whether we had sufficient power to accurately gauge model support, we simulated 1000 datasets in mvMORPH simulations using the function mvSIM and the parameter estimates for the best-fit model in our empirical dataset. The simulated data were then run through all the aforementioned models in mvMORPH to determine whether program would select the correct generating model.

TEST OF LINKED TROPHIC AND MORPHOLOGICAL EVOLUTION

To test whether changes in diet coincided with major changes in body morphology, we determined the frequency with which SIMMAP reconstructed a shift in trophic ecology on the same phylogenetic branch whereon SURFACE or PhylogeneticEM identified a shift in morphological regime. We compared those frequencies to the probability of recovering at least that many matches when distributing the same number of ecological and morphological shifts randomly across the phylogeny (see further description and equation in supplement). The test determines only whether ecology and body shape tend to coevolve on the same branches, and does not explicitly test for convergence.

TESTS OF EVOLUTIONARY DETERMINISM

We included two different tests of evolutionary determinism, or predictability. First, we investigated whether repeated transitions to the same diet resulted in similar body shapes. Potentially convergent lineages were identified using a phylomorphospace approach (Sidlauskas 2008) as implemented in phytools (Revell 2012), as those that independently evolved a trophic ecology and either fell into the same portion of morphospace or diverged from sister taxa in a similar direction. Then, a priori morphospace tests of convergence (C1–C4) implemented in convevol (Stayton 2015) quantified the degree to which convergent taxa evolved to be more similar than their reconstructed ancestors. Trait data were simulated under Brownian motion across the tree for 500 generations to gauge significance.

Second, to determine the degree to which evolution on each continent resulted in similar morphospace occupancy, we compared the Procrustes and Euclidean variances of each continental assemblage. Substantially different variances would indicate nonequivalent evolutionary outcomes, and thus suggest a substantial role for contingency in characiform evolution at the continental scale. The significance of variation between groups was assessed statistically using a permutation technique to generate a distribution by randomizing shape matrix rows relative to group assignment 1000 times (Adams and Otárola-Castillo 2013). We also estimated the expected difference in variance due to differences in ages and branching times among the various South American and African clades under a multivariate Brownian motion model through simulation (see Supporting Information S6).

Results

PHYLOGENY

BEAST reconstructed a well-resolved and well-supported topology for 129 species, supporting previous hypotheses for most relationships (Fig. 3; Supporting Information Fig. S3). High pos-

terior probabilities support all major lineages (>.95), with very high posterior probabilities for most interfamilial relationships (>.99), except for the placement of Erythrinidae, Iguanodectidae, and Parodontidae that had ambiguous support (<.5) due to gene tree conflict. Some intergeneric relationships are also poorly supported (<.95), particularly within the families Anostomidae, Curimatidae, and Alestidae, mostly likely due to low taxon sampling. Fourteen of the 15 families represented by multiple species resolved as monophyletic, with Triportheidae recovered as paraphyletic (Fig. 3). Our topology is broadly congruent with several morphological and molecular phylogenies for Characiformes (Buckup 1993a; Calcagnotto et al. 2005; Mirande 2009; Oliveira et al. 2011; Betancur-R et al. 2018), but see Supporting Information S8 for some differences.

TROPHIC ECOLOGY

Characiforms vary substantially in trophic ecology (see Supporting Information Tables S2 and S7), and converged many times upon all guilds except omnivory, which represents the ancestral condition in all SIMMAP reconstructions (Fig. 4), even when species with uncertain diets were assigned to alternative guilds (see Supporting Information S9). On average, detritivory evolved four (a maximum of five) times, herbivory five (maximum of seven) times, invertivory 10 times (a maximum of 11), and piscivory and insectivory each eight (maximum of nine and 10, respectively) times. Using our coarse classification and limited taxon sampling, the number of species in a guild does not predict the number of times each guild arose. For example, 83% of the species following the second most speciose strategy (detritivory) arose in a single radiation (Curimatidae and two allied families). Each evolution of herbivory yielded only a few modern species. Origins of piscivory and invertivory together account for almost half of the trophic transitions, but subtend clades containing only 17% of the total species diversity.

TIMING AND PATTERNS OF PHENOTYPIC DIVERSIFICATION

The first four principal components summarized respectively 47, 23, 10, and 5% of the variance following Procrustes superimposition, totaling 85% of the variance in landmark position. The remaining components accounted for 3% or less of the total variance, and consecutive vectors had similar eigenvalues. Because the small amounts of isotropic variation indexed by these axes are consistent with the pattern expected of measurement error (Anderson 1963), they were not considered further.

The wireframes in Figure 5 visualize the shape change along each axis. Fishes with a low PC1 score exhibit a moderately elongate body shape, a terminal mouth, anteriorly placed dorsal fin, and posteriorly placed anal fin. As PC1 increases, body depth becomes greater, the mouth becomes more elongate and upturned,
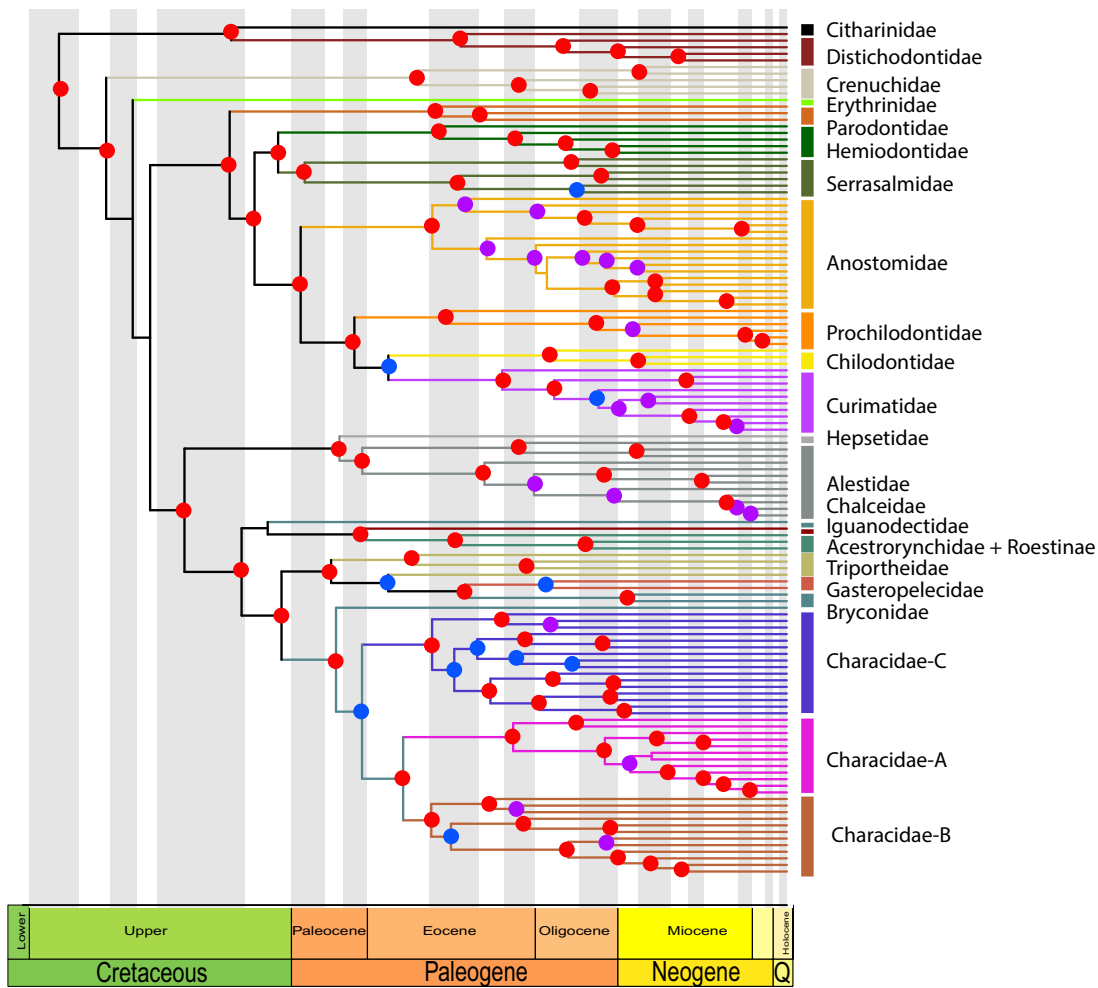
Figure 3. Relationships among sampled species in the order Characiformes through a Bayesian partitioned analyses of the concatenated dataset. Circles at each node represent posterior probability scores. Red represents a posterior probability score of 1, blue represents a score between 0.95 and 0.99, purple represents a score between 0.8 and 0.94, and no circle represents a score below 0.8.

the length of the anal fin increases, and the dorsal and anal fins move posteriorly. Low PC2 scores indicate a deep-bodied fish with a relatively small mouth, an anteriorly placed dorsal fin and posteriorly placed anal fin. As PC2 increases, the body becomes more elongate with the dorsal and anal fins positioned just posterior to the body's midpoint, and the terminal mouth enlarges. A low PC3 score indicates a deep-bodied fish with a short caudal peduncle, small head, but large mouth. As PC3 increases, the body lengthens, the dorsal and anal fins move anteriorly, and the mouth diminishes. Low PC4 scores indicate a deep bodied fish with a small mouth and head. As PC4 increases the body becomes more elongate and the head, mouth, and eyes increase in size.

PCA MORPHOSPACE AND DISPARITY

Characiformes have diversified greatly in body shape (Fig. 6), with members falling into one of four major morphospace regions. Fishes at the top of the morphospace exhibit sagittiform

morphologies, characterized by a slender body with the dorsal and anal fins located posteriorly. This region of morphospace contrasts with the bottom right, populated by compressiform, deep-bodied fishes with the dorsal and anal-fins also located posteriorly. The highest density of families occurs in the middle left, where fishes exhibit a fusiform morphotype with anteriorly placed dorsal fins, posteriorly placed anal fins and terminal or subterminal mouths. Only a handful of species occupy the fourth area at the middle far right. These species differ from other compressiform fishes with posteriorly placed dorsal and anal fins by having upturned versus terminal or inferior mouths.

Body shape varies substantially among, but not within most families. Confamilial species tend toward similar shapes and occupy compact regions of morphospace. Approximately half (48%) of all families exhibit low disparity (Procrustes variance < .0020), or about one fourth that of the family with the highest disparity (Characidae, including all three major subclades, Procrustes
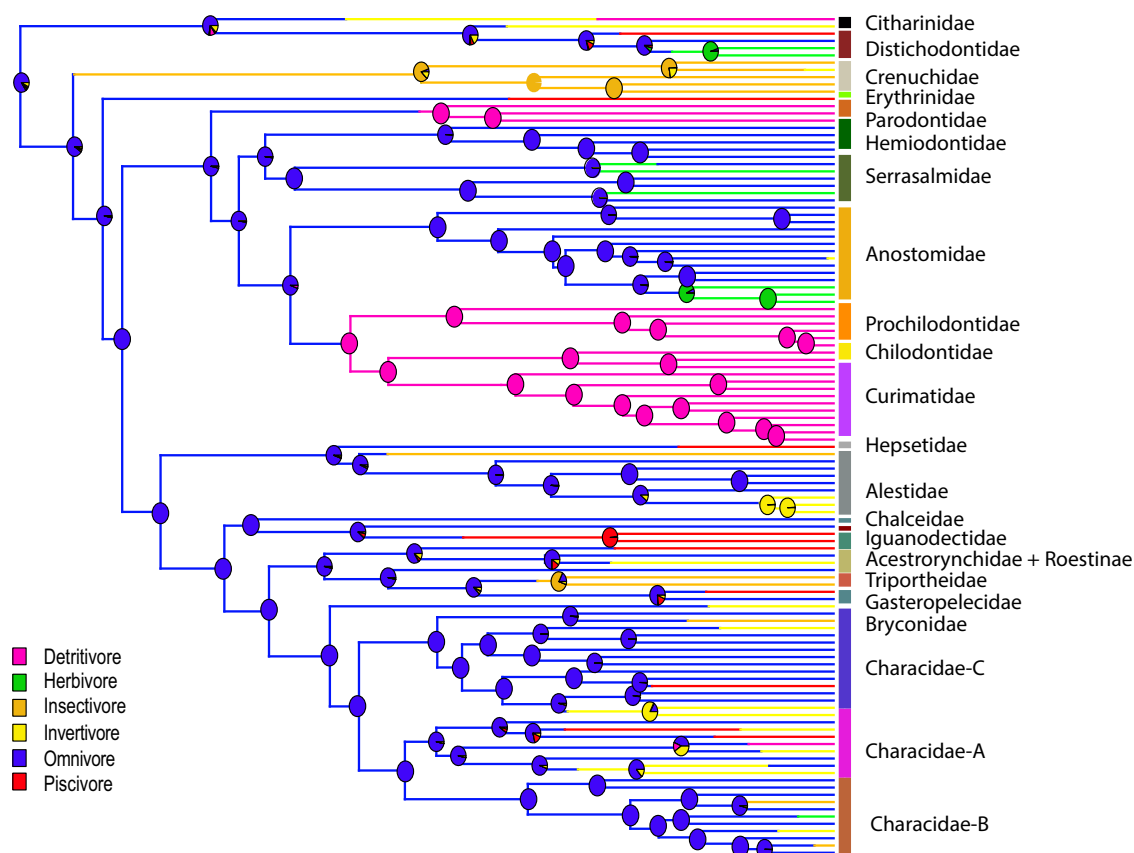
Figure 4. Trophic ecology evolutionary history: summary of 1000 SIMMAP character maps using the fossil-calibrated phylogeny. Colors represent different trophic ecologies; sector of pies at nodes are proportional to the probabilities of each state at that node.
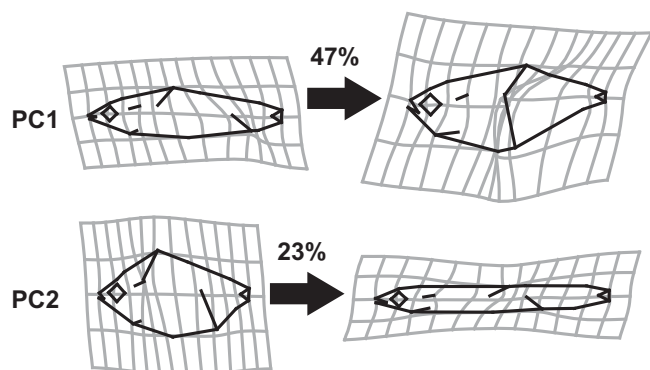

Figure 5. Deformation grids illustrating limits of observed variation of shape change on the two most important principal components. Numerical values represent the percent variance explained by each component.

variance = 0.0072). In the multipeak OU model postulating separate optima for each family, many of these clades have low species diversity, high pull toward to the optimum (average α = 8.06 ± 0.9) and a low rate of evolution (average σ = 0.014 ± .002), suggesting relative morphological and ecological stasis after initial colonization of the morphospace region. In

contrast, 29% of the families exhibited significantly higher levels of disparity (Procrustes variance > .005) than would be expected. All of the lineages with high morphological disparity are relatively species rich and ecologically diverse, such as Characidae considered as a whole, all three of Characidae's major subclades, and to a lesser extent Distichodontidae (Procrustes variance = 0.0059) and Alestidae (Procrustes variance = 0.0054).

FIT OF EVOLUTIONARY MODELS

Both a priori models of Brownian evolution were very unlikely (Table 1), as was the early burst model, implying that Characiformes probably evolved under a more complicated, nonrandom process featuring distinct morphological optima. That said, Ornstein-Uhlenbeck models with separate optima for the Old and New World lineages, or with a single optimum for each trophic ecology were also very unlikely. We found higher support for a priori models with each family possessing a unique optimum, and models with all piscivorous lineages sharing an optimum (OU family + piscivore and OU surface + piscivore; Table 1). However, mvMORPH found the a posteriori model selected by SURFACE to fit best of all, and substantially better than the a priori model hypothesizing a single distinct body shape optimum
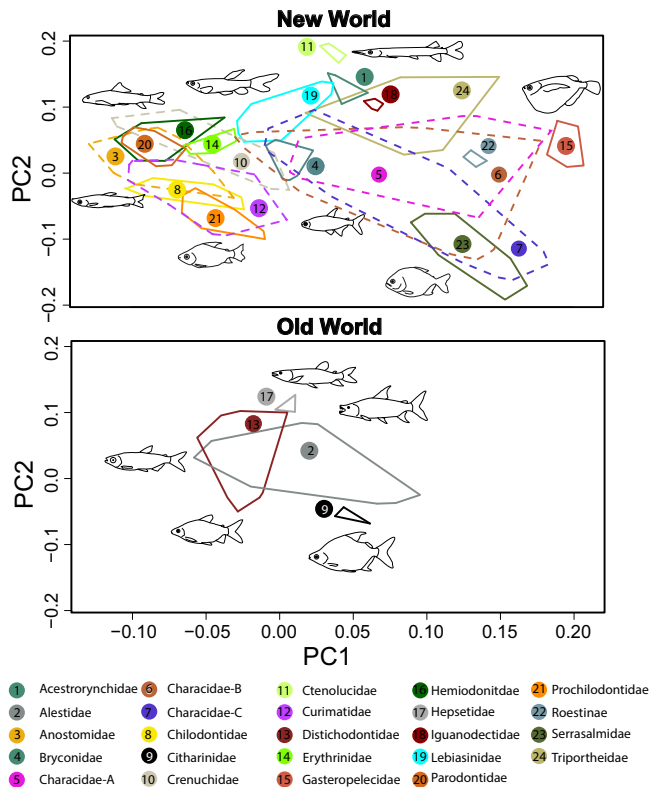
Figure 6. Scatterplot of principal components showing that species in most families cluster closely. New World includes families from South and Central America. Old World includes families from Africa. Morphospace contains all 329 species analyzed. (1) Acestrorhynchidae [2/26], (2) Alestidae [21/116], (3) Anostomidae [86/156], (4) Bryconidae [3/90], (5) Characidae-A [12/23], (6) Characidae-B [12/41], (7) Characidae-C [16/44] (8) Chilodontidae [7/8], (9) Citharinidae [2/8], (10) Crenuchidae [6/87], (11) Ctenoluciidae [2/7], (12) Curimatidae [74/105], (13) Distichodontidae [12/101], (14) Erythrinidae [3/17], (15) Gasteropelecidae [4/9], (16) Hemiodontidae [8/31], (17) Hepsetidae [1/5], (18) Iguanodectidae [1/30], (19) Lebiasinidae [7/77], (20) Parodontidae [8/32], (121) Prochilodontidae [17/21], (22) Roestinae [1], (23) Serrasalmidae [7/95], and (24) Triportheidae [5/23].

Table 1. Results of the multivariate model-fitting analyses for body shape.

Model	P	AIC	AICc	Δ AICc	AICc weight
OU surface	62	−1929	−1910	0	0.989
OU surface + piscivore	66	−1923	−1901	9	0.01
OU sc[*]	50	−1875	−1862	48	0
OU family	86	−1877	−1838	71.7	0
OU family + piscivore	78	−1855	−1823	86.6	0
BMM	84	−1844	−1809	100.8	0
EB	15	−1783	−1782	127.5	0
BM	12	−1769	−1744	165.5	0
OU trophic	38	−1751	−1769	140.8	0
OU continent	22	−1737	−1735	174.6	0

[*]**Model recovered from PhylogeneticEM analysis.**
For each model, the number of parameters (P), the Akaike information criterion (AIC), the small sample corrected AIC (AICc), and the relative fit (ΔAICc) and support (AICc weight) are shown. The best model has the lowest ΔAICc.

for each trophic ecology (Table 1). The SURFACE model was selected as the best model 100% of the time in the 1000 permutations that randomly reclassified each omnivorous species into one of the multiple trophic categories that comprised their diet (Supporting Information Fig. S4).

The SURFACE model includes seventeen optima spread across the phylogeny (Table 1), even when the topology was constrained to that of Oliveira et al. (2011; Supporting Information Table S2). Of the 17 optima identified by SURFACE, 10 evolved on branches subtending families and larger clades in the order (Fig. 7). Most of the optima evolved between 40 and 80 million years ago (Mya), and overall the SURFACE scenario represents a model of substantial body shape diversification in the first half

of characiform history, followed by some later shifts within the species-rich family Characidae.

The PhylogeneticEM results were more conservative, identifying just eight optima. However, like the SURFACE analysis, the majority of the optima (∼85%) identified by PhylogeneticEM evolved in the first 40 million years (My) of characiform evolution with only one evolving within the last 40 my (Fig. 8). In that respect, both the PhylogeneticEM and SURFACE models resemble the a priori models that included an element of early body shape diversification: OU family, OU family + piscivore, and Early Burst. These a priori models, like the PhylogeneticEM, OU surface, and OU surface + piscivore models, were substantially more likely than the model postulating different adaptive peaks for each trophic ecology. Clearly, the model predicting a unique body shape optimum for each trophic ecology did not best fit the data.

The mvMORPH simulation results indicate that our dataset has enough statistical power to separate the best-fitting model from the remainder (Fig. 9). The best-fitting model consistently has a much lower AIC score than any other model, with no overlap between the best fit model and the next best model (Fig. 9).

CO-OCCURRENCE OF MORPHOLOGICAL AND DIETARY SHIFTS

Many shifts in shape optima in the best-fitting models coincided with changes in trophic ecology. For SURFACE, 10 of the 17 body shape optima fell on the same branch as did a shift in trophic ecology. For PhylogeneticEM, five of the eight morphological optima coincided with a shift in trophic ecology. The chance of obtaining
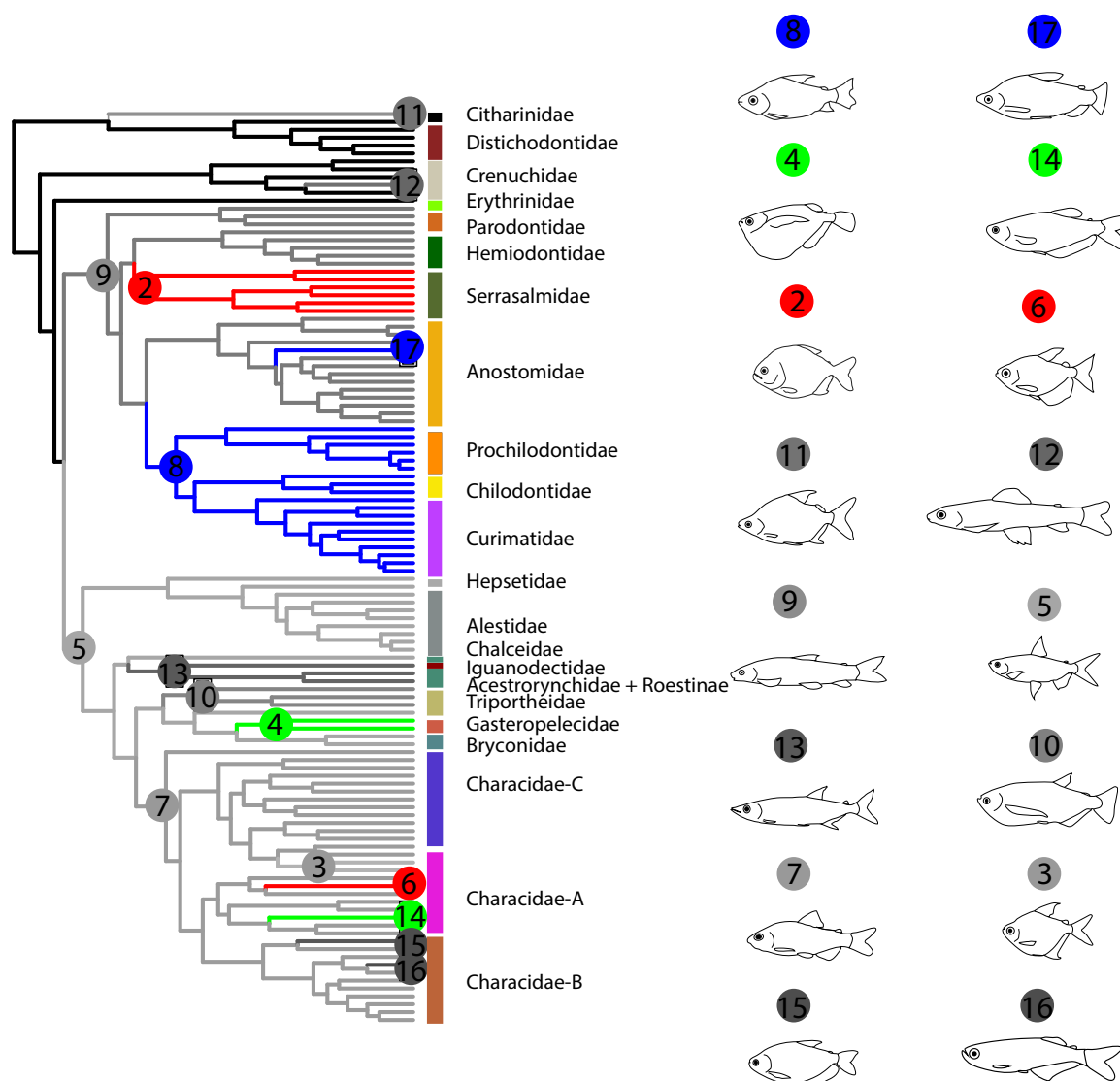
Figure 7. Fossil-calibrated phylogenetic tree for characiform fishes showing optima and regimes for the best-fitting model of body shape evolution assigned based on the SURFACE results. Drawings depict general body shapes representing the optima and do not represent the actual ancestral state.

at least this many co-occurrences by randomizing the ecological and morphological shifts on the phylogeny is 1 in 250,000 for the SURFACE results, and 7 in 10,000 for PhylogeneticEM. Therefore, we conclude that body shape often coevolved with trophic ecology in Characiformes.

CONTINENTAL COMPARISONS AND LEVELS OF CONVERGENCE

The modern African and South American assemblages differ substantially in morphological diversity, despite both including ancient clades. The largest diversification of body shapes occurred in the New World assemblage, which reached twice the disparity (Procrustes variance = 0.0132) of the African assemblage (Procrustes variance = 0.0071), which is a strongly significant

difference via permutation test ($P = 0.001$). Variation in in clade ages and internal branching times cannot explain the unequal disparities; 1000 Brownian simulations indicated an expected variance ratio near unity (0.938), and none of the 1000 simulations yielded a difference even approaching the observed value. The New World assemblage is substantially more speciose than the African, accounting for more than 70% of the species richness in the order. Neotropical families occur throughout the entire morphospace, while the four African families are restricted to the middle (Fig. 6). The species-depauperate African families Hepsetidae and Citharinidae each exhibit very low body shape disparity and a single morphotype. Body shape in the much more speciose Alestidae and Distichodontidae (each sister to one of the other African families) varies more (Procrustes variance = 0.0053 and
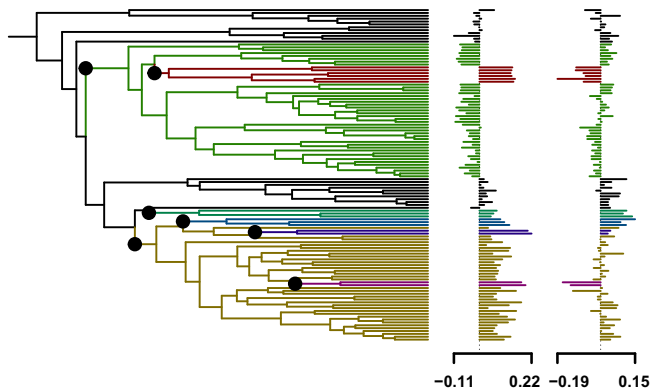
Figure 8. Fossil-calibrated phylogenetic tree for characiform fishes showing optima and regimes for the best-fitting model of body shape evolution assigned based on the PhylogeneticEM results.
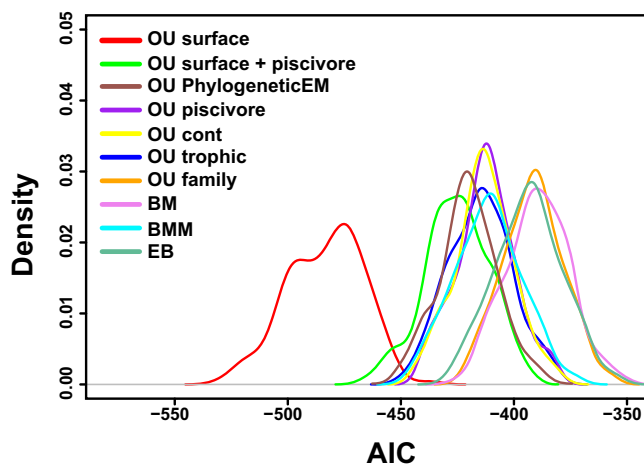

Figure 9. Density plot of AICc scores for the different evolutionary models from the 1000 simulated datasets under the best fit model parameters for body shape evolution.

Table 2. Results from the convevol analysis.

Trophic ecology	C1	C2	C3	C4
Piscivores	0.434*	0.068*	0.008*	0.014*
Detritivore	0.199	0.023	0.003	0.004
Herbivore	0.307	0.071	0.008	0.013
Insectivore	0.081	0.018	0.002	0.003
Invertivore	0.044	0.011	0.001	0.002
Omnivore	0.207	0.033	0.004	0.007

All values with statistically significant *P*-values (*P* < 0.05) indicated with an asterisk.

Only piscivorous lineages converged substantially and significantly. The arrows in Fig. 10 indicate piscivorous lineages evolving toward positive PC2 values (convevol, $P = 0.001$). All piscivorous clades have a more elongate body shape than their nonpiscivorous sister. Their C1 of 0.44 (from convevol) indicates that evolution has closed 44% of the morphological distance among piscivorous taxa. Similar results were found when accounting for trophic uncertainty and using the constrained tree (Oliveira et al. 2011), with piscivorous lineages always found to be statistically convergent (Supporting Information Table S3 and S4). These analyses provide evidence that nonrandom evolutionary processes generated similar body shapes among lineages that feed exclusively on fishes, regardless of the tree topology used.

Discussion

CHARACIFORM BODY SHAPE DID NOT DIVERSIFY RANDOMLY

Though stochastic evolution could conceivably explain the differences among trophically similar linages and between the continental radiations, our model-testing approach revealed Brownian motion to be among the worst-fitting of all candidate models. Random models performed poorly even when rates varied among clades, or in the declining rates scenario described by the early burst model. We conclude that body shape diversity probably evolved under a more complicated model, with lineages attracted to multiple optima distributed throughout characiform morphospace. Examination of the timing at which lineages occupied these optima, the ecological characteristics of the lineages that evolve toward each optimum, and whether species on each continent discovered the same optima can help reveal the major influences guiding the evolution of Characiformes.

MOST CHARACIFORM BODY SHAPE DIVERSITY EVOLVED EARLY

Early in characiform evolution, the incipient lineages leading to the 21 modern families diversified their body shapes remarkably.
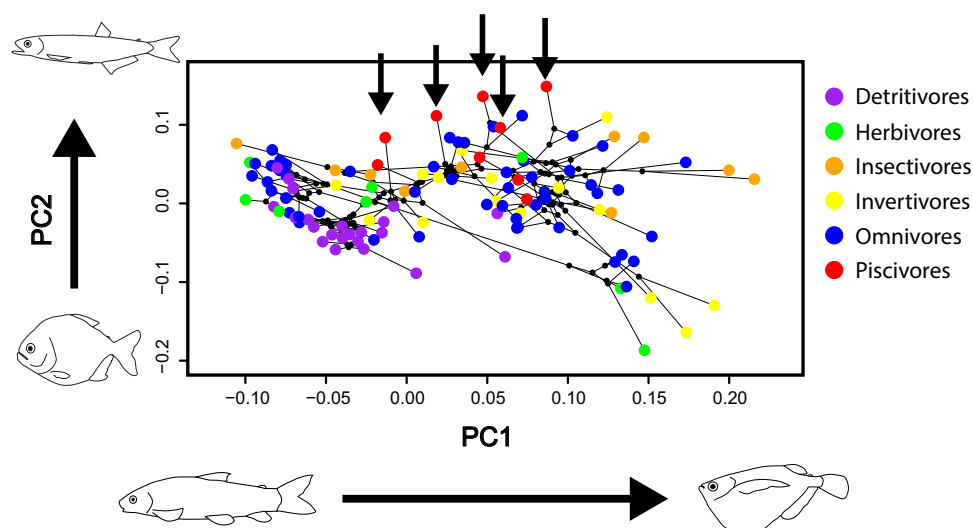
0.0064, respectively), with the latter approaching the variance of the largest Neotropical family (Characidae) despite having many fewer species. However, these clades are also restricted to the center of the morphospace, and have not evolved the extreme body shapes exhibited by the New World lineages at the morphospace periphery.

Though characiforms on both continents exploit all six trophic niches, trophically similar lineages rarely converged morphologically in our dataset. Detritivores, herbivores, insectivores, and invertivores all arose multiple times, yet generally evolved different body shapes scattered throughout the phylomorphospace (Fig. 10). Fishes in each trophic group could be deep bodied or elongate, with large or small heads and terminal, subterminal, or upturned mouths. Most trophic groups, with the exception of piscivorous lineages, exhibited no statistically significant convergence (Table 2; Supporting Information Table S3 and S4).

Figure 10. Phylomorphospace depicting repeated invasion of elongate morphotypes (high PC2 value) by piscivorous lineages.

Our study places the earliest stages of this initial, exuberant radiation between 107 and 87 Mya, likely when early characiforms inhabited a vast tropical lowland on the supercontinent Gondwana (Roberts 1972, 1973), which would separate into Africa and South America around 103 Mya. During the first half of the clade's history, each familial lineage invaded a largely distinct region of morphospace, together spanning most of the modern body shape diversity in the first 40 million years of evolution. Though a few lineages (most notably the ecomorphologically diverse African family Distichodontidae) continued to evolve unique body shapes, as a rule, within-clade diversification was much rarer than among-clade diversification.

This pattern of early diversification matches one of the hallmarks of adaptive radiations observed in other vertebrate lineages (Harmon et al. 2003; Seehausen 2006; Cooney et al. 2017). The OU-surface model selected as optimal, and all other highly supported models include a preponderance of optimum shifts on branches closer to the root of the phylogeny than to the tips. In effect, we reconstruct initial expansion followed by transition to a regime dominated by more restricted evolution around a series of optima. These OU models differ from the relatively simple "early burst" model that Harmon et al. (2010) used to describe adaptive radiation, which did not fit our data well. In the OU models, the presence of multiple optima and a pull toward each governs the evolutionary dynamics more than a simple decrease in the sigma parameter modeling the raw degree of evolutionary change possible. We note that the early burst model predates the implementation of multiple peak OU models in widely available analytical packages (Beaulieu et al. 2012; Ingram and Mahler 2013; Clavel et al. 2015), and suggest that the OU models may more closely approximate the complex landscapes on which adaptive radiations occur. Certainly, the pattern of early characiform

diversification remains fully consistent with an ancient adaptive radiation.

SHIFTS IN TROPHIC ECOLOGY ALONE CANNOT EXPLAIN BODY SHAPE RADIATION IN CHARACIFORMES

Half of all major shifts in characiform body shape coincide with changes in trophic niches, suggesting that dietary diversification sometimes drives characiform body evolution, or vice versa. For example, the remarkable muscular keel that adapts gasteropelecid hatchetfishes to their characteristic leaps out of the water evolves simultaneously with their specialization on insects at the air/water interface, and Africa's citharinids evolved a deep, compressiform body concurrent with becoming obligate detritivores. But, numerous counterexamples exist. The detritivorous/algivorous family Parodontidae shares a body shape with the omnivorous Anostomidae and Hemiodontidae (Géry 1977), the invertivorous Crenuchidae demonstrate two distinct body morphologies with no corresponding shift in diet, and the pirahnas and pacus in Serrasalmidae possess a distinctively robust body plan despite ranging from purely herbivorous members to omnivorous members with large percentages of scales and fishes in their diet (Peterson and Winemiller 1997; Correa et al. 2007; Kolmann et al. 2018). Thus, although characiforms frequently shifted in trophic ecologies, we do not reconstruct those shifts as the sole factor promoting the body shape diversification in the order.

Our results also do not demonstrate the tight convergence in morphology driven by convergence in ecology typically considered a hallmark of adaptive radiation in fishes (Rundle et al. 2000; Rüber and Adams 2001; Muschick et al. 2012). Indeed, our hypothesized OU-trophic model is one of the worst fitting of

all examined, with only piscivores demonstrating statistically significant convergence. The open question then becomes: did this study fail to detect a major part of trophic ecology's influence, or did some other factor also drive body shape diversification in Characiformes?

Several potential explanations exist for a failure to detect convergence in body shape driven by diet. First, there may be an insufficient number of transitions among trophic categories to provide the needed statistical power (Maddison and FitzJohn 2015), particularly when convergence is incomplete. For example, detritivores all group in the bottom left of the morphospace (Fig. 10) and share some obvious similarities in body depth and the dorsal fin position, as well as dissimilarity in the length of the anal fin. That figure also shows a member of the detritivorous Neotropical family Curimatidae moving toward the optimum occupied by the detritivorous African family Citharinidae. The species in question are also remarkably similar in their silvery coloration, body size, small squamation, and other aspects of morphology, and intuitively, most observers would call them convergent. However, there were only four transitions to detritivory, and while all four groups approached each other in morphospace, they do not overlap and their convergence is incomplete. As a result, CONVEVOL returned statistically insignificant results, and SURFACE assigned each of the detritivorous clades to a putatively separate body shape optimum, three of which are occupied only by detritivores and proximate in morphospace. Thus, in the case of detritivory, the small number of transitions may be insufficient to detect morphological convergence. That said, it seems unlikely that our analyses lacked sufficient power in all cases because most diets evolved eight or more times, and because we did detect convergence among piscivores.

Alternatively, our classification system may have failed to capture the true diversity in feeding strategies. For example, the phylomorphospace detected shared morphospace between members of Gasteropelecidae and the characid genus *Poptella*. These convergent species are deep bodied, top water fishes that feed primarily on terrestrial insects (Netto-Ferreira et al. 2007; de Mérona et al. 2008). However, other lineages of characiform insectivores, including members of the family Crenuchidae, are streamlined, benthic fishes feeding primarily on aquatic insects (Géry 1977; Buckup 1993a) and unquestionably convergent on the North American darters (*Etheostoma*), which occupy a similar niche (Winemiller et al. 2015). These two successful groups of characiform invertivores lie on opposite ends of the morphospace, and indicate clearly that there is more than one way for a fish to catch and devour a bug. Yet, the lack of detailed trophic and habitat ecology data across the entire order forced our classification system to combine these two distinct strategies, leading us to find (perhaps unsurprisingly) no global signature of convergence among insectivores.

Because at least some trophic ecologies clearly map to multiple body shape optima, a more nuanced trophic categorization in future studies will likely reveal more convergences. Refinement of the omnivorous category will be particularly important, since this group currently subsumes all characiforms ingesting multiple food types, even though proportions of fish, insect, and plant material vary widely. Better trophic detail will likely reveal ecomorphological convergence between some members of families that overlap in morphospace, such as the largely omnivorous members of the Neotropical Anostomidae, which partially overlap with the trophically diverse African Distichodontidae.

Lastly, our study may be criticized for not measuring the morphology of the characiform trophic apparatus, namely the teeth. In other diverse vertebrate clades that exhibit multiple shifts in trophic ecology, lineages certainly adapt their mouth parts. Darwin's finches adapt the length and depth of their beaks to feed on different food resources (Grant and Grant 2006) and Lake Tanganyika cichlids modify the length and width of the lower pharyngeal jaw (Muschick et al. 2012). Characiformes do vary considerably in tooth morphology, and trophically similar guilds tend to possess similar teeth (Guisande et al. 2012). Conversely, a recent study found no convergence in tooth aspect ratio in two lepidophagous characiform species (Kolmann et al. 2018), suggesting that the relationship between tooth shape and diet may be more complex than Guisande et al. (2012) presented. No study to date has looked at tooth shape diversification across the order, and we refrained from doing so due to the difficulty of locating the homologous landmarks needed to describe dentition mathematically. Nevertheless, it seems certain that tooth morphology and trophic ecology coevolve to some degree, and convergence in this character system offers a plausible explanation for how characiforms were able invade similar trophic niches without converging in body shape.

UNMEASURED HABITAT SHIFTS LIKELY ALSO DROVE SHAPE DIVERSIFICATION

Body shape likely also coevolves with locomotor style and habitat preference (Webb 1982, 1984), and habitat shifts were recently found to drive body shape diversification in continental goodeids (Foster and Piller 2018). Habitat shifts may therefore be the missing drivers of body shape diversification in Characiformes. The results of the SURFACE analysis indicate at least three regime shifts (in Crenuchidae, Gasteropelecidae, and Serrasalmidae) in lineages transitioning across the benthic-pelagic habitat axis, which is a common diversification gradient in other freshwater fishes (Carlson and Wainwright 2010; Hollingsworth et al. 2013; Burress et al. 2016), including multiple adaptive radiations (Schluter 1995; Schluter and Nagel 1995; Hollingsworth et al. 2013). Future analysis of body shape adaptation across macrohabitats in characiform subclades with well-established habitat

transitions and uniform trophic ecology, like Crenuchidae, will offer insights into how habitat transitions may have influenced aspects of body shape evolution. Unfortunately, at the moment reliable habitat data are rarely available for characiforms, and it appears that we are unable to fully categorize their "periodic table of niches" (*sensu* Winemiller et al. 2015). Our ability to test the influence of habitat on body shape evolution will remain limited until macrohabitat sampling improves for many lineages.

CONTINGENCY SHAPED EVOLUTION OF THE CONTINENTAL ASSEMBLAGES

The sundering of the proto-Amazon and Congo river basins also split the radiation of Characiformes. Though the nested position of the African Alestidae + Hepsetidae within the otherwise Neotropical Characoidei suggests that the earliest splits within crown Characiformes predate the end of continental connectivity, complete isolation of the assemblages appears to have been achieved by the middle Cretaceous (Fig. 2), allowing independent evolution in the Old and New Worlds thereafter. Despite the opportunity for similar diversification, they followed very different trajectories, with the four uniquely New World lineages together exploring more morphospace, generating much higher disparity and evolving toward more unique optima than the two African lineages in ensemble.

Those results resemble patterns in Neotropical cichlids, in which Central American species evolved a larger morphological range than their South American counterparts (Arbour and López-Fernández 2016). Like Characiformes, South American cichlid lineages explored large regions of morphospace early. Yet, accelerated phenotypic diversification later in cladogenesis allowed Central American cichlids to eventually diversify more broadly than their South American counterparts (Arbour and López-Fernández 2016). The dissimilar variances produced by geographically separate radiations in both characiforms and Neotropical cichlids suggest that historical contingencies may generally shape geographically broad radiations.

Many contingent factors could explain the differences in diversification between the Old and New World characiform assemblages, including unequal ages of their component clades, dissimilar ecological regimes, variation in lineage diversification dynamics or differences in competition and niche exclusion. Differences ecological regimes or clade age seem unlikely to provide the most important answers. The radiations occupy two halves of what used to be the same river system, and inhabit similar aquatic habitats in tropical regions of sister continents (Roberts 1972). The time-calibrated phylogenies indicate that earliest splits in the two African clades coincide with or slightly postdate the earliest splits in the major South American clades, and null Brownian simulations of morphological diversification indicate an expected variance ratio near unity for the two assemblages. The key more

likely lies in variation in speciation rate or differences in competition, niche exclusion and community composition accrued since the separation of these faunas.

The concurrent diversification of Neotropical cichlids illustrates how competitive exclusion can influence diversity dynamics. The Geophagini, which occupy an unusual benthivorous niche, evolved a riot of morphological diversity in South America, while the closely related Heroini were restricted to the extremes of morphospace (López-Fernández et al. 2013). However, when Heroine cichlids invaded Central America, where geophagines are absent, the lineage diversified more extensively in morphospace than it had on South America, suggesting that competitive exclusion by geophagines restricted the diversification of South American heroins (López-Fernández et al. 2013). Since they have not co-occurred for at least the last 70 million years, New World characiforms could not have restricted their Old World counterparts in that way that geophagin cichlids restricted the heroine cichlids, but some other lineage(s) of fishes present in Africa and not South America certainly could have.

The presence of cypriniform fishes in Africa, but not South America, provides a plausible cause of the massive difference of characiform diversity on these continents. The New World characiforms (along with siluriform catfishes) dominate South American fish communities (Lévêque et al. 2008; Nelson et al. 2016), while Old World characiform lineages compete with cypriniforms, siluriforms, and many other components of the African ichthyofauna (Briggs 2005). The absence of cypriniform fishes in South America contrasts starkly with their otherwise global distribution, in which they are abundant on every other nonoceanic continent (Roberts 1975; Lévêque et al. 2008; Nelson et al. 2016).

Many African cypriniforms appear to have converged on the niche and morphology of certain New World characiformes. For instance, the predatory African cypriniforms in the genera *Raiamas* and *Opsaridium* demonstrate an elongate body form that approaches that of the New World acestrorhynchids and ctenolucids. Members of the hyperdiverse African cyprinid genus *Labeo* are moderately elongate omnivores with inferior mouths, similar in niche and morphology to certain New World anostomids (*Hypomasticus, Leporellus*) and parodontids. Given that ecomorphological similarity, cypriniforms and characiforms could have easily contested for niche space during their concurrent radiations in Africa, lending plausibility to the hypothesis that the discrepancy in Old and New World characiform morphological diversity stems from differences in community structure. Further work, such as construction of a morphospace and trophic database for African cypriniforms, is needed to understand how much they overlap with characiforms in body shape and diet, and whether they likely restricted the diversification of Old World characiforms.

Conversely, it is possible that the variation in disparity results from faster rates of speciation in New World lineages. Lineages that speciate faster typically evolve higher morphological diversity, because rates of speciation and morphological evolution are often positively correlated (Rabosky et al. 2013). Speciation in freshwater fishes is mostly allopatric and driven by vicariance (Coyne and Orr 2004). Large river systems, like the Amazon and Congo Basins, are particularly susceptible to allopatric speciation because of geologic events that increase drainage subdivision and stream capture events (Roberts 1975; Lundberg et al. 1998; Briggs 2005; Albert et al. 2006; Albert et al. 2011). South America and Africa have both experienced multiple uplift and stream capture events (Ribeiro 2006; Swartz et al. 2009; Thomaz and Knowles 2018), likely yielding a rich history of vicariant evolution. Yet, the frequency of drainage isolation on each continent remains unknown and no study has tested for differences in speciation rates between African and South American characiforms. Further work including denser phylogenetic sampling is needed to understand whether variation in lineage diversification dynamics may have created differences in morphological disparity among characiform lineages.

Regardless of underlying cause of the discrepancy, we are left with the pattern of vastly different morphological variance and species richness in the characiform assemblages on the two continents. We acknowledge that some African and South American lineages resemble each other and likely converged deterministically (e.g., the darter-like morphology possessed by the African *Nannocharax* and the South American *Characidium*), and a sufficiently detailed characterization of niche will like reveal more convergence driven by multidimensional niche similarity. However, both assemblages evolved morphologies unmatched in the other, such as the African *Belonophago* and the South American Gasteropelecidae, and the overall differences in diversity are profound. This natural comparison provides a real-world approximation of Gould's famous "replaying life's tape" thought experiment (Gould 1989), and suggests a tendency toward different outcomes on different runs through evolutionary history. Thus, we infer a strong role for contingency in characiform evolution, and perhaps in all continental fish radiations.

SIMILARITIES AND DIFFERENCES BETWEEN THE CONTINENTAL RADIATION OF CHARACIFORMES AND CLASSIC ADAPTIVE RADIATIONS

The characiform continental radiation shares several similarities with classic insular and lacustrine adaptive radiations, most notably the concentration of shifts in morphology and ecology in the first half evolutionary history, and in the frequent co-occurrence of shifts in diet and body shape. However, the large-scale radiation differs from canonical adaptive radiations in the relative scarcity of convergence, and in the temporal scale of diversification.

Though Characiformes invaded the same six trophic categories multiple times, providing ample opportunity for convergence in body shape, only piscivores did so. The remainder of the radiation took good advantage of multiple adaptive pathways to detritivory, insectivory, herbivory, and invertivory. For example, different invertivore lineages evolved different mouth positions, ranging from the unique superior mouths of Anostominae (Myers 1950), to the more common inferior position in many members of Crenuchidae (Buckup 1993b). Our results contrast with the Caribbean *Anolis* lizards (Losos 1992; Kocher et al. 1993; Mahler et al. 2013; Machado-Schiaffino et al. 2015) and the cichlids of the African Rift Lakes (Rüber and Adams 2001), which converge morphologically when adapting to the same ecology. For example, independent lineages of cichlids in Lake Tanganyika evolved identical trophic morphology in response to the same selective pressure in different parts of the lake (Rüber et al. 1999).

The apparent rarity of convergence relative to the classic radiations may arise from different temporal and geographic scale of adaptation. Both *Anolis* lizards and lacustrine cichlids radiated rapidly during independent invasions of geographically restricted "island" ecosystems, and the cichlids experienced little variation in flow regime. In contrast, Characiformes radiated over a longer period of time in a dynamic river ecosystem spanning two continents, and thus likely experienced more habitat variability over time and throughout space. That environmental dynamism apparently provided Characiformes the opportunity to adapt their bodies to numerous fine niches defined not only by diet, but also by preferred position in the water column, optimal water velocity and so forth. With so many other variables in play, Characiformes discovered multiple ways to tap similar food resources.

One trophic strategy, piscivory, provides a counterexample. Characiforms that specialized in eating fishes converged significantly on sagittiform or fusiform bodies with large heads. That convergence is not surprising as elongate fishes with large heads and jaws arise commonly in fishes that pursue or ambush their prey, regardless of habitat preference (Webb 1984; Rüber and Adams 2001; Williams et al. 2015). Many lineages of piscivorous cichlids have similarly evolved elongate, shallow heads, and large streamlined bodies, including members of continental radiations on South America and Africa (Rüber and Adams 2001; Clabaut et al. 2007; López-Fernández et al. 2012; Muschick et al. 2012). In both characiforms and cichlids, these convergences occurred in young and old lineages and on both continents, indicating strong evolutionary determinism during adaptation to a piscivorous lifestyle.

Conclusion

Characiform fishes evolved much of their modern body shape diversify in an early, exuberant period of shape change. During later stages of cladogenesis, fewer novel morphologies arose. Body shape diversification was not random and occurred on a complex adaptive landscape, with morphological change frequently coinciding with dietary transition. The evolution of piscivory drove strong convergence toward elongate bodies and large heads. However, shifts to other trophic ecologies did not always coincide with major changes in body shape, and even when they did, no clear signal of morphological convergence emerged. There appear to be multiple morphological solutions to the demands of each trophic niche, and unmeasured ecological variables, such as shifts in habitat preference, may have also driven characiforms to diversify their body shape so remarkably. The massively different morphospace occupancy of the African and Neotropical portions of the radiation suggests a strong role for contingency in characiform evolution. The assemblage of New World lineages evolved twice as much disparity as the Old World assemblage, and the most extreme body shapes occurred in the Neotropics. Though the mechanism responsible the discrepancy is unknown, the presence of cypriniform competitors in Africa, but not South America, offers a tantalizing plausibility, as does variation in speciation rate. Overall the evolution of body shape in Characiformes exhibits some signatures of an ancient adaptive radiation, but without the ability to more precisely describe trophic ecology and habitat preferences, it is currently impossible to fully reconstruct the mechanisms and processes driving body shape diversification in the order. As future studies permit more precise ecological characterization of each characiform species, and as the phylogeny of Characiformes expands to include more taxa, we anticipate substantial improvement in our ability to detect morphological convergence and infer the evolutionary processes governing the radiation of this major clade of fishes.

AUTHOR CONTRIBUTIONS

M.D.B. and B.L.S. conceived of the project, and wrote the manuscript; M.D.B. performed all data collection and analyses.

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DATA ARCHIVING

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Phylogenetic hypotheses for the major characiform lineages from the current study and Oliveira et al. 2011.

Figure S2. Relationships among sampled species in the order Characiformes returned by Maximum Likelihood analysis of the concatenated dataset in RAxML.

Figure S3. Relationships among sampled species in the order Characiformes through a Bayesian partitioned analyses of the concatenated dataset.

Figure S4. Histogram of AICc scores from mvMORPH analysis when simulating 1000 times across uncertainty index, always showing AICc scores larger than the empirical score (Arrow).

Figure S5. Most likely phylogeny constrained to the interfamilial relationships in Oliveira et al. 2011, showing adaptive optima and regimes for the best-fitting model of body shape evolution assigned based on the SURFACE results.

Table S1. GenBank numbers for tissues used in phylogenetic analysis.

Table S2. Trophic ecology classification and literature reference, including multiple trophic categories for omnivorous taxa when available for each species used in phylogenetic comparative analyses.

Table S3. Priors and parameters used in BEAST.

Table S4. Gene partitions and their models as selected by PartitionFinder 2.0.

Table S5. Results of the multivariate model-fitting analyses for body shape constrained to the Oliveria et al. 2011 topology.

Table S6. Average results from the convevol analysis when simulated 1000 times across the uncertainty index.

Table S7. Results from the convevol analysis with the phylogenetic topology constrained to Oliveira et al. 2011.

Supporting Information