Patterns and rates of ecological diversification among Neotropical cichlid fishes

by

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Abstract

The rapid rise of phenotypic diversity and corresponding ecological roles is an emblematic pattern of cichlid evolution, particularly within clades of the East African Great Lakes. Herein, I evaluate the phenotypic and ecological diversification of Neotropical cichlids through time and among clades using a combination of geometric morphometrics, dietary analysis, and comparative phylogenetic methods. I found that the shape of the lower pharyngeal jaw (LPJ), which is part of a major phenotypic innovation among cichlids (i.e., pharyngognathy), is correlated with dietary patterns among Neotropical cichlids. The evolution of trophic roles such as molluscivory, algivory, and piscivory were associated with modifications in LPJ shape and tooth types. Additionally, LPJ shape evolution was correlated with body shape evolution among Neotropical cichlids; however, this pattern was driven by one of the major lineages (the Heroini) in which the evolution of these traits were highly correlated. This discrepancy among major clades may have arisen due to changes in the adaptive landscape as the Heroini colonized Middle America. Indeed, I detected diversity dependent evolution of LPJ shape following the colonization of Middle America, but not following the colonization of South America. Therefore, dissimilar evolutionary and ecological opportunities provided by the subsequent colonization of South and Middle America likely elicited different constraints during their diversification. Ecosystem-scale colonization events have been similarly important events throughout the evolutionary history of some lineages. I found that following the colonization the Paraná and Uruguay Rivers, distantly related pike cichlids (*Crenicichla*) diversified *in situ* and in

parallel into similar novel ecomorphs associated with trophic specializations, namely molluscivory, crevice feedings, and periphyton grazing. These results indicate that phenotypic and ecological diversity among Neotropical cichlids has varied dramatically among clades, through time, and in response to ecological opportunities provided by the colonization of new environments.

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General Introduction

Cichlid fishes are a textbook example of adaptive radiation, parallel evolution, and ecologically-based speciation (Kocher 2004; Seehausen 2015). In particular, the clades that inhabit the East African Great lakes (Tanganyika, Malawi, and Victoria) have rapidly diversified *in situ* and in parallel into hundreds of species that exhibit bewildering morphological, functional, and behavioral diversity (Salzburger et al. 2005; Seehausen 2015). This biodiversity arose via complex interactions between processes such as sexual selection and hybridization as well as fortuitous ecological opportunities during their evolutionary history. For example, the ~700 species endemic to Lake Victoria arose over the last 150,000 years via hybridization between divergent lineages from the Nile and Congo Rivers (Meier et al. 2017), which increased genetic variation that have allowed the descendant lineage to overcome evolutionary constraints and explore new regions of the adaptive landscape (Seehausen 2004). In addition to these prominent examples of adaptive radiation, numerous smaller radiations also inhabit small lakes throughout Africa and Middle America (Schliewen 2001; Elmer et al. 2014; Martin et al. 2015; Ford et al. 2016). All of these lake-dwelling clades have diversified along predictable environmental gradients, namely the benthic-to-pelagic habitat axis (Hulsey et al. 2013; Elmer et al. 2014; Kusche et al. 2014; Machado-Schiaffino et al. 2015; Kautt et al. 2016) and the hard-shelled to soft-bodied prey axis (Kidd et al. 2006; Muschick et al. 2012; Burress et al. 2015).

Patterns of speciation and ecological diversification have been different among Neotropical cichlids for myriad reasons such as discrepancies in habitat types, the role of sexual selection, hybridization, and ecological opportunity (Seehausen 2015; Burress 2015). Cichlids colonized South America via trans-Atlantic dispersal from Africa (Friedman et al. 2013; Matschiner et al. 2016). Diversification in locomotor and trophic-associated functional morphology occurred quickly following the origin of the Neotropical clade (López-Fernández et al. 2013; Arbour and López-Fernández 2014; Feilich 2016; Burress 2016). After this initial bout of ecological diversification, many Neotropical cichlid lineages have diversified without much morphological or ecological changes, which has largely been interpreted as the existence of several adaptive peaks upon which these lineages are evolutionary ‘stuck’ (López-Fernández et al. 2013; Arbour & López-Fernández 2014; Burress 2015).

In the following Chapters, I evaluate patterns of phenotypic evolution among Neotropical cichlids, particularly among major clades and through time, and contrast these findings with those observed in African cichlids. In Chapter 1, I evaluate the trophic implications of pharyngeal jaw diversification by quantifying relationships between diet and pharyngeal jaw shape and pharyngeal tooth types. In Chapter 2, I compare body shape and pharyngeal jaw shape evolution among major clades and through time. In Chapter 3, I evaluate relationships among pharyngeal jaw size, shape, and size of the pharyngeal jaw-associated musculature and test for diversity dependence in each trait to assess the degree of trophic niche filling following the sequential colonization of South and Middle America. In Chapter 4, I assess the parallel phenotypic evolution and trophic diversification between the Paraná and Uruguay River species flocks of pike cichlids (*Crenicichla*) and compare them to prominent examples of parallel evolution such as anole lizards and African cichlids.

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Chapter 1: Ecological diversification associated with the pharyngeal jaw diversity of Neotropical cichlid fishes

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Summary

1. Innovations can facilitate bursts of diversification by increasing access to novel resources and the attainment of novel functional designs. Pharyngognathy, exhibited by highly diverse groups such as wrasses and cichlid fishes, is hypothesized to increase foraging capacity and efficiency.

2. Here, I test the hypothesis that pharyngeal jaw shape and tooth morphology is adaptive in an ecologically diverse radiation of Neotropical cichlid fishes that spans North, Central, and South America.

3. I partitioned species into generalized trophic guilds using published stomach content analyses and quantified shape variation of the lower pharyngeal jaw using geometric morphometrics. Additionally, I tested for convergence in LPJ shape and trophic guild by mapping the phylogeny onto the principal components and testing for shifts towards similar evolutionary regimes.

4. Major LPJ shape variation included the length and orientation (i.e., narrow or wide) of the lateral processes and length of the medial process, which varied based on the proportion of fishes and plants consumed. Pharyngeal tooth number, diversity, and the frequency of tooth types were not evenly distributed among trophic guilds. There were seven distinct evolutionary regimes that converged upon four optima.

5. Pharyngeal jaw diversification is associated with the exploitation of novel resources among Neotropical cichlids such that pharyngeal specialization has increased access to otherwise poorly

accessible resources, such as resources that are difficult to crush (e.g., hard shelled organisms) and assimilate (e.g., algae).

Introduction

Throughout the history of life, innovations have periodically generated bursts of diversification. Many bony fishes exhibit gill arches that are modified to assist with prey processing (Liem 1973). In some cases, the pharyngeal arches have been modified into a functional set of jaws (i.e., pharyngeal jaw apparatus). A series of modifications to the pharyngeal apparatus have been hypothesized to increase foraging capacity, versatility, and efficiency (Liem 1973; Hulsey 2006; Wainwright *et al*. 2012) and is associated with some of the most species-rich and functionally diverse lineages of fishes, perhaps most notably marine wrasses and freshwater cichlids (Wainwright *et al.* 2012). Oral and pharyngeal jaws are developmentally decoupled such that they are derived from the first (in part) and seventh pharyngeal arches, respectively (Fraser *et al.* 2009). However, both sets of jaws operate in tandem such that the oral jaws are involved in food acquisition (i.e., grasping prey) while the pharyngeal jaws process food (i.e., tearing and/or crushing prey; Liem 1973). This functional decoupling is hypothesized to represent a major release of the oral jaws from demands associated with processing food (Liem 1973) and may have facilitated the trophic diversification of cichlid fishes.

Among cichlids, the pharyngeal apparatus consists of two independent upper bones and two lower bones that are sutured together into a single structure (Fig. 1). These bones have various degrees of unicuspid and bicuspid teeth that may be conical, flattened, or molariform (Fig 1; Casciotta & Arratia 1993; Hulsey 2006). Well-developed musculature modulates these bones to allow for considerable manipulation of prey, ranging from crushing hard-shelled organisms (i.e., snails and bivalves) to sifting minute edible items from mouthfuls of sediment, which would not be feasible tasks for oral jaws (Drucker & Jensen 1991; Hulsey 2006). A muscular sling contracts, pulling the lower pharyngeal jaw (LPJ) dorsally against the upper jaws, which are stabilized by and articulate with the neurocranium (Wainwright *et al*. 2012). LPJ shape and dentition are plastic (Huysseune 1995; Muschick *et al*. 2011; Gunter *et al*. 2013) and continuous pharyngeal tooth replacement allows for maintenance of tooth size, shape, and number (Huysseune 1995). Thus, LPJ shape and dentition may respond to functional demands imposed by specific prey items (Hulsey *et al*. 2008).

The shape and dentition of the LPJ has been associated with the trophic characteristics of species. For example, using comparisons among species pairs, reinforcement of the LPJ and molariform teeth are associated with durophagy (i.e., specialization on hard-shelled organisms; Hulsey 2006). Hypertrophied LPJs enable more crushing force (Meyer 1989), thereby reducing handling time associated with manipulating hard-shelled prey (Hulsey *et al*. 2005). Robust, molariform teeth are often located along the posterior midline where most structural stress is concentrated during mastication of difficult to crush prey (Hulsey *et al*. 2008). Durophagy has been a key axis of divergence during speciation of some river and lake dwelling cichlids (Kidd *et al*. 2006; Burress *et al*. 2013a) and is a frequent source of polymorphism (Hulsey *et al.* 2008). Among other guilds, associations between form and function of the pharyngeal jaws are less established. Many types of pharyngeal dentition are adapted for piscivory, such as recurved unicuspid, beveled, and bicuspid crenulate teeth (Witte & Barel 1976). Among piscivores, where prey does not require crushing force, but is instead consumed whole, the LPJ is often reduced and the teeth are adapted for merely grasping prey (Hellig *et al*. 2010; Burress *et al*. 2013a). Reduced LPJ morphology has also been linked to shifts toward utilization of pelagic resources

(Barluenga *et al*. 2006). Links between pharyngeal jaw morphology and ecological guilds such as omnivory, herbivory, and zooplanktivory, are hypothetical, but many potential associations have recently been proposed (Burress 2014).

Cichlids exhibit fast rates of speciation and phenotypic diversification among fishes (Rabosky *et al*. 2013; Near *et al*. 2013). Pharyngognathy is hypothesized to represent one of a few key innovations that facilitated the ecological diversification and subsequent proliferation of cichlids (Liem 1973; reviewed in Burress 2014). This dynamic apparatus emerged via functional and morphological reorganization and subsequent integration of existing muscles and bones, thus functional specialization may occur without altering the modularity of the various components (Liem 1973). Therefore, small structural modifications in shape or dentition may be sufficient for dramatic and rapid movement across regions of the adaptive landscape (Liem 1973). The adaptive value of pharyngeal shape and dentition is well documented among specific lineages or populations cichlids (Hulsey 2006; Hulsey *et al*. 2008; Burress *et al*. 2013a); however, this has not been explicitly tested using a large clade within a single analytical framework (Burress 2014). Here, I formally test this facet of the hypothesized utility of pharyngeal diversification: that LPJ shape and tooth morphology is associated with resource exploitation across trophic guilds in a transcontinental adaptive radiation of Neotropical cichlid fishes.

Methods

I dissected the lower pharyngeal jaw of 146 individuals from 45 Neotropical cichlid species accessioned in the Auburn University Museum of Natural History. The lower pharyngeal jaw was photographed in occlusal view using a mounted Nikon D5100 digital camera. I used 17 landmarks that describe the orientation and length of the medial and lateral processes, size of the dentigerous area, and curvature of the lower pharyngeal jaw (Fig. S1). Sliding landmarks were equally spaced along the lateral and posterior margins (Fig. S1). Sliding landmarks are not associated with a homologous structure, but are used to quantify the curvature of an object. Photos were digitized and landmarked using tpsUTIL (Rohlf 2004) and tpsDIG2 (Rohlf 2006), respectively. I used Procrustes fit to generate a consensus shape and remove variation associated with size and rotation using tpsRelw (Rohlf 2007). I then used these coordinates to generate phylogenetic principal components using the phyl.pca command, which is part of the phytools package in R (Revell 2009). Phylogenetic PCA generates variables that describe major axes of shape variation after the removal of phylogenetic correlation (Revell 2009). See below for details of the phylogeny used in this analysis.

I partitioned species into generalized trophic guilds using existing literature (Table 1): obligate and facultative piscivores, invertivore (i.e., soft bodied), sifting and non-sifting omnivores, molluskivores, and herbivores. Obligate piscivores consume almost exclusively fishes, whereas facultative piscivores consume primarily fishes (i.e., >55% by volume) but may consume lesser fractions of invertebrates. Sifting and non-sifting omnivores were separated based on descriptions of their feeding behavior and morphologies (Lopez-Fernandez et al. 2012; Arbour & Lopez-Fernandez 2013; Lopez-Fernandez et al. 2014). Invertivores, molluskivores, and herbivores were coded based on invertebrates, mollusks, and plant material representing their primary prey item, respectively. The use of categories to define trophic patterns that are continuous is admittedly limited and subjective. For example, omnivory is ubiquitous and varies widely among species (Burress 2014). Thus, owing to this limitation, I also used continuous variables (i.e., proportions of prey items) based on detailed stomach content analyses (Table 1). Itemized proportions were pooled into three generalized categories: plants, invertebrates, and fishes that were conserved across most published analyses. Values were taken directly from text, Tables, or estimated from Figures if necessary. If results were partitioned across age classes, seasons, or habitats, all such categories were pooled, except with respect to age classes of piscivores due to the well-established ontogeny of piscivory (Winemiller 1989; Burress *et al*. 2013b). In such cases, only adult classes were considered. I used linear regressions to test if the proportion of these prey items could predict lower pharyngeal jaw shape (i.e., pPCs). Pharyngeal dentition was quantified by counting the number of teeth using a dissecting microscope. Intact, broken, and missing teeth were included in counts. I compared the number of pharyngeal teeth among trophic guilds using Tukey HSD post hoc tests. All statistics were performed in R. I also recorded the presence of specialized dentition types based on those described by Casciotta and Arratia (1993): 1) posteriorly recurved unicuspid, 2) anteriorly recurved unicuspid, 3) anteriorly recurved bicuspid, 4) crenulate bicuspid, 5) beveled, and 6) molariform. All species had (at least) generic conical unicuspid or bicuspid dentition and were thus not considered specialized, unless they were enlarged, recurved, and positioned along the posterior margin. Terms used in reference to the different regions and aspects of the LPF are illustrated in Fig. S2.

To evaluate LPJ shape and dietary convergence (or divergence) among Neotropical cichlids, I estimated the phylogeny and divergence times using BEAST (Drummond *et al*. 2012) based on the input file from López-Fernández *et al*. (2013a; doi:10.5061/dryad.34621), which included five loci (cytb, 16S, ND4, RAG2, and S7 intron 1) for 166 species of cichlids (originally from López-Fernández *et al*. 2010). Settings were unchanged such as clades were constrained to be monophyletic, speciation priors, models of sequence evolution across gene partitions, divergence time calibrations, settings of the uncorrelated relaxed clock model (Drummond *et al*. 2012), and MCMC settings. Analysis was performed using BEAST v1.8.0 and

BEAGLE library v2.1 on the CIPRES Web Portal (Miller *et al*. 2010; Ayres *et al*. 2011; Drummond *et al*. 2012). Convergence was assessed with Tracer v1.5 and the maximum clade credibility tree was computed from the posterior distribution of trees with 10% burnin using TreeAnnotator v1.7.5. The tree was then pruned using the ape package in R.

To estimate ancestral states, I mapped the phylogeny onto pPC1 and pPC2 (i.e., phylomorphospace) using Mesquite v2.75 (Maddison & Maddison 2011). In this procedure, internal (i.e., ancestral) node values are estimated using weighted squared-change parsimony (Maddison 1991; Revell 2007). The resulting phylomorphospace depicts both the magnitude and the direction of shape change along each branch. Lastly, I estimated the evolutionary history of trophic guild diversification using maximum likelihood (mk1 model) character state reconstruction in Mesquite. Maximum likelihood analyses find the ancestral states (e.g., internal nodes) that maximize the probability that the observed character states (e.g., terminal nodes) would evolve under a stochastic model of evolution (Schluter *et al*. 1997; Pagel 1999). The data matrix was coded using the previously described trophic guilds (Table 1). This reconstruction was then overlaid onto phylomorphospace so that the evolution of LPJ shape and trophic guild could be interpreted simultaneously.

To access the adaptive landscape, and quantify convergence, I used SURFACE analysis (Ingram & Mahler 2013), which uses stepwise Akaike Information Criterion to locate regime shifts (k) on the phylogeny, then identify whether these shifts are towards convergent regimes. This process involves iteratively adding regime shifts to a Hansen model, then iteratively removing shifts to identify convergent regimes (k'). The reduction in complexity (k-k') corresponds to the number of regimes that can be collapsed into an existing regime (i.e., convergence; Δk). Distinct LPJ shape evolutionary regimes were visualized by overlaying the convergent and nonconvergent regimes onto the phylogeny. Additionally, these regimes, and estimated optima, were projected onto LPJ morphospace to visualize the adaptive landscape associated with Neotropical cichlid LPJ diversity. To test if convergence was significantly different than could result from a null expectation, I generated 100 simulated datasets under a 'null' model using the surfaceSimulate function (Ingram & Mahler 2013). I then tested for convergence (Δk) using these simulated data and calculated the probability of the observed data based on the null distribution.

Results

Pharyngeal shape

Shape analysis resulted in two biologically meaningful pPCs: pPC1 described 62.1% of the total variation, and represents variation between narrow to broadly spaced lateral processes and pPC2 described 20.8% of the total variation, and represents variation between long and short lateral processes with small and large dentigerous areas, respectively. Invertivores and herbivores are associated with the extremes of positive pPC1 values (i.e., widely spaced lateral processes; Fig. 2a). Invertivores, herbivores, and non-sifting omnivores have converged in this region of morphospace. Molluskivores occupy a unique region of morphospace along the extremes of both positive pPC1 and negative pPC2 values (e.g., *Thorichthys* and *Herichthys*; Fig. 2a), reflecting their short, widely spaced lateral processes and large dentigerous areas. Omnivores occupy a large region of morphospace that overlaps all other guilds except molluskivores (Fig. 2a). Piscivores exhibit two discrete LPJ morphologies, those associated with negative pPC1 and near-zero pPC2 values (e.g., *Cichla* and *Petenia*) and those associated with positive pPC1 and pPC2 values (e.g., *Crenicichla*, *Acaronia*, *Caquetaia*, and *Parachromis*; Fig.

2a). The zooplanktivore (e.g., *Chaetobranchus flavescens*) had a LPJ shape similar to that of some piscivores (Fig. 2a). The proportion of fishes consumed significantly predicted pPC1 (R^2=0.198; $F_{1,44}$=10.61; P=0.0022) and exhibited a non-significant trend with pPC2 (R^2=0.078; $F_{1,44}$=3.66; P=0.062) such that piscivory tends to increase with longer lateral processes and small dentigerous areas, respectively (Fig. 3). The proportion of plants consumed also predicted pPC1 (R^2=0.158; $F_{1,44}$=8.08; P=0.0069), but did not predict pPC2 (R^2=0.047; $F_{1,44}$=2.12; P=0.15) such that herbivory is associated with short, widely spaced lateral processes (Fig. 3). However, the proportion of invertebrates consumed did not predict pPC1 (R^2=0.002; $F_{1,44}$=0.082; P=0.776) or pPC2 (R^2=0.006; $F_{1,44}$=0.246; P=0.622) (Fig. 3). PC1 and PC2 likely represent continua between high and low degrees of processing potential (i.e., tearing) before ingestion and high and low potential for crushing force, respectively.

Pharyngeal dentition

The number of pharyngeal teeth varied among trophic guilds, with obligate piscivores (e.g., *Cichla*) having significantly more teeth than other guilds (Fig. 4a). Sifting omnivores had significantly more pharyngeal teeth than non-sifting species (Fig. 4a). Invertivores and molluskivores had the least numbers of teeth (Fig. 4a). I observed all pharyngeal tooth types discussed by Casciotta and Arratia (1993) (Fig. 1). Pharyngeal tooth diversity (Fig. 4b) varied among trophic guilds, such that dentition types were not evenly distributed among guilds (Fig. 4c). Facultative piscivores had the most diverse pharyngeal dentition. Beveled unicuspid and bicuspid crenulate teeth were exclusively associated with facultative piscivores (Fig. 4c) and were positioned along the lateral and posterior margins, respectively (Fig. 1). Obligate piscivores (e.g., *Cichla*) and a zooplanktivore (e.g., *Chaetobranchus flavenscens*) had poor pharyngeal tooth diversity, possessing exclusively posteriorly recurved unicuspid and anteriorly recurved bicuspid dentition, respectively (Fig. 4b,c). Invertivores, molluskivores, omnivores, and herbivores had similar diversity (Fig. 4b), but differed in the frequency of tooth types (Fig. 4c). Many species of all guilds had anteriorly recurred teeth along the posterior margin. Among piscivores, these were typically unicuspid and conical or weakly laterally compressed. These teeth among omnivores and herbivores were bicuspid and strongly laterally compressed into blade-like structures.

Evolutionary regimes and convergence

The evolutionary relationships recovered in the MMC tree (Fig. S3) were consistent with those of López-Fernández et al. (2013) and Říčan et al. (2013) and indicates several cases of convergence in both trophic guild and LPJ morphology. Omnivory (i.e., sifting or non-sifting) was estimated as the ancestral trophic state of all major clades (Fig S3). Molluskivory evolved independently at least twice, within *Crenicichla* and the *Thorichthys*+*Herichthys* clade (Fig. S3). Piscivory evolved independently at least six times (Fig. S3). Invertivory and herbivory also appear to have evolved multiple times among disparate lineages (Fig. S3). Ancestral state reconstruction of LPJ shape suggests that the ancestral LPJ shape is associated with extant omnivorous (e.g., sifting and non-sifting) species and ancestral nodes estimated to be omnivorous (Fig. 5). Additionally, shifts in dietary patterns, such as towards piscivory, herbivory, and molluskivory, appear to coincide with shifts toward LPJ shapes along the periphery of phylomorphospace (Fig. 5). There were seven evolutionary regimes in LPJ shape diversification (Table 2; Fig. 6), which resulted in shifts towards four optima estimated using SURFACE analysis (Table 2; Fig 6b,c). Most species exhibited a nonconvergent regime associated with widely-spaced lateral processes (i.e., positive pPC1 values; Fig. 6a,b). A second nonconvergent regime occurred within the clade containing *Geophagus* and *Gymnogeophagus* (Fig. 6a). There were also two cases of convergent regimes. First, *Petenia splendida*, *Chaetobranchus flavescens*, and three *Cichla* species exhibit shifts towards convergent regimes, namely LPJ morphologies characterized by elongate, narrowly-spaced lateral processes (Fig. 6a,b,d). Secondly, *Symphysodon discus*, *Apistogramma hoignei*, *S. daemon*, and *S. mapiritensis* exhibit shifts towards convergent regimes associated with relatively short, widely spaced lateral processes, and convex lateral margins (Fig. 6b,d). The null distribution resulted in 0 to 4 cases of convergence (Δk), with a mean of 1 case of convergence ($\Delta k = 1.0$; Fig. 6c). Five (of 100) simulated datasets resulted in Δk values equal to or larger than that of the observed data. Therefore, the observed data resulted in convergence that exceeds expectations under the null model (P=0.05).

Discussion

Morphology-to-ecology linkages

Throughout the history of life, structural or functional innovations have periodically facilitated a burst of diversification. Pharyngognathy has been hypothesized as one such crucial innovation (Liem 1973; Wainwright et al. 2012; Burress 2014). Here, variation in lower pharyngeal jaw morphology and dentition was associated with trophic function at broad evolutionary and ecological scales. The proportion of fishes and plant material consumed predicted important shape variation in the lower pharyngeal jaw, suggesting they may elicit functional demands that have led to selection on LPJ shape. The capacity to generate crushing force was also a major source of variation among Neotropical cichlids. Many trophic functions require intermediate degrees of crushing force and prey manipulation prior to ingestion. For example, molluskivores, herbivores, invertivores, and facultative piscivores formed a continuum along pPC2 representing the transition from high to low functional demands associated with pre-ingestion prey processing. On one extreme, molluskivores possessed a hypertrophied LPJ with short lateral and medial processes, and robust molariform teeth necessary for processing hard-shelled organisms (Hulsey 2006). These features allow the pharyngeal apparatus to tolerate the stress incurred during shell crushing (Hulsey et al. 2008). The musculature that articulates the pharyngeal bones is also well developed among these species (Liem, 1973). In contrast, herbivory requires minimal crushing force but may have functional demands associated with prolonged pharyngeal processing necessary to facilitate assimilation of nutrient-poor plant material (Burress 2014). Densely packed dentition may facilitate this process amongst herbivores, which consume large fractions of algae, diatoms and/or vegetative detritus (e.g., leaves and twig fragments). For example, *Hypsophrys nematopus* grazes large fractions of algae and diatoms from rock surfaces (Winemiller *et al*. 1995; Burcham 1988). Many, tightly spaced conical teeth may facilitate rupturing algae and diatom cells by acting as a mill that grinds material (Hulsey *et al*. 2005), and subsequently allow better assimilation (Xie 2001). In contrast, many herbivorous Afrotropical cichlids are physiologically specialized and equipped for algivory such that they have long digestive tracts and specific digestive enzymes (Sturmbauer *et al*. 1992) and often possess flattened LPJ teeth to merely pack material before ingestion (Liem 1973). Thus, modification of pharyngeal dentition and the digestive system may represent different solutions to managing poor quality food resources exploited by herbivorous Neotropical and Afrotropical cichlids, respectively.

Omnivory is ecologically complex and functionally demanding because an organism feeds at multiple trophic levels and consumes prey that vary greatly in nutrient content and digestibility (Rudnick & Resh 2005). Indeed, omnivores had highly variable LPJ morphologies, of which the non-sifting species partially overlapped with facultative piscivores, invertivores, and herbivores in morphospace. Thus, this LPJ shape may represent a generalized shape suitable for many trophic functions such that different trophic niches may be utilized by merely modifying the types and number of pharyngeal teeth. For example, evolutionary shifts from non-sifting omnivory to herbivory consists of increasing tooth number frequency of tooth types, rather than significant modification of LPJ shape. Indeed, this region of morphospace (i.e., positive pPC1 values) represents an adaptive optimum. In contrast, sifting omnivores occupy a unique region of morphospace with narrowly spaced lateral processes and long medial processes. This LPJ morphology may have coevolved with these species' laterally compressed bodies (i.e., narrowly-spaced lateral processes) and large buccal cavities (i.e., long medial processes) associated with sifting behaviour and mouth brooding, respectively (López-Fernández *et al*. 2012; Arbour & López-Fernández 2013). Because foraging via benthic sifting and mouth brooding co-occur in many groups such as *Geophagus* and *Satanoperca*, LPJ shape may be largely the result of integration with other phenotypic traits associated with foraging and reproductive behavior. If this is the case, selection favored LPJ phenotypes that maximize the space available for sifting food and brooding young. Many of these sifting species (i.e., *Gymnogeophagus* and *Geophagus*) exhibit regime shifts toward an optima associated with narrowly-spaced lateral processes of intermediate length with slightly concave lateral margins. The remaining sifting omnivores (e.g., *Satanoperca*), along with some non-sifting omnivores (i.e., *Apistogramma* and *Symphysodon*), exhibit regime shifts that converge upon an optimum associated with narrowly-spaced lateral processes of intermediate length with slightly convex lateral margins.

Sifting omnivores also possessed more pharyngeal teeth than non-sifters, and also tended to have bicuspid dentition. Benthic sifting is often considered an adaptive quality among Neotropical cichlids (López-Fernández *et al*. 2013a; 2014), and may encompass species that intentionally or inadvertently ingest plant material while targeting animal prey buried underneath sediments and detritus (López-Fernández *et al*. 2014). López-Fernández *et al*. (2014) found that sifting species were not more effective at physically unearthing buried prey, thus the functional advantages of LPJ specialization may be manifested after prey is taken into the mouth. The pharyngeal jaw functions much like a rake, separating edible materials from sediment (i.e., winnowing), which are expelled through the gill openings or the mouth (Drucker & Jensen 1991). Densely packed pharyngeal teeth may facilitate this process by increasing winnowing efficiency or versatility by allowing smaller items to be separated from sediment. The benthivory adaptive peak (López-Fernández *et al*. 2013a; 2014) is further refined here considering the existence of two distinct LPJ shape optima among these species. As described above, these optima can be distinguished by the concave or convex curvature of the lateral margins, and highlight the complexity of the adaptive landscape such that a previously identified adaptive peak based on behavior and external morphology (López-Fernández *et al*. 2013a; 2014) may encompass two distinct trophic optima as revealed by regimes of LPJ evolution.

Piscivory is often considered an adaptive peak among fishes (Collar *et al*. 2009) and specifically among Neotropical cichlids (López-Fernández *et al*. 2013a). Among Neotropical cichlids, piscivory is associated with two discrete LPJ morphologies: long, narrowly spaced lateral processes (e.g., *Cichla* and *Petenia*) and long, widely spaced lateral processes (e.g., *Acaronia*, *Caquetaia*, *Crenicichla*, and *Parachromis*). These groups represent obligate and facultative piscivores, respectively. *Cichla* have poorly developed musculature associated with the pharyngeal apparatus (Liem 1973) and pharyngeal dentition that is adapted purely for food transport (i.e., grasping), with no provisions for processing prey prior to swallowing (i.e., tearing or crushing). *Cichla*, along with another obligate piscivore (*Petenia*) and a zooplanktivore (*Chateobranchus*), exhibit regime shifts that converge upon a LPJ optima. Piscivory and zooplanktivory likely share a reduced need for pharyngeal processing considering they swallow prey whole (Winemiller 1989; Burress *et al*. 2015). In contrast, facultative piscivores consume important fractions of secondary prey items and have fewer and more diversely shaped and sized pharyngeal teeth that may facilitate processing this wider variety of prey items (i.e., insects and crustaceans). For example, these species often possess large conical teeth along the LPJ midline, particularly medially at the posterior margin. Functionally, such enlarged dentition may limit pharyngeal gape (Wainwright, 1991; Burress et al. 2015), but may facilitate exploitation of various prey types that require processing such as tearing or crushing actions (Burress 2014). One such prey item are large littoral crustaceans, which supplement the diets of many of these species (Winemiller *et al*. 1995; Montaña & Winemiller 2009; Burress 2012). Having the functional plasticity necessary to feed on different prey types would be particularly favorable when resource availability fluctuates or is unpredictable (Winemiller 1990; Burress *et al*. 2015).

Phenotypic plasticity can be observed and experimentally induced in cichlid fishes (Huysseune 1995; Hulsey *et al*. 2005; 2008; Muschick *et al*. 2011). Phenotypic plasticity is observed at a scale (i.e., population) that is finer than considered in this study (i.e., species); nonetheless, plasticity in lower pharyngeal jaw shape may confound some of the aforementioned interpretations, particularly those associated with foraging along the soft-to-hard shelled prey axis. Most literature dealing with phenotypic plasticity in cichlid LPJs pertains to exploitation of soft- and hard-bodied prey (Huysseune 1995; Hulsey *et al*. 2005; Hulsey 2006), thus phenotypic plasticity could be particularly confounding in the conclusions drawn about molluskivory. However, there were no obvious mismatches between the dietary literature and LPJ morphologies of the species examined. Nonetheless, plasticity in LPJ shape and dietary patterns likely contribute some of the variation observed in this study and may explain some of the overlap among trophic guilds (Witte et al. 1997).

Phylogenetic considerations

The sister group of Neotropical cichlids, African cichlids (Pseudocrenilabrinae), also exhibit diverse lower pharyngeal jaw shapes that appears to be associated with their diet and diversification (Muschick *et al*. 2012; 2014). Although, LPJ diversification has been studied at broad scales (Muschick *et al*. 2012; 2014), diet has not been explicitly linked to LPJ morphology. Nevertheless, African cichlids exhibit many of the same LPJ modifications as Neotropical cichlids. For example, piscivores have reduced LPJs with elongate lateral and medial processes and recurved teeth (Liem 1973; Hellig *et al*. 2010), whereas molluskivores have hypertrophied LPJs with short processes and molariform teeth (Huysseune 1995). Pharyngeal dentition among African cichlids is also characterized by myriad tooth types including many that are observed in Neotropical cichlids, such as unicuspid and bicuspid teeth that can be molariform, flattened, recurved, and/or blade-like in shape (Liem 1973). Thus, many of the morphology to trophic linkages described herein are likely generalizable across cichlids (Burress 2014).

The sister group of cichlids, the engineer blennies (*Pholidichthys*), have modified pharyngeal arches, but do not exhibit pharyngognathy (Wainwright et al. 2012; Near et al. 2013). Similar to cichlids, they have two independent upper pharyngeal jaw bones and the 5th ceratobranchials are fused into a single structure (i.e., the LPJ; Springer & Freihofer 1976). However, in contrast, their pharyngeal dentition is simple and non-specialized, consisting of conical recurved teeth (Springer & Freihofer 1976). The Pholidichthyidae consists of only two species, suggesting that merely exhibiting components of phayngognathy is not sufficient for impressive diversification. More disparately related groups, such as the Labridae (wrasses), Embiotocidae (surfperches), and Pomacentridae (damselfishes) exhibit more diverse pharyngeal jaw morphologies and correspondingly more species diversity (Wainwright et al. 2012). Surfperches and damselfishes have perhaps the most similar pharyngeal jaw arrangement to cichlids, such that they have muscular slings that allow for a biting mechanism and a fused lower jaw element (Wainwright et al. 2012). However, pharyngognathy likely evolved independently among these groups (Wainwright et al. 2012).

Conclusions

Modification of LPJ shape and dentition results in numerous trophic adaptations, most notably associated with the evolution of piscivory, benthic sifting, herbivory, and molluskivory. Thus, increasing access to nutrient sources, and subsequently ecological niches, that would not otherwise be available. For example, nutrients from hard-shelled organisms such as mollusks or nutrient-poor materials such as algae are exploitable due to specializations of LPJ shape and dentition. LPJ shape (Hulsey *et al*. 2008) and dentition (Hulsey *et al*. 2005) may affect the efficiency (i.e., handling time) associated with exploitation of specific prey items or may preclude exploitation altogether (Burress *et al*. 2015). Similar LPJ morphologies and dentition have evolved independently in association with piscivory and sifting omnivory. Thus, LPJ shape and tooth morphology may have an adaptive value within an ecologically diverse and speciose radiation of Neotropical cichlids. Indeed, ecological diversification has played a key role in cichlid speciation. For example, divergence in LPJ shape has been associated with ecological speciation events in Neotropical lakes (Barluenga *et al*. 2006; Elmer *et al*. 2010) and rivers (Burress *et al*. 2013a) and adaptive radiation in the East African Great Lakes (Muschick *et al*. 2012). Furthermore, pharyngeal dentition is more diverse than that of the premaxilla and dentary (i.e., oral dentition) among Neotropical cichlids (Casciotta & Arratia 1993) and may have played an important role in their proliferation (Burress 2014), much like craniofacial diversification among Afrotropical cichlids (Hulsey *et al*. 2010). Here, I have extrapolated existing knowledge of the form to function linkage of the LPJ from intraspecific (Hulsey 2006; Hellig *et al*. 2010), species pair (Kidd *et al*. 2006; Hulsey et al. 2008), and congeneric (Burress *et al*. 2013a; Burress *et al*. 2015) contexts to a lineage that represents a major teleost adaptive radiation (López-Fernández *et al*. 2013a; McMahan *et al*. 2013) that spans North, Central, and South America. Pharyngognathy has likely facilitated the exploitation of novel resources during the rapid and extensive diversification of cichlid fishes.

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Table 1. Forty-five Neotropical cichlid species examined in this study and references used to categorize species into generalized trophic guilds. Sub-guilds consist of obligate (O) and facultative (F) piscivores and sifting (S) and non-sifting (NS) omnivores. Abbreviations correspond to Fig. 2.

Species	*N*	Abbreviation	Guild	Reference
Acarichthys heckelii	5	Ac_he	Omnivore-NS	Hawlitscheck *et al*. 2013
Acaronia nassa	4	Ac_na	Piscivore-F	Mérona and Rankin de Mérona 2004
Amphilophus citrinellus	2	Am_ci	Omnivore-NS	Elmer *et al*. 2010
Andinoacara pulcher	4	An_pu	Omnivore-NS	Winemiller *et al*. 1995
Apistogramma hoignei	2	Ap_ho	Omnivore-NS	López-Fernández *et al*. 2012
Archocentrus centrarchus	1	Ar_ce	Omnivore-NS	Winemiller *et al*. 1995
Astatheros robertsoni	2	As_ro	Omnivore-S	Winemiller *et al*. 1995
Astronotus ocellatus	5	As_oc	Invertivore	López-Fernández *et al*. 2012
Australoheros facetus	2	Au_fa	Omnivore-NS	Yafe *et al*. 2002
Biotodoma wavrini	4	Bi_wa	Omnivore-NS	López-Fernández *et al*. 2012
Caquetaia kraussii	3	Ca_kr	Piscivore-F	Winemiller *et al*. 1989
Chaetobranchus flavescens	5	Ch_fl	Zooplanktivore	Mérona & Rankin de Mérona 2004
Cichla monoculus	2	Ci_mo	Piscivore-O	Mérona & Rankin de Mérona 2004
Cichla orinocensis	1	Ci_or	Piscivore-O	López-Fernández *et al*. 2012
Cichla temensis	5	Ci_te	Piscivore-O	López-Fernández *et al*. 2012
Cichlasoma orinocense	4	Ci_on	Invertivore	López-Fernández *et al*. 2012
Cichlasoma urophthalmus	2	Ci_ur	Omnivore-NS	Hinojosa-Garro *et al*. 2013
Crenicichla lugubris	5	Cr_lu	Piscivore-F	López-Fernández *et al*. 2012
Crenicichla minuano	6	Cr_mi	Molluskivore	Burress *et al*. 2013a
Crenicichla scottii	5	Cr_sc	Piscivore-F	Burress *et al*. 2013b
Crenicichla wallacii	1	Cr_wa	Invertivore	López-Fernández *et al*. 2012
Cryptoheros nigrofasciatus	3	Cr_ni	Herbivore	Winemiller *et al*. 1995
Cryptoheros spilurus	4	Cr_sp	Herbivore	Cochran-Biederman & Winemiller 2010
Dicrossus filamentosus	3	Di_fi	Invertivore	López-Fernández *et al*. 2012
Geophagus abalios	5	Ge_ab	Omnivore-S	López-Fernández *et al*. 2012
Geophagus dicrozoster	4	Ge_di	Omnivore-S	López-Fernández *et al*. 2012
Geophagus steindachneri	3	Ge_st	Omnivore-S	López-Fernández *et al*. 2012
Geophagus surinamensis	2	Ge_su	Omnivore-S	Winemiller *et al*. 1995
Guianacara stergiosi	3	Gu_st	Omnivore-NS	López-Fernández *et al*. 2012
Gymnogeophagus rhabdotus	2	Gy_rh	Omnivore-S	Yafe *et al*. 2002
Herichthys cyanoguttatus	2	He_cy	Molluskivore	Hulsey *et al*. 2006
Heros severus	5	He_se	Omnivore-NS	López-Fernández *et al*. 2012
Hoplarchus psittacus	5	Ho_ps	Omnivore-NS	López-Fernández *et al*. 2012
Hypsophrys nicaraguensis	1	Hy_ni	Herbivore	Higham *et al*. 2007
Hypsophrys nematopus	1	Ne_ne	Herbivore	Burcham 1988
Mesonauta festivum	4	Me_fe	Invertivore	Winemiller *et al*. 1989
Mikrogeophagus ramirezi	4	Mi_ra	Invertivore	López-Fernández *et al*. 2012
Parachromis dovii	1	Pa_do	Piscivore-F	Winemiller *et al*. 1995
Paraneetroplus maculicauda	1	Pa_ma	Herbivore	Cochran-Biederman & Winemiller 2010
Petenia splendida	3	Pe_sp	Piscivore-F	Cochran-Biederman & Winemiller 2010
Satanoperca daemon	5	Sa_da	Omnivore-S	López-Fernández *et al*. 2012
Satanoperca mapiritensis	3	Sa_ma	Omnivore-S	López-Fernández *et al*. 2012
Symphysodon discus	1	Sy_di	Omnivore-NS	Crampton 2008
Thorichthys meeki	7	Th_me	Molluskivore	Cochran-Biederman & Winemiller 2010
Tomocichla tuba	4	To_tu	Herbivore	Winemiller *et al*. 1995

Table 2. Summary of regime shifts and convergence in lower pharyngeal jaw morphology of Neotropical cichlids from the best-supported Hansen model from SURFACE analysis.

Parameter	Value	Interpretation
k	7	# of regime shifts
k'	4	# of distinct regimes
Δk	3	$k-k'$, the reduction in complexity when accounting for convergence
c	5	# of shifts towards convergent regimes occupied by multiple lineages
k'_{conv}	2	# of convergent regimes reached by multiple lineages
c/k	0.71	Proportion of shifts that are towards convergent regimes

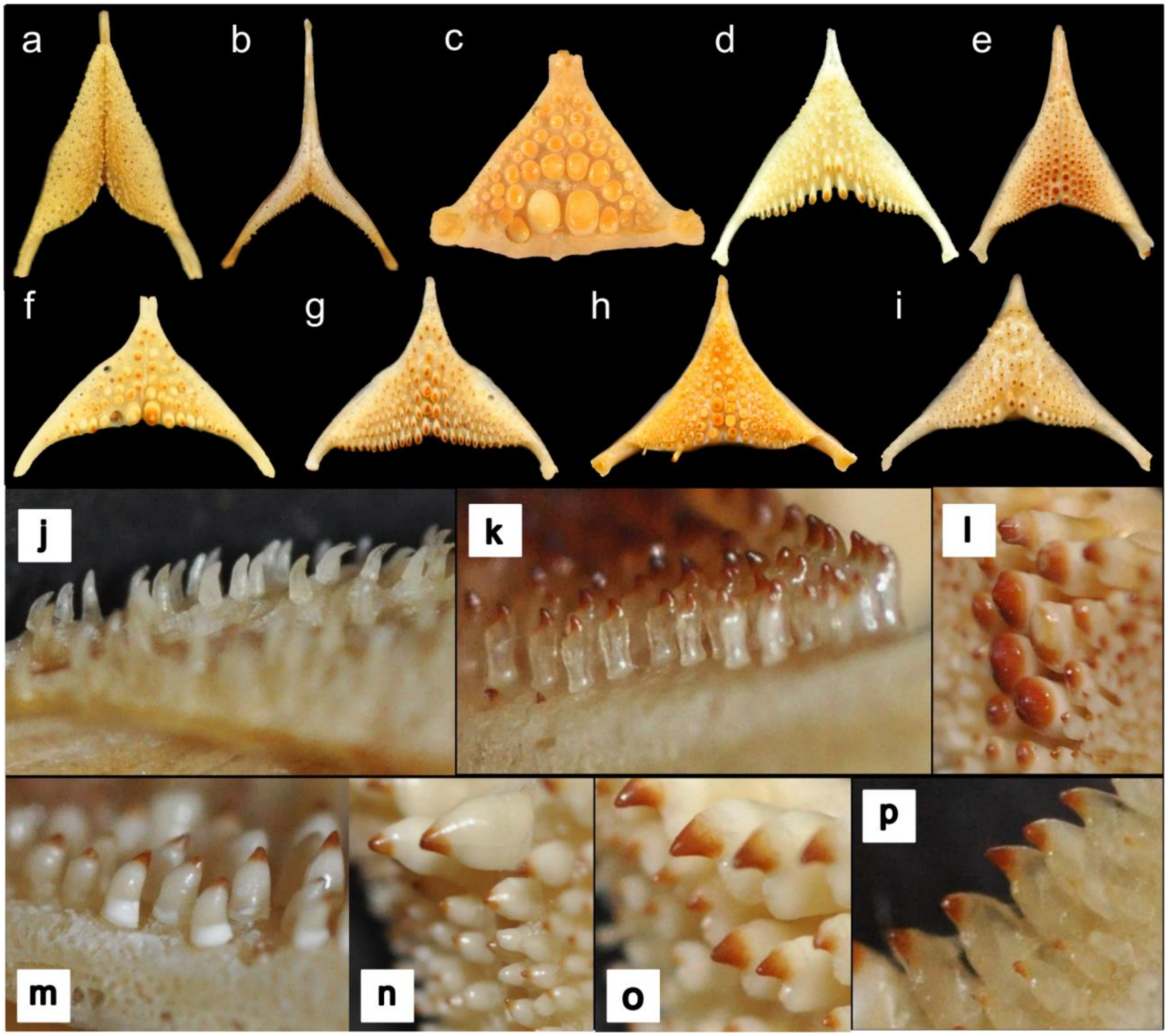

Figure 1. Representative lower pharyngeal jaw diversity among Neotropical cichlids: (a) *Cichla intermedia*, (b) *Chaetobranchus flavescens*, (c) *Crenicichla minuano*, (d) *Parachromis dovii*, (e) *Geophagus abalios*, (f) *Crenicichla reticulata*, (g) *Tomocichla tuba*, (h) *Paraneetroplus maculicauda*, and (i) *Aequidens diadema*. Pharyngeal tooth types observed among Neotropical cichlids (adapted from Casciotta and Arratia 1993): (j) unicuspid, recurved; *Cichla temensis*; (k) generic bicuspid; *Geophagus abalios*; (l) molariform; *Herichthys cyanoguttatus*; (m) beveled; *Crenicichla scottii*; (n) generic unicuspid, conical; *Crenicichla scottii*; (o) bicuspid, recurved; *Parachromis dovii*; and (p) bicuspid, crenulate; *Crenicichla lugubris*. Images are not to scale.

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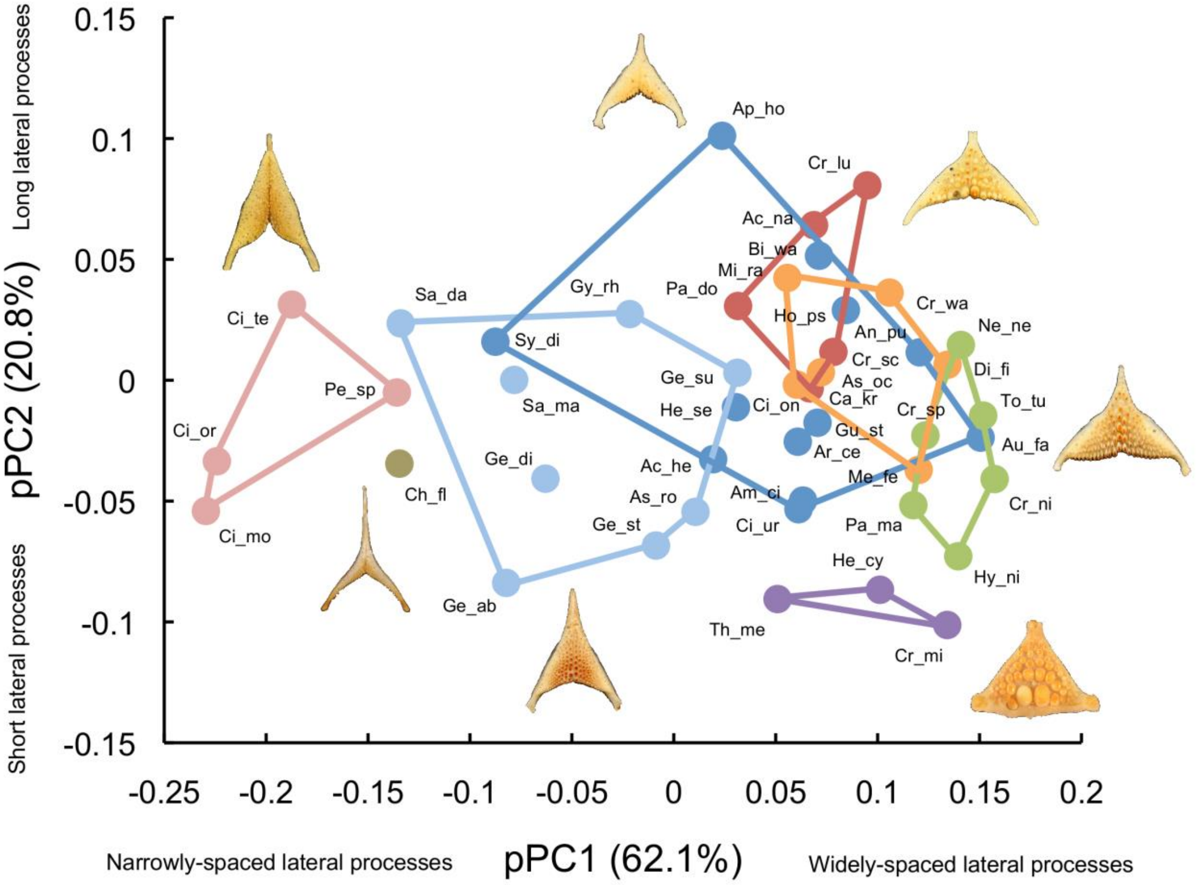

Figure 2. Size- and phylogeny-corrected shape variation (a) of the lower pharyngeal jaw among 45 Neotropical cichlid species. Colours correspond to generalized trophic guilds: obligate piscivore (pink), facultative piscivore (red), invertivore (orange), sifting omnivore (light blue), non-sifting omnivore (dark blue), molluskivore (purple), herbivore (green), and zooplanktivore (brown). Images are meant to serve as examples of jaws associated with the extremes of morphospace.

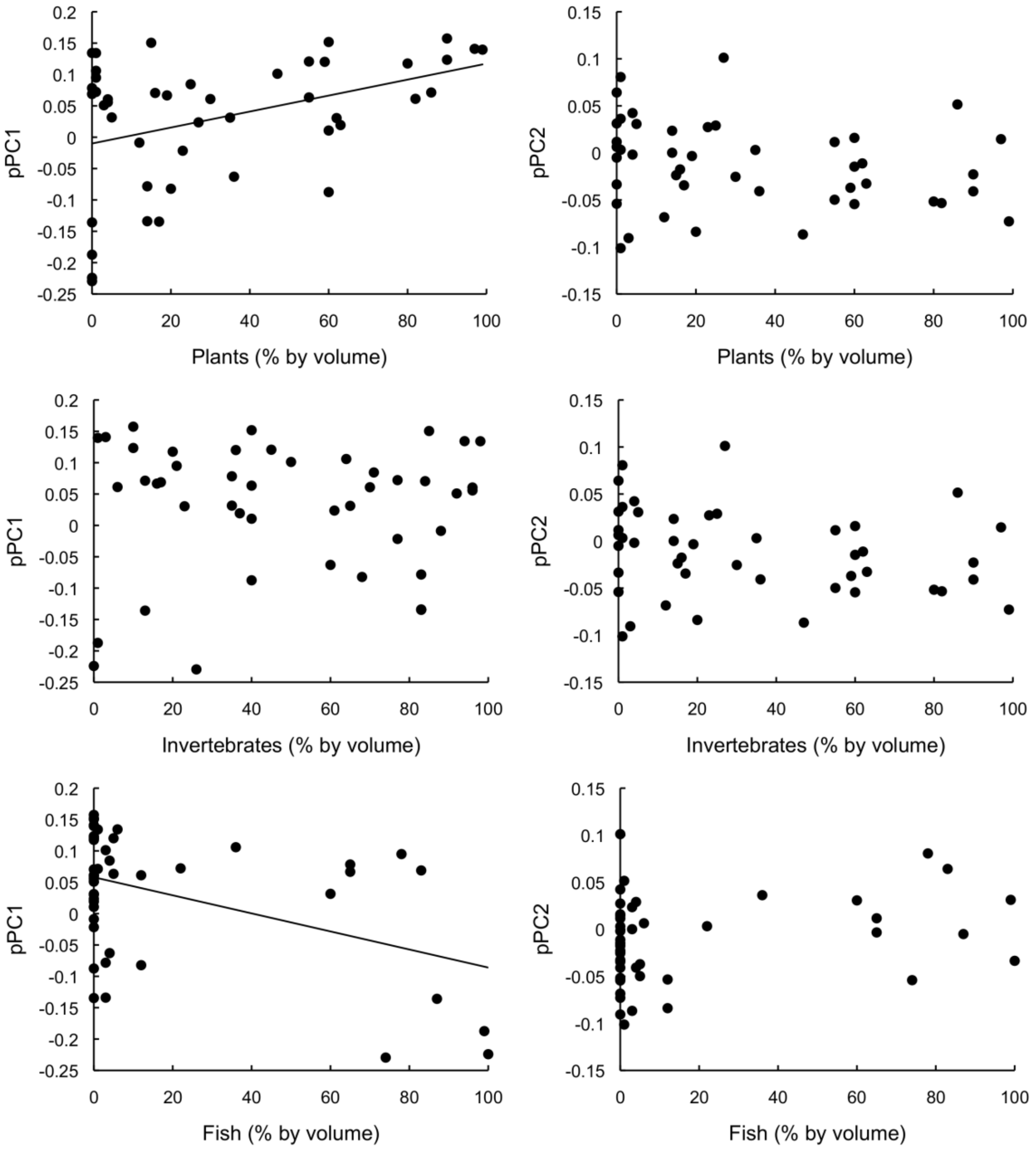

Figure 3. Relationships between diet and phylogenetic principal components (pPC) that reflect major shape variation among the lower pharyngeal jaws of Neotropical cichlid fishes. Lines denote significant relationships. Positive and negative values of pPC1 are associated with widely and narrowly spaced lateral processes, respectively. Positive and negative values of pPC2 are associated with long and short lateral processes, respectively.

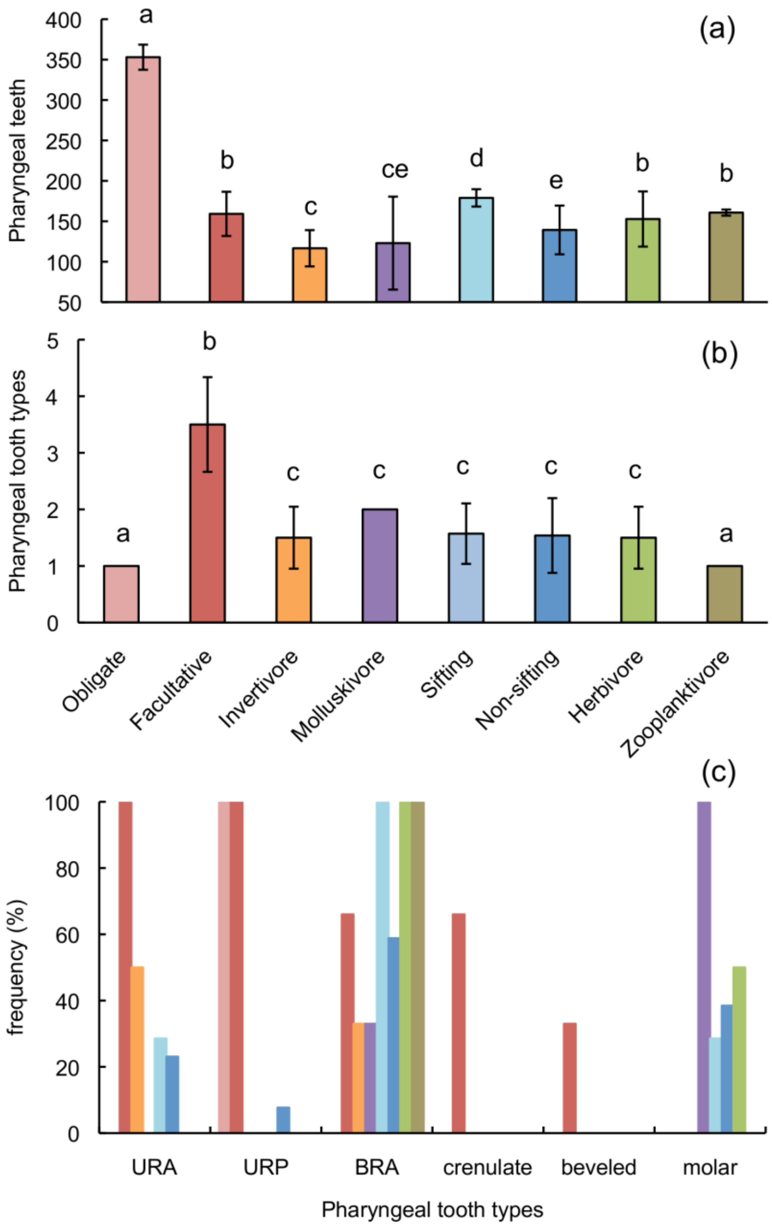

Figure 4. Mean (±SD) pharyngeal tooth number (a), tooth types (b), and frequency (c) among generalized trophic guilds. Tooth types are adapted from Casciotta and Arratia (1993) and include 1) unicuspid, recurved anteriorly (URA), 2) unicuspid, recurved posteriorly (URP), 3) bicuspid, recurved anteriorly (BRA), 4) bicuspid crenulate, 5) beveled, and 6) molariform. See text for discussion of tooth types. Letters denote significant comparisons based on Tukey HSD post hoc tests. Colour codes are consistent among panels.

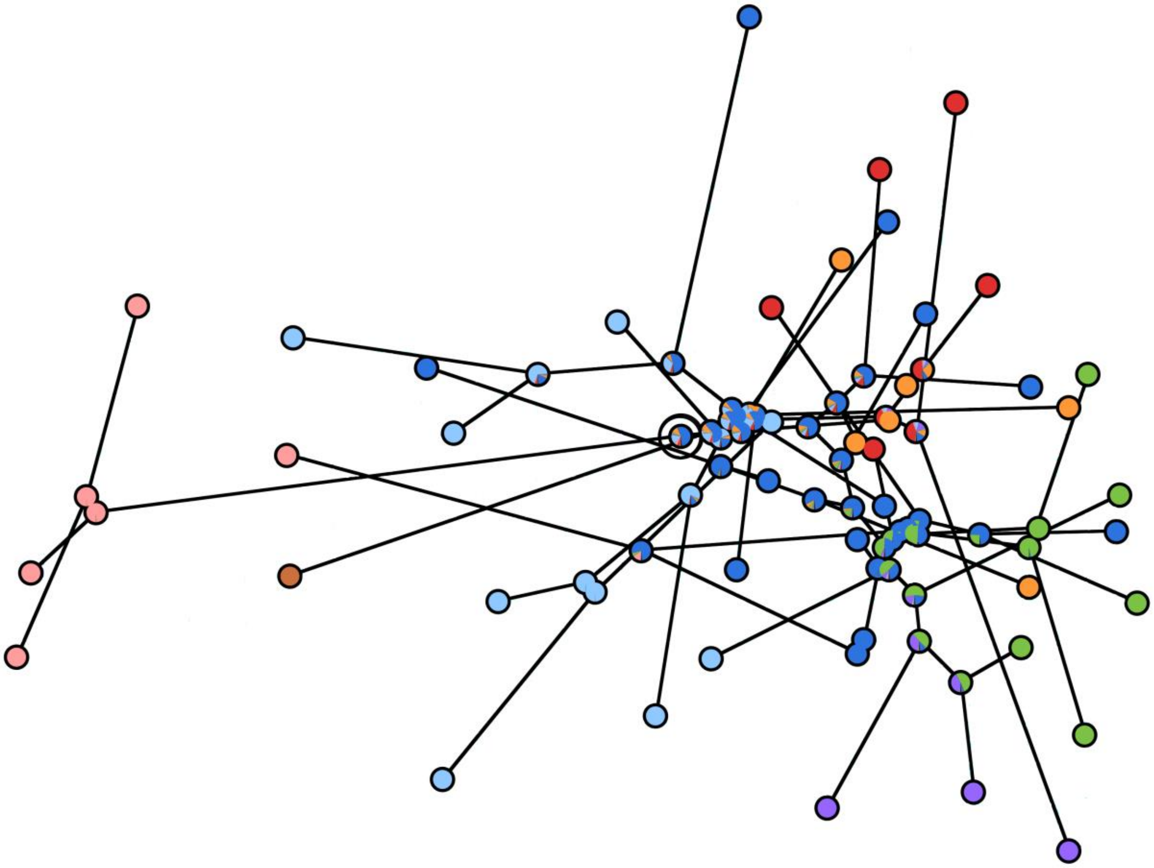

Figure 5. Phylomorphospace depicting the ancestral state reconstruction of LPJ shape and trophic guild. Branches reflect the magnitude and direction of changes in LPJ shape. Values of internal nodes were calculated using weighted square-change parsimony (Maddison 1991; Revell 2007). Pie diagrams depict the likelihood of the ancestral trophic state based on maximum likelihood (mk1 model) character state reconstruction (Maddison & Maddison 2011). Colour codes correspond to those in Figure 2.

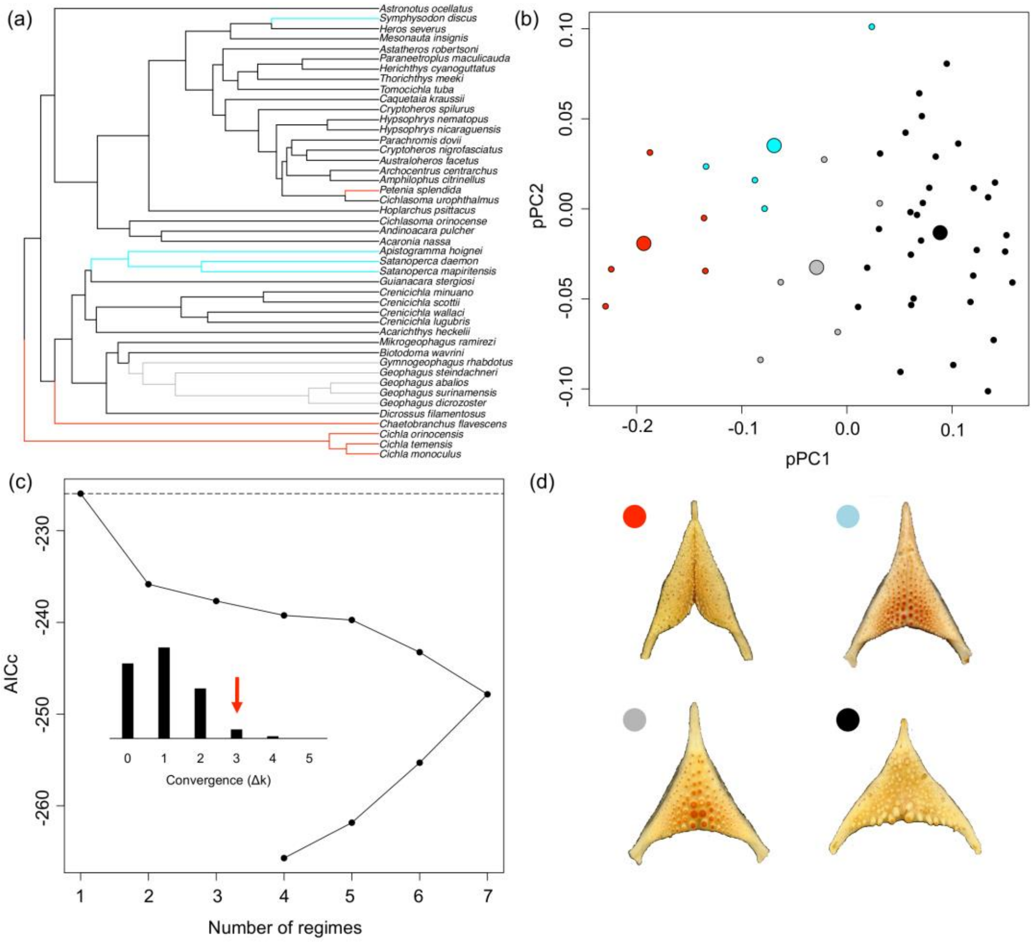

Figure 6. Results from SURFACE analysis of lower pharyngeal jaw shape among Neotropical cichlid fishes. (a) Phylogeny, with convergent (coloured) and nonconvergent (greyscale) regimes estimated from the best-fit model. (b) Trait values (phylogenetic principle components) that describe lower pharyngeal jaw shape for each species (small circles) and estimated optima (large circles). Regime colours match those in the phylogeny. (c) Change in AICc during the forward and backward phases of the analysis. Dashed line represents the AICc value for an Ornstein-Uhlenbeck model of evolution. The frequency distribution of null models is inset. The red arrow indicates the position of the observed data (*P*=0.05). (d) Representative lower pharyngeal jaws near each optima.

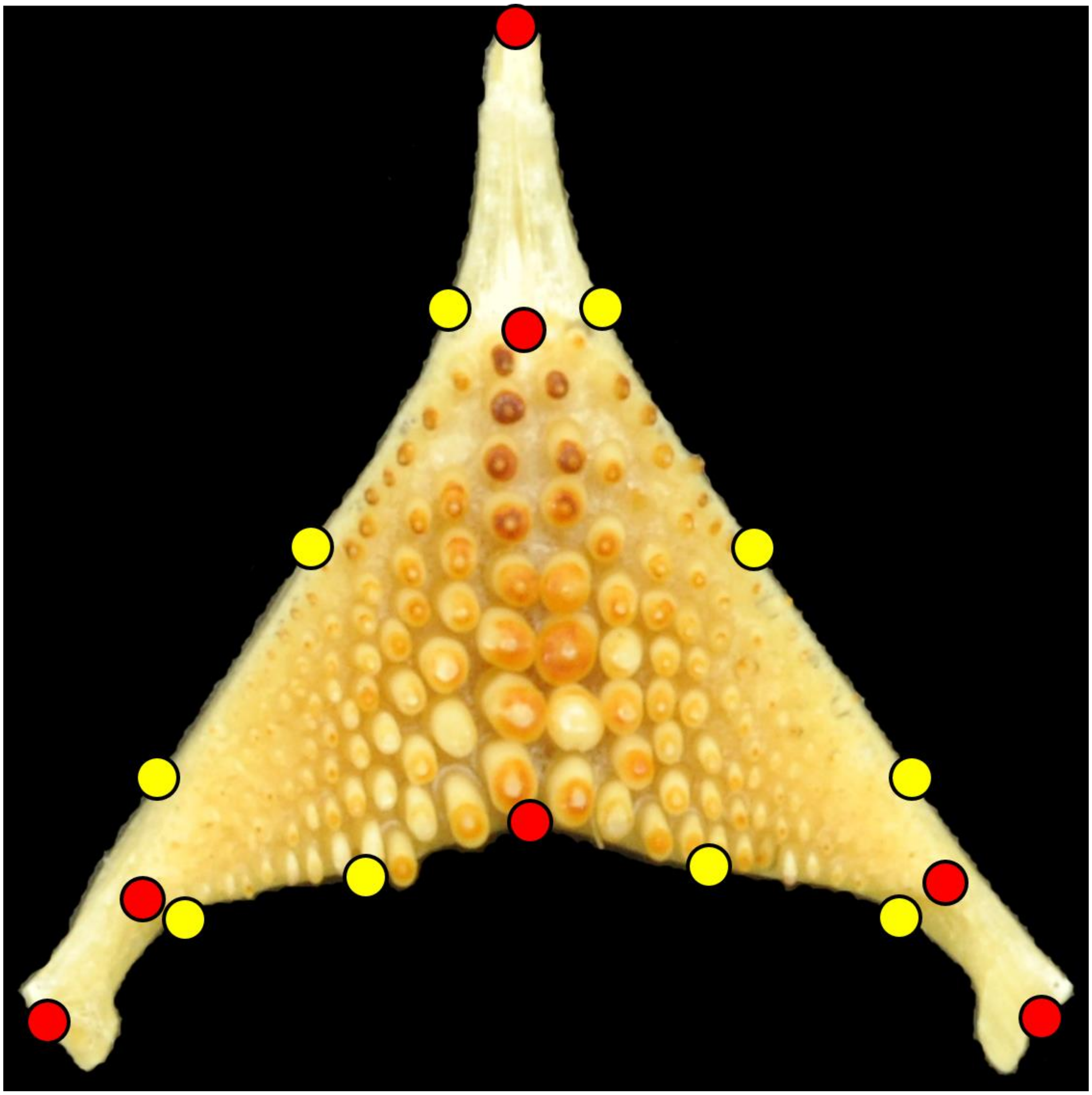

Figure S1. Landmark scheme used to quantify the shape of the lower pharyngeal jaw. Red points depict homologous landmarks and yellow points depict sliding landmarks.

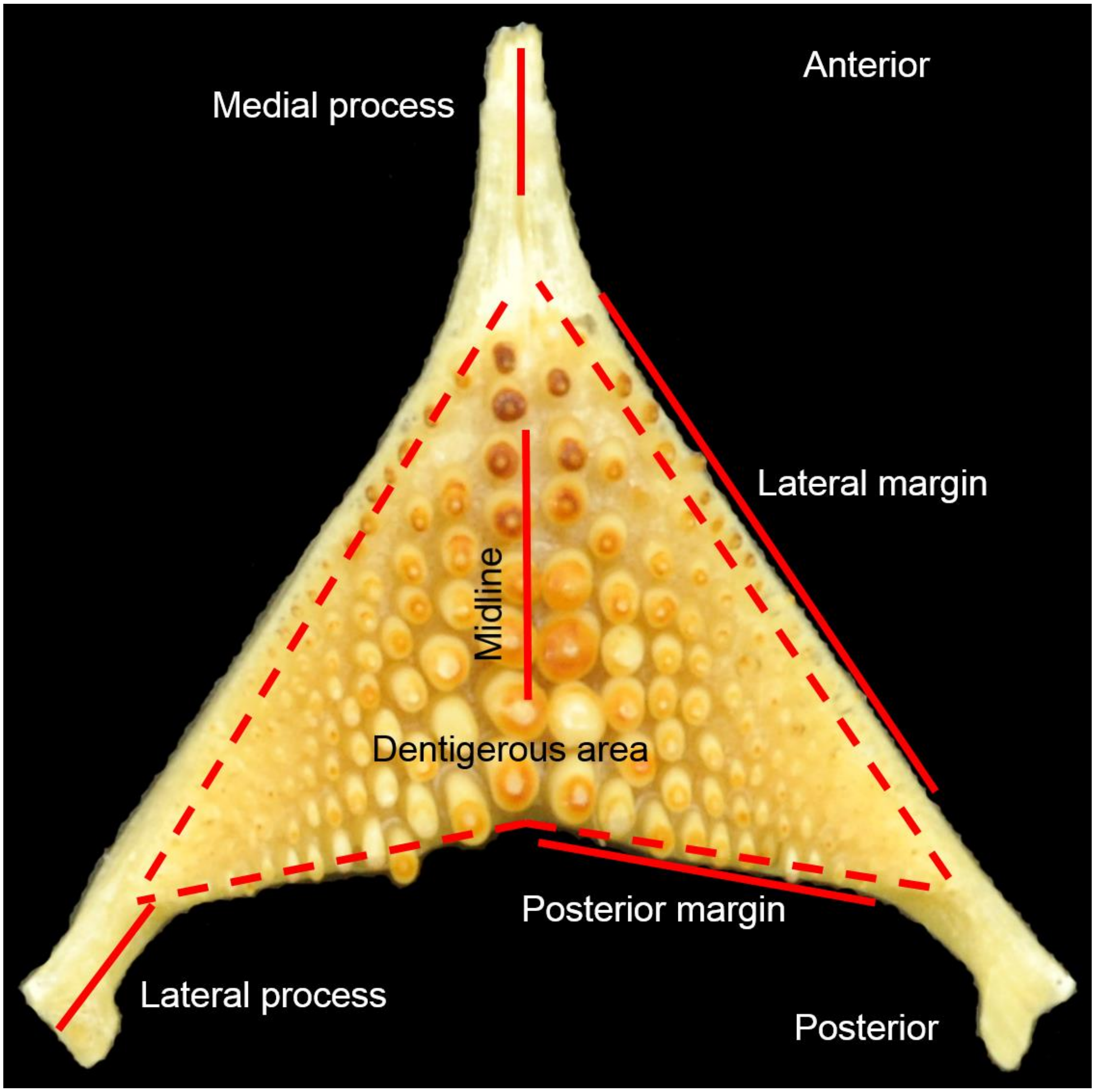

Figure S2. Depictions of the regions and aspects of the lower pharyngeal jaw referenced in the manuscript text.

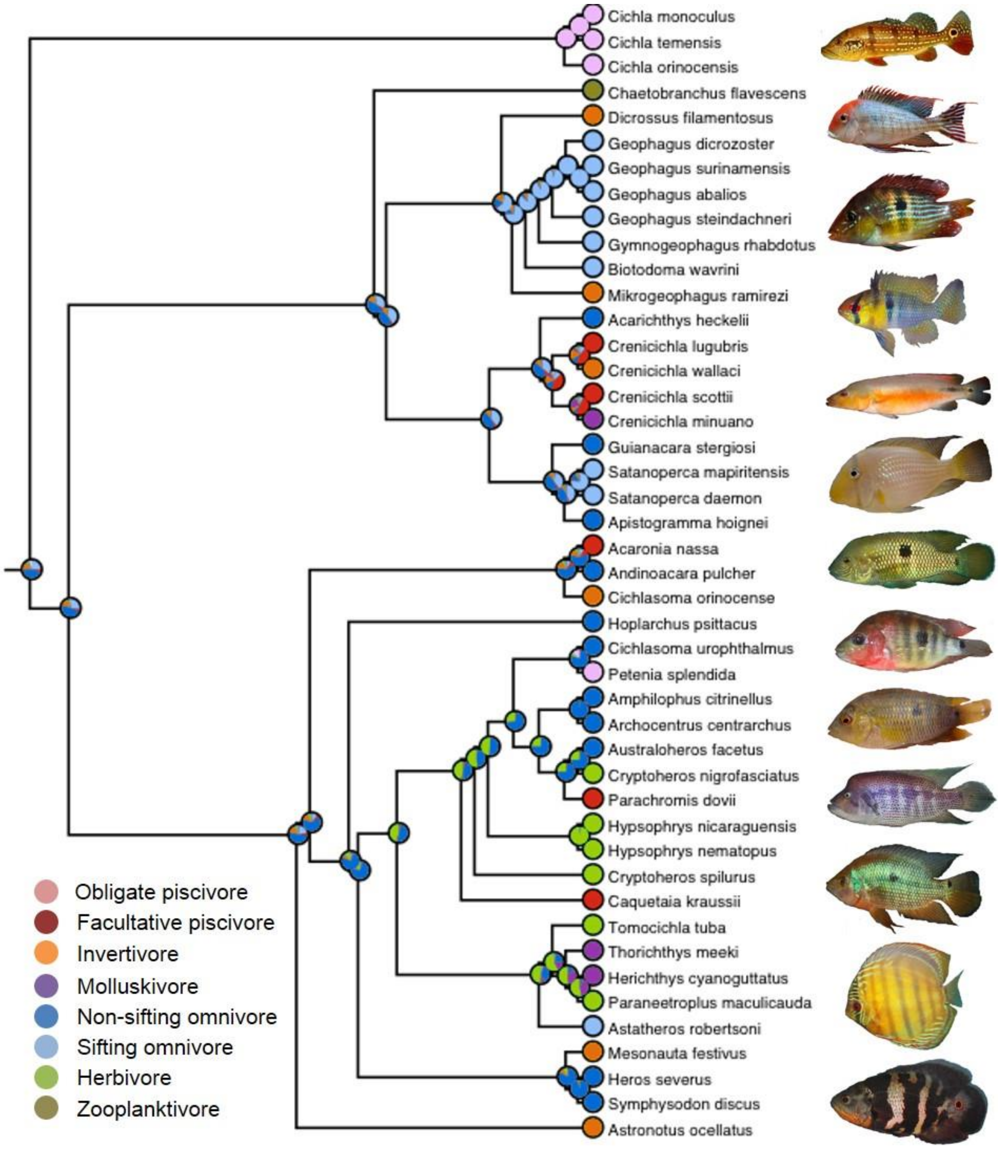

Figure S3. Maximum likelihood ancestral state reconstruction showing the evolution of trophic guild among Neotropical cichlids. Pie diagrams show the likelihood of each character state at each node. Phylogeny and divergence times are based on a pruned reconstruction of the maximum clade credibility tree from López-Fernández et al. (2013). Taxa are coded according to trophic guild (see Table 1 for references used to classify guilds). Images by the author and courtesy of Jeff Rapps. This reconstruction is plotted onto phylomorphospace in Figure 5 of the manuscript.

Chapter 2: Iterative convergent radiations revealed by pharyngeal jaw evolution following the colonization of the Americas by cichlid fishes

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Abstract

Key innovations have periodically led to salient leaps in functional capacity, efficiency, and/or versatility. A series of modifications to the pharyngeal arches that resulted in a second functional set of jaws are hypothesized to represent a major innovation that promoted diversification among cichlid fishes. Here, we test the degree of coevolution among pharyngognathy-associated traits across a phylogeny of 97 Neotropical cichlids and test opposing hypotheses about the time-dependent nature of pharyngeal jaw evolution following the subsequent colonization of South and Middle America by cichlid fishes. Masses of the lower pharyngeal jaw (LPJ) and muscular sling (MS), which closes the pharyngeal jaws, were highly correlated across the phylogeny. In contrast, LPJ and MS masses were uncorrelated with LPJ shape. The masses of pharyngeal bones and musculature are likely coevolved in association with the mechanistic generation of crushing force and subsequent tolerance of force-induced stress, whereas the shape of pharyngeal bones may be associated with more specialized trophic roles. We found that rates of morphological evolution accelerated through time, particularly in LPJ and MS masses after the colonization of Middle America. Furthermore, morphological disparity deviated greatly from expectations from BM near present day due to the evolution of convergent morphologies associated with repeated diversification along a functional axis characterized by the inferred crushing force potential of the pharyngeal jaws. These results indicate that cichlids have iteratively diversified into similar

ecomorphs and may constitute numerous parallel adaptive radiations that arose following their successive colonization of South and Middle America.

Introduction

Throughout the history of life, key innovations have periodically generated bursts of diversification and altered the trajectory of lineages across the adaptive landscape in response to novel ecological opportunities. Ray-finned fishes possess two sets of jaws. In addition to the oral jaws that consist of the mandible and upper jaw (maxilla and premaxilla), they possess a second set that is unique to ray-finned fishes, which consists of pharyngeal arches that are modified to assist with prey processing (Liem 1973; Wainwright 2005). In most cases, these pharyngeal jaws are composed of paired toothed upper and lower jaw bones; however, a series of modifications (i.e., pharyngognathy) to this complex have been hypothesized to represent a major functional innovation that increased trophic capacity, versatility, and efficiency (Liem 1973; Hulsey 2006; Wainwright et al. 2012; Burress 2016; Wainwright and Longo 2017). Pharyngognathy is a derived condition characterized by three modifications relative to the widespread primitive condition: (i) the left and right lower pharyngeal jaw (LPJ) bones are sutured together into a single structure, (ii) the upper pharyngeal jaw bones articulate directly against the neurocranium via a synovial joint, and (iii) a muscular sling suspends the LPJ directly from the neurocranium and generates the biting mechanism by contracting and drawing the LPJ upward into the upper pharyngeal jaws (Liem 1973; Wainwright et al. 2012). Although, pharyngeal jaws allow for prey acquisition and processing to be delegated to different muscle-skeleton systems (Liem 1973), pharyngognathy has advanced the capacity of fishes to process poorly accessible prey items such as hard-shelled, structurally complex, or difficult to digest prey (Wainwright et al. 2012; Burress

2016). Indeed, pharyngognathy may have facilitated the evolution of trophic roles that would otherwise be unlikely evolutionary outcomes (Hulsey 2006; McGee et al. 2015; Burress et al. 2015; Burress 2016).

There are many aspects of the pharyngeal apparatus that function in concert during mastication. These potentially include i) the shape of the pharyngeal bones, ii) mass of the pharyngeal bones, and iii) mass of the associated musculature that operates the biting motion of the pharyngeal jaws. Individually, these elements may impose constraints upon force generation and subsequent stress tolerance, but sum to determine the functional repertoire of the complex (Liem 1973). The upper pharyngeal jaws are generally ovoid and have simple dentition, whereas the LPJs are highly variable in shape and degree of atrophy/hypertrophy, and have diverse tooth types (Casciotta and Arratia 1993). Additionally, LPJ shape is associated with trophic patterns at population-, genus-, and family-scales among cichlids (Hulsey et al. 2005; Hulsey 2006; Burress et al. 2015; Burress 2016). Well-developed musculature modulates the pharyngeal jaws to allow for considerable manipulation of prey, ranging from crushing hard-shelled organisms (i.e., snails and bivalves), sifting minute edible items from mouthfuls of sediment, and rupturing algae cells, which would not be feasible tasks for oral jaws (Drucker and Jensen 1991; Hulsey 2006; Burress 2016). There are ten muscles associated with the pharyngeal jaws; however, a single muscle is mostly responsible for activating the jaw-like motion utilized during mastication (Liem 1973; Wainwright et al. 2012). This muscle, the MS, suspends the LPJ directly from the neurocranium. The major source of movement among the pharyngeal jaw elements occurs when the MS contracts, pulling the LPJ dorsally against the upper pharyngeal jaws, which are stabilized by and articulate with the neurocranium (Wainwright et al. 2012).

Here, we evaluate three aspects of the pharygognathy that have well-established inferred functional associations with trophic patterns among cichlids: LPJ shape, LPJ mass, and MS mass (fig. 1). We focus on two objectives. First, we evaluate the degree of coevolution among these three traits across the Neotropical cichlid phylogeny. Biting force generated by the MS imposes structural stress upon the LPJ during mastication (Hulsey et al. 2008); therefore, we predict that the masses of the LPJ and MS should be highly correlated to maintain efficient coordination and functionality while processing prey. Secondly, we test opposing hypotheses about the temporal patterns of adaptive radiation. A central hypothesis of adaptive radiation is that traits exhibit diversity-dependent evolution such that rates of morphological evolution slow through time as niches become saturated (Schluter 2000; Freckleton and Harvey 2006; Losos 2011). In theory, such early bursts occur in response to ecological opportunity that gradually wanes over time (Schluter 2000; Glor 2010). In this case, we expect morphological divergence early in clades evolutionary history to result in morphological variation that is partitioned within clades rather than among clades (Schluter 2000; Harmon et al. 2003). Early bursts in morphology have been shown in anole lizards and cichlid fishes (Harmon et al. 2003; López- Fernández et al. 2013) and extensively throughout the fossil record (Foote 1994; Ruta et al. 2006; Hughes et al. 2013), but have otherwise rarely been observed in nature (Harmon et al. 2010). Alternatively, iterative radiations should exhibit faster rates of morphological evolution toward the present as lineages colonize similar regions of the adaptive landscape in response to ecological opportunity (Frédérich et al. 2013). In this scenario, we expect rates of morphological evolution to increase through time and for morphological disparity to partition among clades rather than within clades as a result of exploring similar morphologies during their evolutionary history. Iterative adaptive radiations have been shown in other pharyngognathous fishes (Frédérich et al. 2013) as well as the fossil record (Slater 2015). We used several comparative methods to test these hypotheses about the evolution of pharyngeal jaw-associated traits following the colonization of the Americas by cichlid fishes.

Methods

Phylogenetic inference

We estimated the phylogeny and divergence times using BEAST (Drummond et al. 2012). We modified the BEAST input file originally used by López-Fernández et al. (2013) (doi:10.5061/dryad.34621), which was based on the data and phylogeny published in López-Fernández et al. (2010). López-Fernández et al. (2013) included five loci (Cyb4, 16S, ND4, RAG2, and S7 intron 1) for 166 cichlid species. We made two modifications to the input file. First, we included 12 additional species that were not in the original analysis, using sequences from other published studies. We aligned these sequences to the López-Fernández et al. alignment using the ClustalW alignment algorithm implemented in Geneious 6.1.8 (Larkin et al. 2007; Kearse et al. 2012). Second, López-Fernández et al. (2013) defined a number of clades as monophyletic, but to better assess the phylogenetic relationships given the additional taxa, we removed clade constraints for monophyly except for four strongly-supported clades, including the ingroup (Cichlinae) and the nodes with fossil constraints (Cichlasomatini, Heroini, and *Gymnogeophagus*). This necessitated increasing chain length to reach convergence, so each chain was run for 200 million generations, logging every 20,000 generations. Other settings were the same as in López-Fernández et al. (2013), including speciation priors, models of sequence evolution across gene partitions, divergence time calibrations, and setting of the uncorrelated relaxed clock model (Drummond et al. 2006). Analysis was performed using BEAST v1.8.1

(Drummond et al. 2012) on the Auburn University CASIC HPC cluster . We ran three independent chains. Convergence (ESS for parameters > 200) was assessed with Tracer v1.5, and the maximum clade credibility (MCC) tree was computed from the posterior distribution of trees by combining the results of all three chains and excluding burn-in using Log Combiner v1.7.5 and TreeAnnotator v1.7.5.

Quantifying pharyngeal jaw-associated traits

We dissected the LPJ of 307 individuals from 97 Neotropical cichlid species accessioned in the Auburn University Museum of Natural History. Only specimens that represent adult size classes were used to minimize confounding effects of ontogeny. The LPJs were cleaned using dilute bleach, rinsed in deionized water, and then photographed in dorsal view using a mounted Nikon D5100 digital camera camera (Nikon Corporation, Tokyo, Japan). We used four homologous landmarks positioned at the ends of the medial and lateral processes and at the posterior midline (fig. S1). Additionally, 18 sliding landmarks were positioned along the lateral and posterior margins (fig. S1). Of these sliding landmarks, six were equally spaced between the ends of the medial and lateral processes and three sliding landmarks were equally spaced along each posterior margin between the end of the lateral process and posterior midline (fig. S1). Sliding landmarks are not associated with a homologous structure but instead trace the curvature of an object between two homologous landmarks. Photos were consolidated using tpsUtil (Rohlf 2004), then digitized and landmarked using and tpsDIG2 (Rohlf, 2006). Landmarks were superimposed, aligned, and principal components were generated using tpsRelw (Rohlf 2007). Size, rotation, and translation were accounted for during the superimposition procedure. The tps program suite can be freely accessed at http://life.bio.sunysb.edu/morph. Lastly, LPJs were air dried until asymptotic mass. The MSs were lyophilized until asymptotic mass. Dry weights of the LPJs and MSs were then measured to the nearest 0.000001 grams. All statistics were performed in R (R Development Core Team). LPJ mass (R^2=0.496; F=93.66; P<0.0001) and MS mass (R^2=0.219; F=26.58; P<0.0001) were significantly positively correlated with body size, thus we calculated residuals using regressions with body size to account for magnitude (Burress et al. 2015). These size-relative values were used in all subsequent analyses.

Phylogenetic signal

To evaluate how well the phylogeny explained trait values, we tested for phylogenetic signal, the tendency for closely-related species to resemble one another, using Blomberg’s K (Bloomberg et al. 2003) and Pagel’s λ (Pagel 1999). Brownian motion should produce k-values equal to 1, whereas K values >1 and <1 indicate that there is more and less phylogenetic signal, respectively, than expected from BM. Likewise, λ-values close to 0 indicate no phylogenetic signal, whereas λ-values equal to 1 indicated that phylogenetic signal is consistent with that expected from BM. We calculated K and λ using the phylosig function implemented in the PHYTOOLS package (Revell 2012) for R.

Models of morphological evolution

To evaluate patterns and modes of morphological diversification, we assessed the fit of several macroevolutionary models to the cichlid phylogeny using the fitContinuous function implemented in the GEIGER package (Harmon et al. 2008). As null models, we fit Brownian motion and white noise evolutionary models. Brownian motion (Felsenstein 1973) describes the extent to which trait covariance increase proportional to time (i.e., the extent of shared ancestry among species). White noise is a non-phylogenetic model that describes the extent to which data come from a single normal distribution without trait covariance among species. Additionally, we tested two opposing time-dependent models of evolution. First, the early-burst model (Blomberg et al. 2003; Harmon et al., 2003; 2010) describes the extent to which the rate of evolution decreases through time. Second, the delta model (Pagel 1999) describes the extent to which the rates of evolution are temporally distributed early or late in the history of a clade (i.e., comparatively fast or slow recent evolution). Lastly, we fit an Ornstein-Uhlenbeck model (Butler and King 2004), which describes a random walk with a tendency to evolve towards trait optima over time. We compared the relative fits of these five models of evolution by comparing their log-likelihoods, AIC scores, and AIC weights. Model fit improvement was determined based on two AIC units.

Morphological evolutionary rates, disparity, and convergence

To evaluate degrees of coevolution among traits across the phylogeny, we calculated phylogenetically independent contrasts (Felsenstein 1985) for each trait using the pic function implemented in APE (Paradis et al. 2004) and tested for correlations using linear models using the lm function. Standardized independent contrasts are Brownian rate parameters for the portion of the phylogeny at which they are calculated (McPeek 1995). To assess the time-dependent nature of trait evolution, we used the node-height test (Freckleton and Harvey 2006), which regresses the absolute magnitude of rate contrasts against the height of the node (i.e., time since root) at which they were calculated. Brownian motion should result in no correlation between node height and rate contrasts; however, a significant positive correlation between absolute rate contrasts and node height would indicate that that rates of morphological evolution have increased through time, whereas a significant negative correlation would indicate that rates of morphological evolution have slowed through time. We calculated these tests separately for the South American and Middle American portions of the phylogeny to assess the potential influence of each colonization event. To assess the probability of spurious significant relationships, we simulated 100 data sets via BM across the phylogeny using the surfaceSimulate function implemented in SURFACE (Ingram and Mahler 2013). We then performed node-height tests on these simulated data sets to produce a null distribution of effect sizes (r; correlation coefficient) that could result from BM and provide an expected rate of spurious significant correlations resulting from BM.

To assess the degree to which morphological disparity is partitioned within or among clades, we calculated the morphological disparity index (MDI) to quantify the difference between observed subclade disparity through time and that expected from a null BM model (Harmon et al. 2003). Negative MDI values indicate lower than expected subclade disparity (i.e., more divergent) and positive MDI values indicate higher than expected subclade disparity (i.e., more convergent) than expected under BM. MDI statistics were calculated over the first 80% of the tree to reduce the influence of incomplete sampling of the tip species (Harmon et al. 2003). MDI statistics were calculated using the dtt function implemented in GEIGER (Harmon et al. 2008). We then visualized trait evolution through time by generating phenograms (Evans et al. 2009) that depict the inferred evolutionary history of traits through time using the fancyplots function implemented in PHYTOOLS (Revell 2012). Lastly, we evaluated the degree of trait convergence relative to that of traits simulated under BM using the runSurface and surfaceSimulate functions, respectively, implemented in SURFACE (Ingram and Mahler 2013). SURFACE uses AIC to identify regime shifts on the phylogeny by fitting a multi-peak

Orenstein-Uhlenbeck model of evolution during successive backward and forward phases (Ingram and Mahler 2013). Regime shifts (*k*) towards trait optima are identified during the forward phase and regimes shifting towards common optima are then collapsed into convergent regimes (*k*') during the backward phase of the analysis. The degree of convergence is quantified by calculating the difference in the number of regimes and the number of convergent regimes (*k*-*k*'). We generated null distributions for three parameters to which we compared parameter estimates from the observed data: the number of regime shifts (*k*), the number of shifts towards convergent regimes occupied by multiple lineages (*c*), and the degree of convergence (Δk). We visualized the evolutionary history of each trait by mapping them onto the phylogeny using the contMap function implemented in PHYTOOLS (Revell 2012). The states at internal nodes were estimated using ML and were extrapolated along each branch following Felsenstein (1985).

Results

Our analyses included 97 species, including species well-distributed across the major Neotropical lineages (fig. 2*A*). The species included a wide array of trophic guilds that are representative of known trophic diversity and presumably impose diverse functional demands on the pharyngeal jaw apparatus while foraging (Burress 2016). PC1 explained 79.0% of the variation in LPJ shape and described variation in the orientation of the lateral processes (fig. 2*B*). PC2 explained 12.6% of the variation in LPJ shape and described variation in the length of the medial and lateral processes (fig. 2*C*). LPJ mass and MS mass varied by 2.6 and 2.1 orders of magnitude among species, respectively (table S3).

Shape and mass correlations

Phylogenetically independent contrasts of LPJ mass and MS mass were significantly correlated (R^2=0.53; F=106.0; P<0.0001; fig. 3). Phylogenetically independent contrasts of LPJ masses were not correlated with PC1 contrasts (R^2=0.035; F=3.36; P=0.07; fig. S2*A*) or PC2 contrasts (R^2=0.0001; F=0.01; P=0.92; fig. S2*B*). Phylogenetically independent contrasts of MS masses were also not correlated with PC1contrasts (R^2=0.03; F=2.45; P=0.12; fig. S2*C*) or PC2 contrasts (R^2=0.012; F=1.14; P=0.29; fig. S2*D*). Both variables that described LPJ shape, PC1 and PC2, exhibited phylogenetic signal; however, the size-relative masses of the LPJ and MS did not (table 1).

Models of morphological evolution

The best fit model of morphological evolution for the primary axis of LPJ shape variation (PC1) was OU; however, the fit was not significantly better than delta or BM based on AIC scores (table 2). The best fit for the secondary axis of LPJ shape variation (PC2) was delta; however, the fit was not significantly better fit than OU (table 2). For the size-relative masses of the LPJ and MS, the best fit models of morphological evolution was delta and OU, which had similar AIC scores (table 2). Of the two time-dependent models of morphological evolution, the delta model, which describes the tendency for the rate of evolution to increase over time, consistently fit better for all four traits than the EB model, which describes the tendency for the rate of morphological evolution to slow through time (table 2). Furthermore, estimates of the delta parameter was greater than 1 for all four traits, indicating that recent evolution has been relatively fast (PC1=1.84; PC2=2.67; LPJ mass=2.99; MS mass=2.99).

Rates of morphological evolution

The primary axis of LPJ shape variation (PC1) exhibited no correlation between rate contrasts and node height after the colonization of South America (R^2=0.006; *F*=0.14; *P*=0.71) or Middle America (R^2=0.065; *F*=0.1.94; *P*=0.17; fig. 4*A*). The second axis of shape variation (PC2) also exhibited no correlation between rate contrasts and node height after the colonization of South America (R^2=0.019; *F*=1.25; *P*=0.27), but exhibited a significant positive relationship following the colonization of Middle America (R^2=0.157; *F*=5.23; *P*=0.03; fig. 4*B*). The size-relative mass of the LPJ exhibited a significant positive relationship between rate contrasts and node height in both South America (R^2=0.097; *F*=6.91; *P*=0.011) and Middle America (R^2=0.295; *F*=11.69; *P*=0.002; fig. 4*C*). Likewise, the size-relative mass of the MS exhibited a significant positive relationship between rate contrasts and node height in both South America (R^2=0.083; *F*=5.80; *P*=0.019) and Middle America (R^2=0.255; *F*=9.57; *P*=0.005; fig. 4*D*). Effect sizes indicate that the rate of increase in morphological evolution through time increases after the colonization of Middle America (fig. 4). One hundred data sets simulated across the phylogeny using BM resulted in two significant relationships between rate contrasts and node height (*P*=0.02); however, none of the significant effect sizes from the observed data (fig. 4) were sampled during the analysis of the simulated data (r; range -0.31-0.24).

Disparity and convergence in pharyngeal jaw traits

Disparity-through-time analyses suggest that the evolution of both LPJ shape variables, PC1 (MDI=0.127; *P*=0.243) and PC2 (MDI=0.147; *P*=0.165), exhibit subclade disparity consistent with that expected from BM throughout the history of the clade (fig. 5*A*,*B*). Neither shape variable (PC1 and PC2) exhibits an early burst (i.e., lower than expected disparity) that would suggest that disparity was partitioned among clades or any obvious signature corresponding with the colonization of South America or Middle America (fig. 5*A*,*B*). Similarly, the size-relative masses of the LPJ (MDI=0.616; *P*=0.0104) and MS (MDI=0.431; *P*=0.0104) subclade disparity evolved consistent with expectations from BM early in the clades history (~0.0-0.4 relative time); however, observed disparity is higher than that expected from BM since approximately the colonization of Middle America (~0.45-1.0 relative time), indicating that disparity is increasingly distributed within rather than among clades over time (fig. 5*C*,*D*). Likewise, the inferred evolutionary history of traits through time based on BM ancestral reconstruction suggests that variance of both shape variables (PC1 and PC2) increased proportional to time, whereas the size-relative masses of the LPJ and MS appear to expand more recently (i.e., ~0.7-1.0 relative time; fig. 5*E*).

Simulations from SURFACE provided null distributions for the expected number of evolutionary regime shifts, number of shifts towards convergent regimes, and the degree of convergence among pharyngeal jaw traits (fig. 6). Both LPJ shape variables (PC1 and PC2) exhibited similar numbers of shifts in evolutionary regimes as expected from BM, whereas the size-relative masses of the LPJ and MS exhibited more evolutionary regimes than expected from BM (fig. 6*A*). Furthermore, shifts in both size-relative masses of the LPJ and MS evolutionary regimes tended to be towards convergent regimes at higher rates than expected from BM (fig. 6*B*), resulting in comparatively high proportion of shifts towards convergent regimes in those traits. Indeed, the degree of convergence (i.e., number of convergent regimes evolving towards trait optima) in LPJ shape variables (PC1 and PC2) was consistent with that expected from BM; however, the degrees of convergence in size-relative masses of the LPJ and MS either deviated (MS) or nearly deviated (LPJ) from expectations from BM (fig 6*C*). These cases of convergence arose from the repeated patterns of atrophy and hypertrophy of the LPJ and MS across the Neotropical cichlid phylogeny (fig. 6*D*,*E*).

Discussion

Trophic implications of pharyngeal jaw trait coevolution

Pharyngeal jaws have myriad functions including winnowing edible food from mouthfuls of sediment (Drucker and Jensen 1991), crushing hard-shelled organisms such as snails (Hulsey 2006), and grinding cellulose-rich material to facilitate digestion (Xie 2001; Burress 2016). LPJ shape and dentition are plastic (Huysseune 1995; Muschick et al. 2011; Gunter et al. 2013), and continuous tooth replacement allows for maintenance of tooth size, shape, and density (Huysseune 1995). Thus, LPJ shape, size, dentition, and associated musculature may respond in unison to functional demands imposed by specific prey items (Hulsey et al. 2008). Indeed, we found that the size-relative masses of the LPJ and MS were highly correlated; however, these traits were uncorrelated with LPJ shape. In addition to the complex multidimensional nature of shape, this decoupling of pharyngeal elements may be explained by complex relationships among LPJ shape, LPJ mass, and trophic function. For example, among piscivores, the LPJ is often atrophied and has elongate lateral processes (Liem 1973; Barel et al. 1977; van Oijen 1989; Burress et al. 2015; Hellig et al. 2010); however, there are two distinct LPJ shapes exhibited by piscivores: 1) widely-spaced lateral processes (i.e., among *Acaronia*, *Caquetaia*, *Crenicichla*, and *Parachromis*) and 2) narrowly-spaced lateral processes (i.e., among *Cichla* and *Petenia*; Burress 2016). Regardless of LPJ shape, all piscivores exhibit atrophy of their LPJ and MS (Liem 1973; Barel 1983). Presumably, there is little need for generating biting force for merely grasping and positioning prey that is consumed whole (Winemiller 1989; Wainwright 1991;

Burress et al. 2013). Piscivores probably rely on sharp, recurved pharyngeal teeth for this function (Casciotta and Arratia 1993; Burress 2016). Furthermore, algivores (i.e., *Cryptoheros*, *Herotilapia*, *Hypsophrys*, and *Neetroplus*) and benthic sifters (i.e., all *Geophagus* and *Satanoperca*) also exhibit drastically different LPJ shapes (Burress 2016), yet both guilds have relatively reduced LPJs and MSs in terms of their size-relative masses. Algivores tend to have small, sharp, conical teeth (Casciotta and Arratia 1993), and therefore may utilize specialization of their pharyngeal teeth to grind and rupture algae cells (Burress 2016), rather than rely on generation of crushing force by the MS. Similarly, benthic sifters, which primarily use their LPJs to winnow edible items from mouthfuls of sediment (Drucker and Jensen 1991), likely do not require much biting force for this purpose. Similar modification to LPJ and MS masses occur in association with numerous trophic functions and across multiple LPJ shapes.

Functional constraints and LPJ-MS coupling

The two principle constraints associated with pharyngeal jaws are biting force and pharyngeal gape (Wainwright 1998; Wainwright 1991; Wainwright et al. 2012; McGee et al. 2015). Biting force generated by the MS imposes structural stress upon the LPJ during mastication (Hulsey et al. 2008). Therefore, the mass of the LPJ and MS should be intimately correlated to maintain functional efficiency that scales across different demands for biting force. Indeed, the size-relative masses of the pharyngeal bones and muscles were strongly correlated. Crushing force plays a central role in constraining trophic patterns, namely along the soft-bodied to hard-shelled resource axis (Wainwright 1988). Demands for both the capacity to generate sufficient crushing force and the structural pharyngeal jaw integrity to tolerate stress incurred during crushing functions has implications for dietary breadth and attainment of novel resource dimensions

(Wainwright 2005; Wainwright 1998; Hulsey 2006). For example, exploitation of durophagy is largely made possible due to the mechanical potential of pharyngeal jaws, particularly the improved biting force capacity and efficiency associated with pharyngognathy (Liem 1973; Galis and Drucker 1996). Wrasses, surfperches, and cichlids feed extensively upon hard-shelled crustaceans and molluscs (Liem 1986; Wainwright 1988; Burress 2016) and parrotfish pharyngeal jaws may facilitate crushing coral (Bellwood 1995). Although there are examples of fishes crushing molluscs with their oral jaws, the vast majority of species process these items in the pharyngeal jaws (Wainwright 2005), and therefore the extreme bite force dimension of dietary niches is essentially constrained by pharyngeal jaws.

The capacity for generating biting force is likely not evenly distributed across different LPJ shapes. LPJs with narrowly-spaced lateral processes exclusively exhibit reduced bone and muscle masses, suggesting poor ability to exert crushing force (Liem 1973). Species that exhibit these LPJ shapes have trophic characteristics that require little biting force such as piscivores (e.g., *Cichla*) and zooplanktivores (e.g., *Chaetobranchus*: Burress 2016). In contrast, LPJs with widely-spaced lateral processes exhibit an array of bone and muscle masses, indicating that this LPJ shape may perform more variable functions. Indeed, species that have this LPJ shape includes diverse trophic characteristics that have different demands for biting force generation such as piscivores (e.g., *Crenicichla*), algivores (e.g., *Cryptoheros*), and molluscivores (e.g., *Thorichthys* and *Herichthys*; Burress 2016). Thus, some LPJ morphologies may be more functionally constrained than others via their relative restricted or dynamic associations with bone and muscle masses.

Pharyngognathy facilitates attaining processing-intensive trophic functions, yet may constrain transitions into trophic roles with minimal demand for mechanical processing by the pharyngeal jaws (McGee et al. 2015). For example, piscivory requires little from the pharyngeal jaws because prey are often consumed whole rather than processed prior to ingestion (Winemiller 1989; Burress et al. 2015), and the mere function of grasping prey can be performed by the oral jaws. In this case, pharyngeal jaws impose constraints via pharyngeal gape that limits exploitation of some prey sizes (van Oijen 1982; Wainwright 1988; Burress et al. 2015). Additionally, pharyngeal jaws may reduce efficiency such that processing time is increased (McGee et al. 2015). In some extreme cases, some piscivorous lineages exhibit partial reversal of a hallmark characteristic of pharyngognathy in response to these functional constraints. For example, juvenile *Cichla* have unsutured LPJs, permitting the fifth ceratobranchials to spread apart laterally (Stiassny and Jensen 1987) and thereby subvert constraints imposed by pharyngeal gape. Alternatively, some species may relax limitations from pharyngeal gape via atrophied LPJs or low-profile pharyngeal dentition (Burress et al. 2015).

Pharyngeal jaw-associated trait evolution

We found no support for a single adaptive radiation via an early burst of morphological diversification in cichlid fishes. Subclade disparity was not partitioned among clades early in the evolutionary history of the clade that would indicate that subclades diverged early into novel regions of morphospace. Likewise, we found that an EB model of morphological evolution consistently fit poorly among other candidate models, indicating that the evolution of pharyngeal jaw-associated traits has not slowed through time. Rather, we present evidence that the observed pharyngeal jaw trait diversity evolved via recent iterative bouts of diversification into convergent ecomorphs. Late in the evolutionary history of the clade, subclade disparity increasingly became partitioned within clades, indicating that subclades were evolving into similar suites of ecomorphs. Furthermore, we found that mixtures of OU, delta, and white noise models of morphological evolution best fit pharyngeal jaw-associated trait evolution among Neotropical cichlids. Lastly, node-height tests indicate that rates of trait evolution, particularly of the size-relative masses of the LPJ and MS, have increased over time. Node-height tests and DTT also exhibit a clear signature associated with the colonization of Middle America and subsequent shift towards faster rates of evolution of convergent ecomorphs. These results are consistent with multiple adaptive radiations diversifying recently and rapidly into convergent morphologies rather than a single adaptive radiation arising via an early burst of morphological evolution (Freckleton and Harvey 2006; Harmon et al. 2010; Frédérich et al. 2013; Slater 2015). These adaptive radiations are likely associated with repeated diversification along the soft-body to hard-shelled prey axis evidenced by the widespread diversification and inferred function of size-relative masses of the LPJ and MS across the Neotropical cichlid phylogeny. This pattern of morphological evolution is consistent with the independent evolution of trophic guilds with varied demands for crushing force among Neotropical cichlids such as piscivory and molluscivory (Burress 2016).

The colonization of Middle America from South America likely played a key role in the repeated adaptive radiation of cichlids. This colonization event involved trans-Atlantic dispersal via island hopping and utilization of land bridges (Chakrabarty 2004; Říčan et al. 2013; Tagliacollo et al. 2015) and may have occurred via successive waves by different clades (Říčan et al. 2013) that may have provided similar novel ecological opportunities among divergent lineages. These ecological opportunities likely favored the repeated evolution of convergent morphologies. Several Middle American species with well-developed LPJs and MSs also have well-developed oral jaw-associated musculature and thus appear to have invested in generation of biting force by both their oral and pharyngeal jaws (Arbour and López-Fernández 2014). Many of these species are frugivorous and may require oral and pharyngeal biting force to efficiently negotiate thick husks and seeds (i.e., *Astronotus*, *Hoplarchus*, and *Vieja*; Winemiller et al. 1995; Burress 2016) as well as molluscivores that must generate sufficient biting force to crush shells (Hulsey 2006; Hulsey et al. 2008). This handful of trophic guilds are widely distributed across the Neotropical cichlid phylogeny, but may have arose independently more frequently along Middle American lineages (Burress 2015; 2016) and thereby explain the clear signature of the trans-Atlantic colonization of middle in our DTT and node-height analyses.

Broader evolutionary implications

The series of modifications to the pharyngeal arches emphasized herein (i.e., pharyngognathy) have evolved independently many times among fishes (Wainwright et al. 2012), including the wrasses (Labridae), damselfishes (Pomacentridae), and surfperches (Embiotocidae). The shape, size, and organization of pharyngeal bones and the attachment sites and sizes of muscles are highly variable across these groups (Wainwright 2005; Wainwright et al. 2012; Burress 2016). The masses of the pharyngeal jaw-associated muscles are more variable than oral jaw-associated muscles among labrid fishes (Wainwright et al. 2004), suggesting that functional diversity of the pharyngeal jaws are potentially greater than that of the oral jaws (Wainwright 2005). The developmental, structural, and functional decoupling of the oral and pharyngeal jaws permits potential autonomy in their evolution. For example, the pharyngeal jaws have become specialized for mechanistic processing of prey items such as crushing, grinding, and winnowing (Drucker and Jensen 1991; Hulsey 2006; Burress 2016). In contrast, the oral jaws have mechanistically specialized for generation of suction or prey acquisition (i.e., scraping or grasping actions; Liem 1973; Wainwright 2005). Thus pharyngognathous fishes may exhibit complex evolutionary histories and unique trajectories across regions of the adaptive landscape unavailable to other lineages of fishes. Furthermore, Frédérich et al. (2013) found that damselfishes morphological diversity evolved via iterative convergent adaptive radiations based measurements of the oral jaws (mandible and premaxilla). In combination with our results for cichlid fishes, these studies indicate that recent adaptive radiations characterized by the iterative evolution of convergent morphologies may be a general theme among pharyngognathous fishes and therefore, many groups that constitute important components of freshwater and marine reef communities.

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Table 1. Results from tests for phylogenetic signal in pharyngeal jaw-associated traits across the Neotropical cichlid phylogeny: lower pharyngeal jaw (LPJ) shape (PC1 and PC2) and size-relative masses of the LPJ and muscular sling (MS).

Trait	K / P	λ / P
PC1	0.950 / 0.001	0.884 / <0.001
PC2	0.676 / 0.001	0.844 / <0.001
LPJ	0.257 / 0.492	0.00067 / 1.0
MS	0.293 / 0.268	0.049 / 0.513

Table 2. Results from fitting morphological diversification models to pharyngeal jaw traits across the Neotropical cichlid phylogeny: lower pharyngeal jaw shape (PC1 and PC2), lower pharyngeal jaw mass (LPJ mass), and muscular sling mass (MS mass). Models for each trait are listed in order of best-fit based on AIC scores. Fitted models include Brownian motion (BM), early burst (EB), Ornstein-Uhlenbeck (OU), white noise (white), and delta (delta).

Trait	Model	log-likelihood	AIC	dAIC	wtAIC
PC1	OU	107.97	-209.93	0	0.452
	delta	107.36	-208.71	1.22	0.245
	BM	106.25	-208.51	1.42	0.221
	EB	106.25	-206.51	3.42	0.081
	white	79.62	-155.23	54.7	<0.001
PC2	delta	107.36	-374.81	0	0.502
	OU	190.32	-374.63	0.18	0.459
	BM	186.52	-369.04	5.77	<0.001
	EB	186.52	-367.04	7.77	0.010
	white	173.87	-343.74	31.07	<0.001
LPJ mass	white	170.28	-336.56	0	0.677
	OU	170.54	-335.07	1.49	0.323
	delta	154.47	-302.94	33.62	<0.001
	BM	140.77	-277.54	59.02	<0.001
	EB	140.77	-275.54	61.02	<0.001
MS mass	white	556.18	-1108.37	0	0.563
	OU	556.93	-1107.86	0.51	0.437
	delta	543.45	-1080.90	27.47	<0.001
	BM	530.34	-1056.68	51.69	<0.001
	EB	530.34	-1054.68	53.69	<0.001

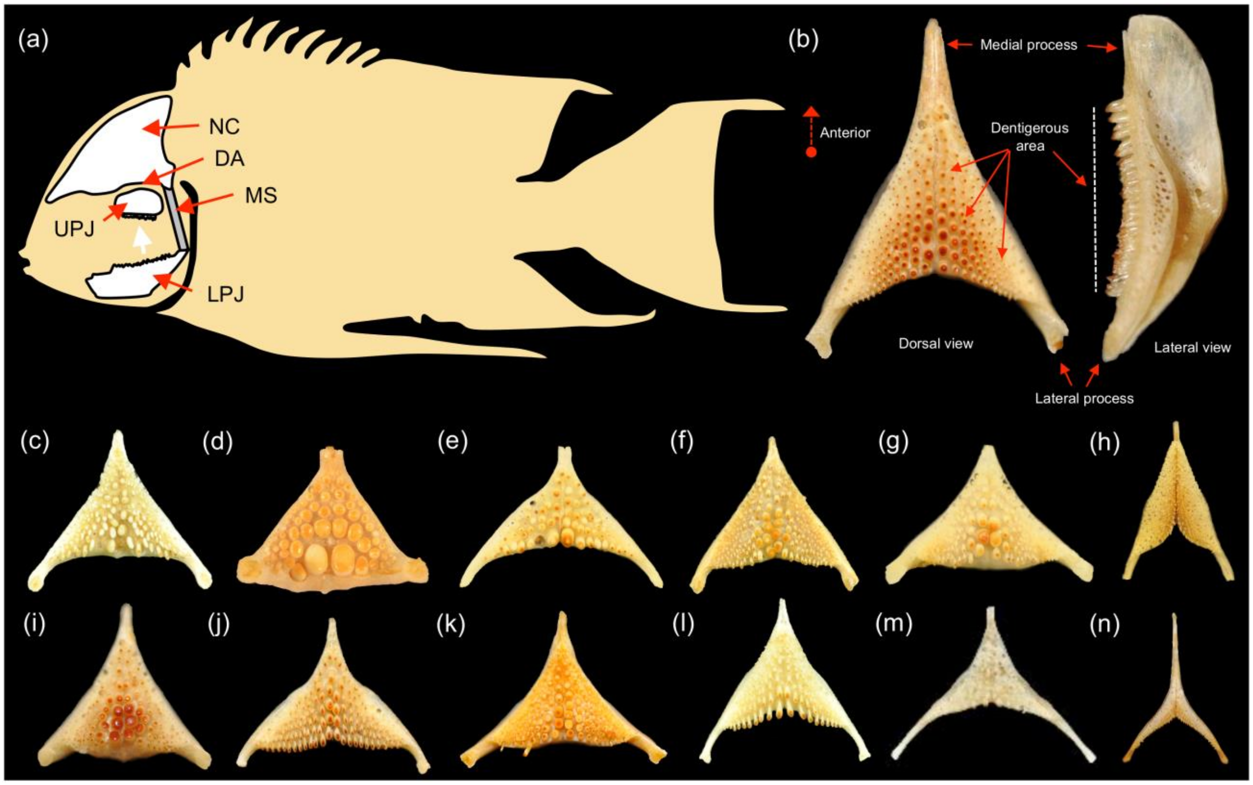

Fig. 1. Diagram depicting the major pharyngognathous elements of a general cichlid (a): neurocranium (NC), upper pharyngeal jaw (joined second and third infrapharyngobranchials; UPJ), diarthrotic articulation (DA), lower pharyngeal jaw (sutured fifth ceratobranchials; LPJ), and muscular sling (fourth levator externus; MS). The white arrow depicts the movement of the LPJ during mastication. Diagram depicting the major features of the lower pharyngeal jaw (b) in dorsal (*Geophagus abalios*) and lateral view (*Parachromis friedrichstahlii*). Representative cichlid LPJ diversity: (c) *Astronotus ocellatus*, (d) *Crenicichla minuano*, (e) *Crenicichla reticulata*, (f) *Mayaheros urophthalmus*, (g) *Australoheros scitulus*, (h) *Cichla intermedia*, (i) *Thorichthys aureum*, (j) *Talamancaheros sieboldi*, (k) *Vieja maculicauda*, (l) *Parachromis dovii*, (m) *Crenicichla celidochilus*, and (n) *Chaetobranchus flavescens*. Images are not to scale.

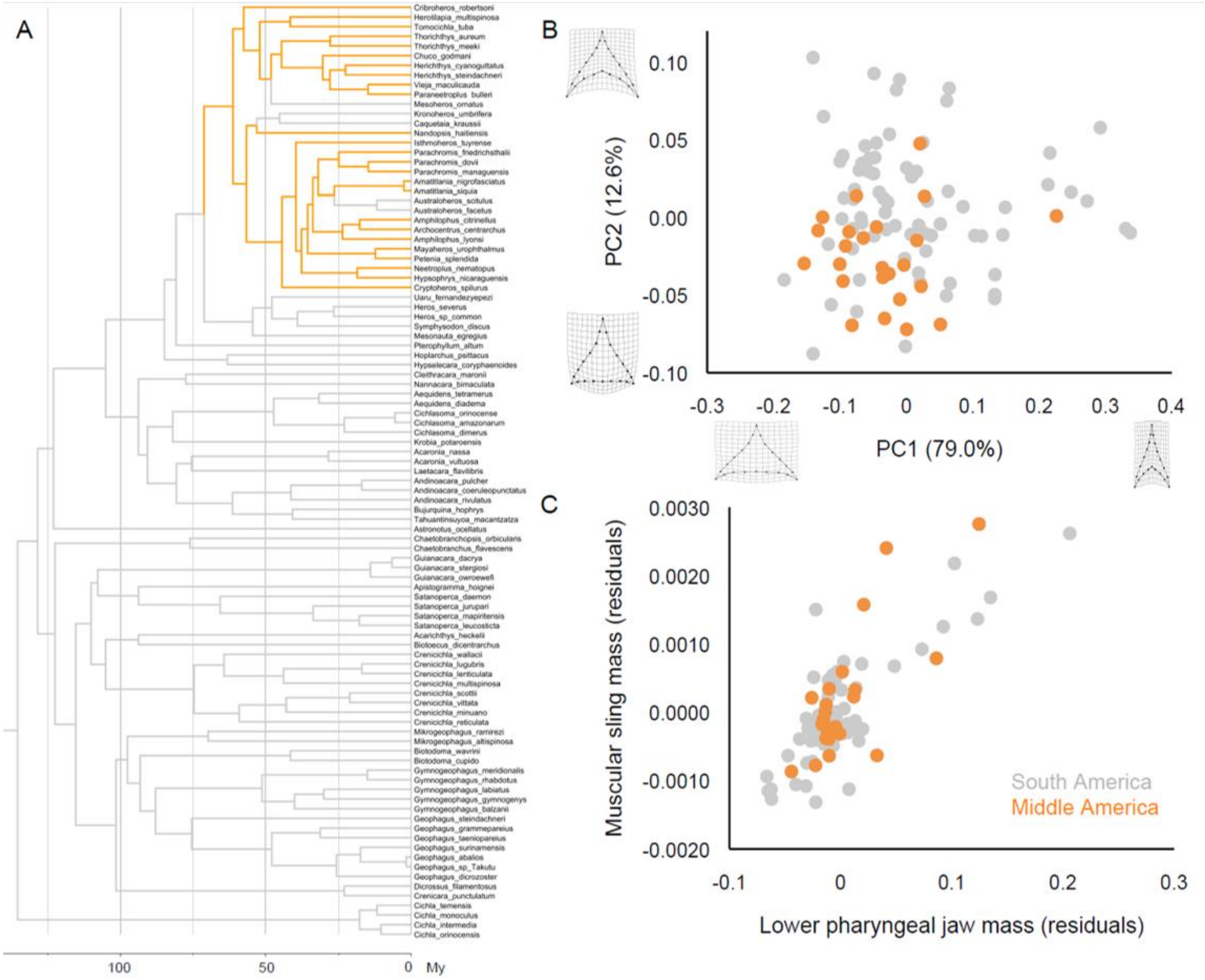

Fig. 2. Phylogeny (*A*) and pharyngeal jaw-associated traits used in the study: shape diversity of the lower pharyngeal jaw (LPJ; *B*) and the size-relative masses of the LPJ and muscular sling (MS; *C*) among 97 Neotropical cichlid species. Wireframes depict the shapes associated with the extremes of each axis. Each point represents a species mean and are color-coded based on geographic origin.

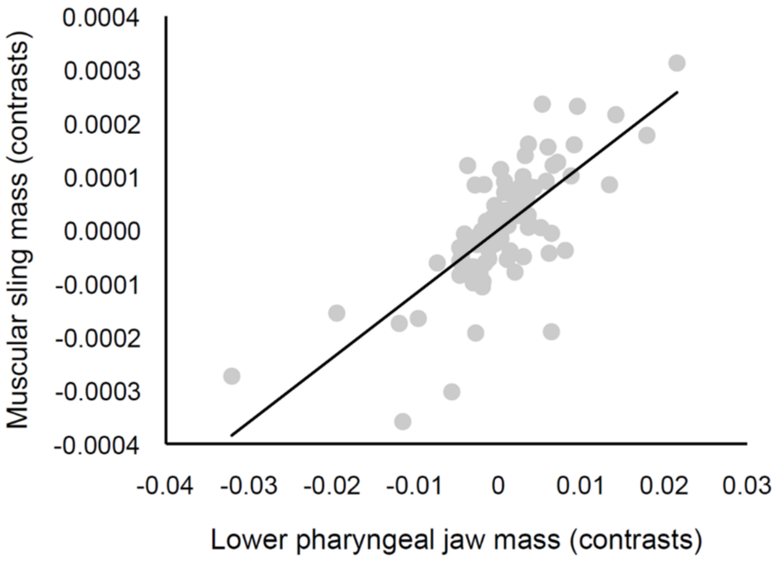

Fig. 3. The correlated evolution of size-relative masses of the lower pharyngeal jaw and muscular sling across the Neotropical cichlid phylogeny.

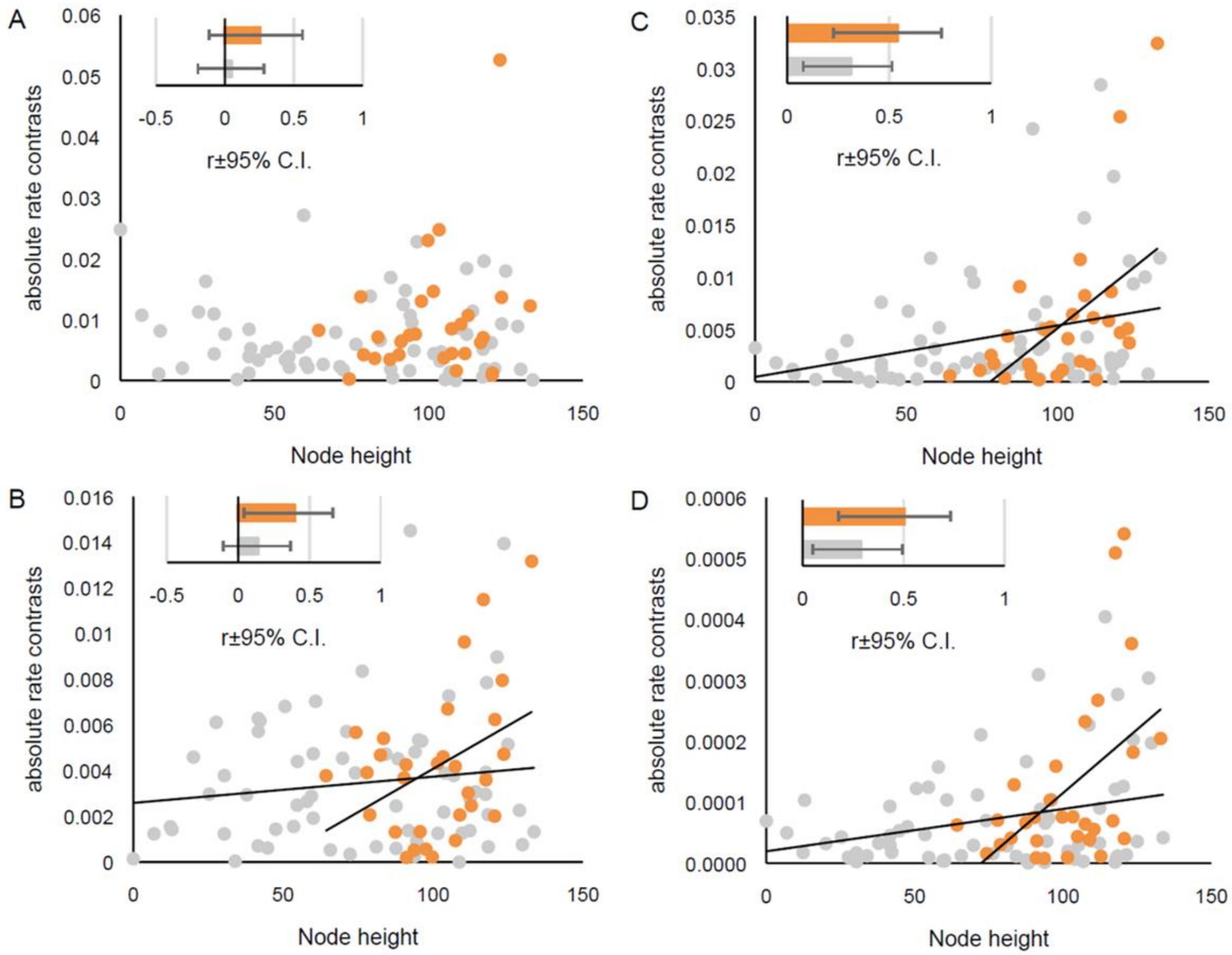

Fig. 4. Relationships between the absolute rate contrasts of lower pharyngeal jaw (LPJ) shape (PC1, *A*; PC2, *B*), size-relative LPJ mass (*C*), and size-relative muscular sling (MS) mass (d) as a function of the ages of the nodes at which they were calculated. Best-fit lines depict significant relationships. Color codes denote nodes associated with South (gray) and Middle (orange) American clades. Effect sizes (r±95% C.I.) for each test are inset.

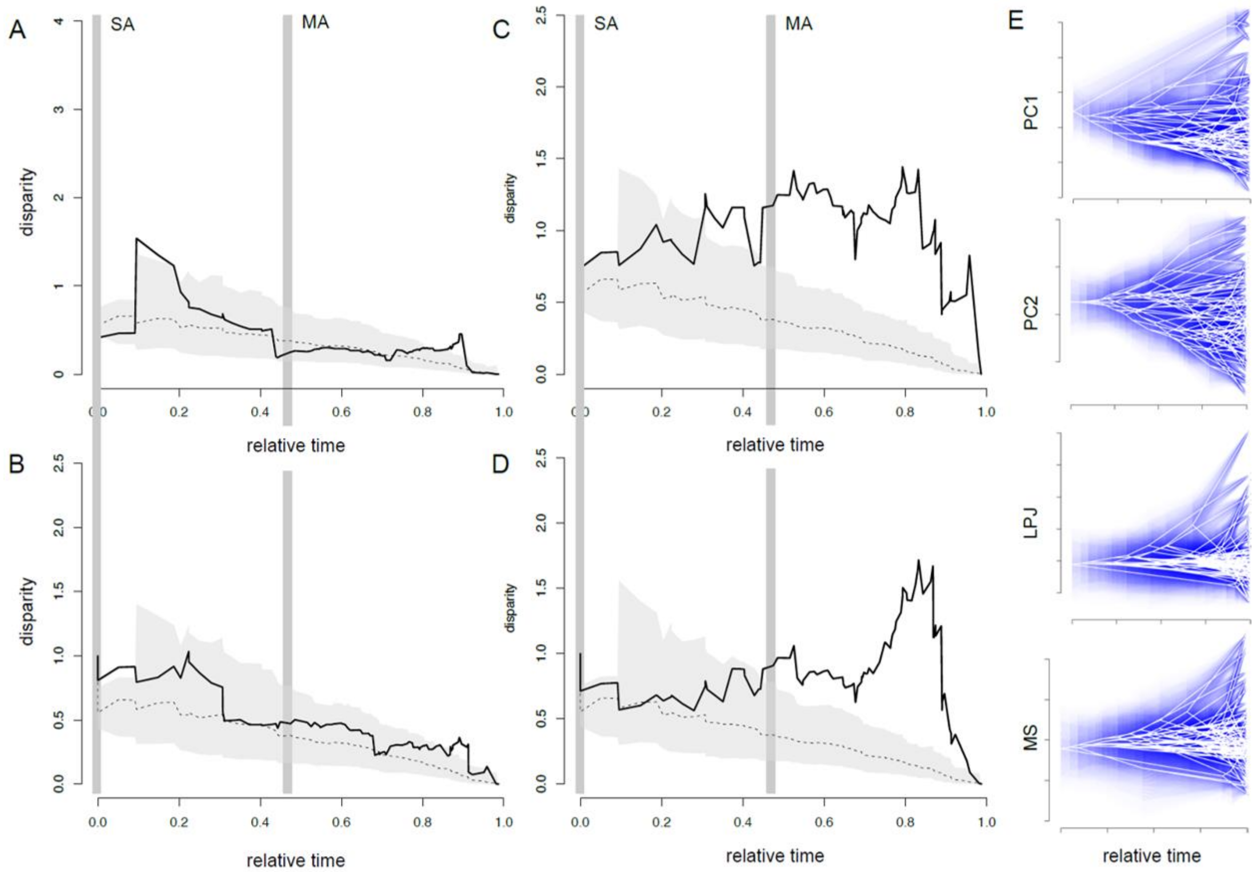

Fig. 5. Disparity-through-time analyses for pharyngeal jaw associated traits across the Neotropical cichlid phylogeny: lower pharyngeal jaw (LPJ) shape (PC1, *A*; PC2, *B*), size-relative LPJ mass (*C*), and size-relative muscular sling (MS) mass (*D*). The approximate point in relative time that corresponds to the colonization of South and Middle America are denoted by the vertical lines. Inset are the inferred evolutionary histories of each trait (*E*).

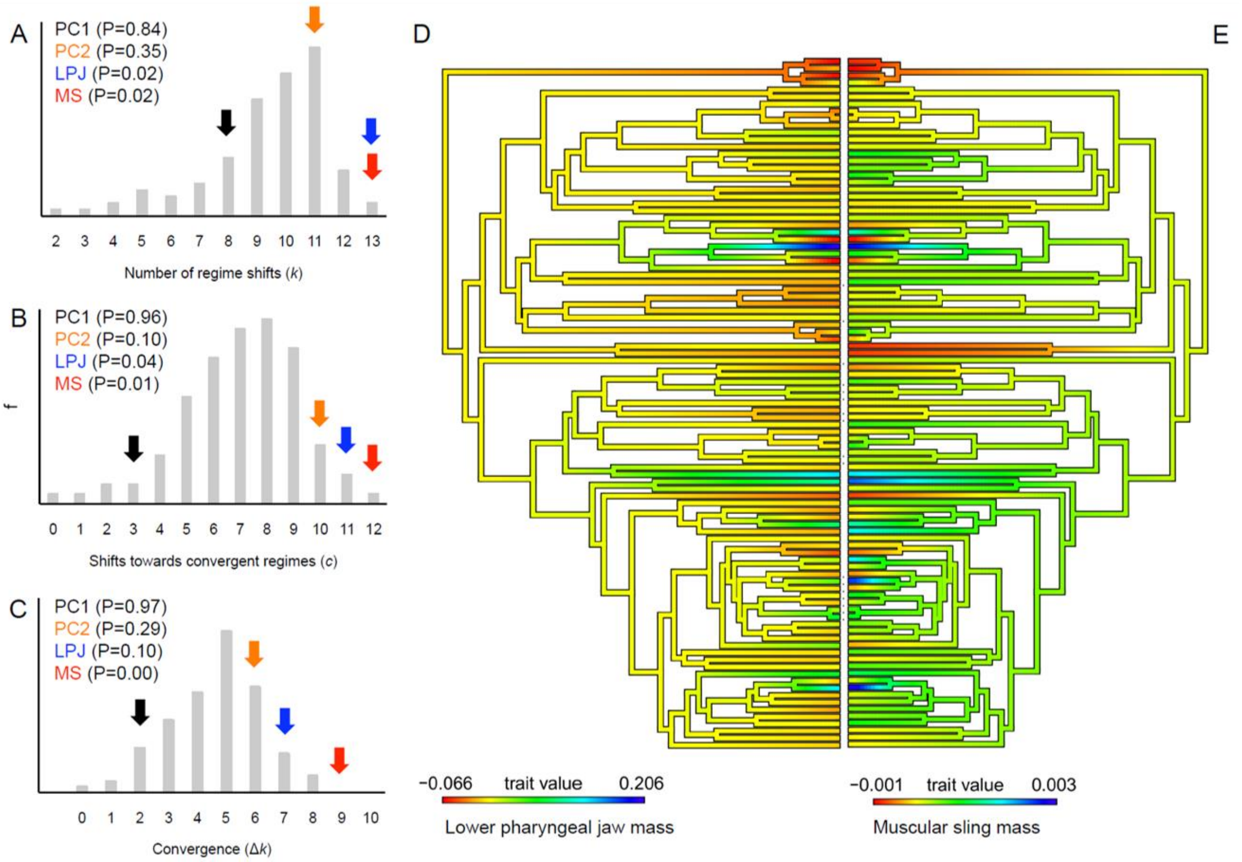

Fig. 6. Evolutionary history of convergence in pharyngeal jaw-associated traits across the Neotropical cichlid phylogeny: lower pharyngeal jaw (LPJ) shape and size-relative masses of the LPJ and muscular sling (MS). Relative parameter estimates from observed data relative to null distributions from 100 data sets simulated under BM: the number of regime shifts (*A*), the number of shifts towards convergent regimes (*B*), and the degree of convergence (*C*). The p-values denote the probability of sampling the observed parameters from the simulated null distribution. Size-relative masses of the LPJ (*D*) and MS (*E*) mapped across the Neotropical cichlid phylogeny.

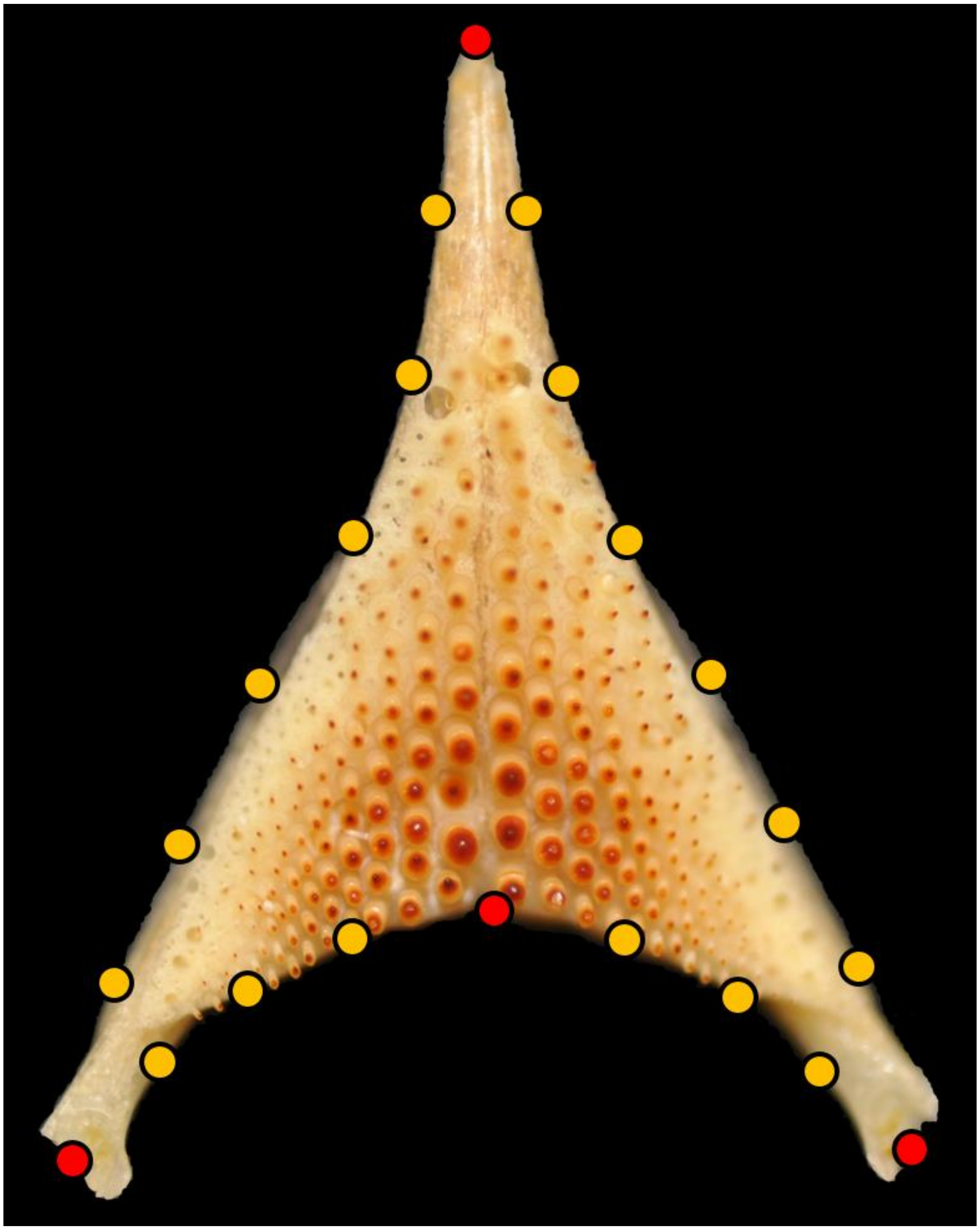

Figure S1. Landmark scheme used to quantify lower pharyngeal jaw shape: homologous landmarks (red dots) and sliding landmarks (yellow dots).

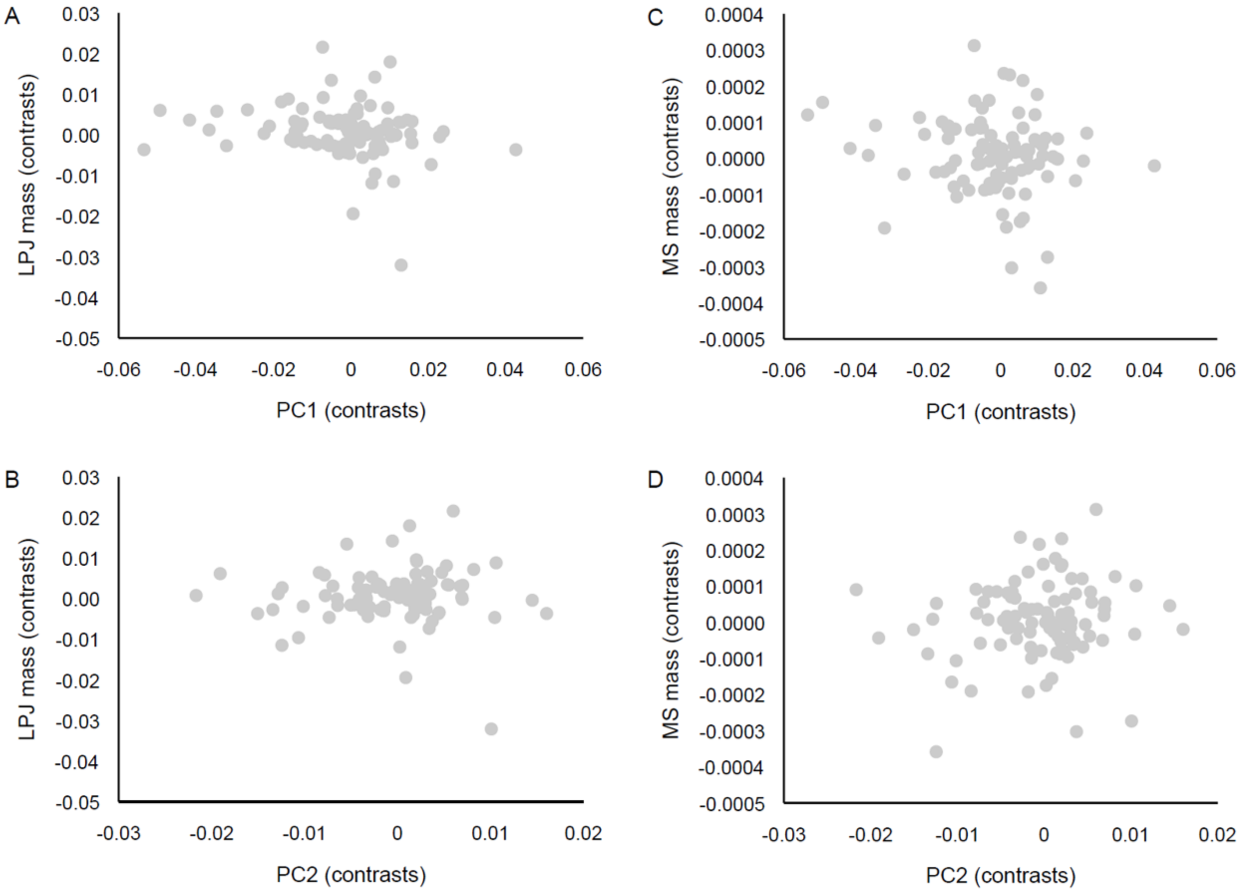

Figure S2. Relationships among measures of lower pharyngeal jaw (LPJ) shape (PC1 and PC2) and the size-relative masses of the LPJ and muscular sling (MS). Values are phylogenetically independent contrasts.

Chapter 3: Body and pharyngeal jaw shape evolution are correlated across the Neotropical cichlid phylogeny

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Abstract

The rise of ecological roles and their associated adaptations are often unevenly distributed across clades and through time. Such disparities are hallmarks of adaptive radiations. We investigate the patterns of body and pharyngeal jaw shape diversification across the Neotropical cichlid phylogeny and the sequence of their evolution through time, and discuss these patterns in the context of adaptive radiation and the stages model of adaptive radiation. Body shapes were more clade-specific than pharyngeal jaw shapes; however, both the Heroini and Geophagini exhibited unique morphologies. Body and pharyngeal jaw shape evolution were correlated among the cichlines; however, this relationship is driven by the Heroini, which was the only tribe that exhibited correlated body and pharyngeal jaw shape evolution. Additionally, body shapes diversified prior to pharyngeal jaw shapes among the Geophagini, but may have diversified simultaneously in other clades. These results suggest that the major clades, the Heroini and Geophagini, have explored different evolutionary and ecological opportunities throughout their histories. Within the Geophagini, body shape diversification may reflect habitat-associated adaptation, whereas pharyngeal jaw shape diversification reflects trophic adaptations. Within the Heroini, habitat- and trophic-associated adaptations may have been coupled, perhaps in response to their colonization of Middle America. Patterns of body and pharyngeal jaw evolution among Neotropical cichlids varies among clades and through time.

Introduction

Adaptive radiation is the rise of ecological roles and their associated adaptations (Simpson 1953; Schluter 2000; Givnish 2015). Adaptive radiations are elicited by ecological opportunities associated with colonization of novel environments or the evolution of key innovations that facilitate movement into novel regions of the adaptive landscape (Schluter 2000; Losos 2010). Adaptive radiations are hypothesized to have generated much of the morphological, functional, ecological, and species diversity on Earth (Simpson 1953; Schluter 2000). Darwin's finches on the Galapagos archipelago (Lack 1947), anole lizards on the Caribbean islands (Losos 2009), and cichlid fishes in the East African Great Lakes (Danley & Kocher 2001; Kocher 2004) are prominent examples of extant adaptive radiations. Adaptive radiation may be constrained by diversity-dependence or ecological regulation resulting from reduced availability of ecological niches (Rabosky 2009; Losos 2010). Additionally, adaptive radiation requires some degree of geographic and ecological cohesion such that adaptive opportunities are shared (Glor 2010). Thus, many models of adaptive radiation are somewhat limited in geographic breadth (i.e., confined to islands or lakes). However, adaptive radiation has recently been invoked to explain lineages that span multiple continents (López-Fernández *et al*. 2013).

Cichlid fishes represent a classic example of adaptive radiation due to their functional, morphological, and behavioral diversity, coupled with their rapid proliferation (Burress 2015; Seehausen 2015). There has been considerable research on adaptive radiations of cichlid fishes among the East African Great Lakes (i.e., Lakes Malawi, Tanganyika, and Victoria), and more recently in the Neotropics (López-Fernández *et al*. 2013; Arbour & López-Fernández 2013; Burress 2016). Adaptive radiation may occur as a sequence of stages such that habitat- and trophic-associated adaptations are temporally discrete (Streelman & Danley 2003; Kocher 2004). Thus, diversification and adaptation to available ecological niches is based on different adaptive traits or trait complexes throughout the process of adaptive radiation (Kocher 2004; Streelman & Danley 2003). The stages model was initially proposed for cichlid fishes (Danley & Kocher 2001 and later generalized to vertebrates (Streelman & Danley 2003).

Extensive diversification of body and pharyngeal jaw morphology among cichlids reflects their adaptation to heterogeneous environmental conditions throughout their evolutionary history (reviewed in Burress 2015). Body morphology is often best predicted by trophic niche (López-Fernández *et al*. 2013; Clabaut *et al*. 2007; Muschick *et al*. 2014); however, body morphology may represent multiple types of diversification and/or specialization. Head shape is presumably a predominately trophic trait, whereas trunk morphology responds mainly to selection pressures exerted by habitat (Kocher 2004; Muschick *et al*. 2014). For example, body depth is often associated with macrohabitat specialization, such as along the benthic-pelagic axis, in cichlids in Lake Malawi (Hulsey *et al*. 2013) and Nicaraguan crater lakes (Elmer *et al*. 2010). Head shape (i.e., craniofacial morphology) is associated with feeding, including what and how prey are consumed (Gerking 1994). Craniofacial diversification associated with functionally different foraging strategies (i.e., suction versus biting) was important in the adaptive radiation of African cichlids (Hulsey *et al*. 2010) and American cichlids (López-Fernández *et al*. 2013).

Alternatively, the pharyngeal apparatus may serve as a key innovation that facilitated the utilization of novel regions of the adaptive landscape by cichlid fishes (among others; Burress 2015; Wainwright *et al*. 2012), particularly the exploitation of hard-shelled organisms (i.e., molluscs), difficult to digest prey (i.e., algae), and piscivory (Burress 2016). Pharyngeal jaws may reduce handling time via generation of crushing force (Hulsey *et al*. 2005), and they may promote assimilation via mastication and subsequent rupturing of algae cells (Burress 2015; 2016). The pharyngeal apparatus may have provided a functional release of the oral jaws from demands associated with prey processing (Liem 1973; Wainwright *et al*. 2012), subsequently allowing craniofacial diversification associated with prey acquisition. However, pharyngeal jaws may also impose functional constraints, particularly via gape among piscivores, such that exploitation of some resource may be compromised (McGee *et al*. 2015; Burress *et al*. 2015). It is unknown if adaptation of body and pharyngeal morphology occurred sequentially (i.e., habitat-followed by trophic-adaptation) or the degree to which their diversification is evolutionarily and ecologically correlated.

Neotropical cichlids (Cichlinae) are a monophyletic group, sister to African cichlids (McMahan *et al*. 2013; Friedman *et al*. 2013). Cichlines inhabit North, Central, and South American lakes and rivers (López-Fernández *et al*. 2010), and include 540 valid species (Eschmeyer & Fong 2015). Most diversity is distributed among three tribes: the Heroini (~150 spp.), Geophagini (~250 spp.), and Cichlasomatini (~90 spp.; López-Fernández *et al*. 2010). The Heroini and Geophagini have been hypothesized to represent adaptive radiations (McMahan *et al*. 2013; López-Fernández *et al*. 2010; Říčan *et al*. 2016). There is considerable variation in body shape, body size, functional adaptations, and pharyngeal jaws exhibited by the tribes (López-Fernández *et al*., 2013; Steele & López-Fernández 2014; Arbour & López-Fernández 2014; Burress 2016).

We investigated body and pharyngeal jaw shape evolution among 97 species of Neotropical cichlid fishes. Our objectives were twofold. First, we test if body and lower pharyngeal jaw (LPJ) shape evolution are correlated across the Neotropical cichlid phylogeny. Second, we test if the stages model of adaptive radiation, which postulates that habitat- and trophic-associated adaptation occurs sequentially (i.e., Danley & Kocher 2001; Streelman & Danley 2003), is generalizable to the temporal pattern of body and LPJ shape evolution during the evolutionary history of Neotropical cichlids. We assessed these patterns of shape evolution across cichlines as well as among major clades because previous work has indicated that the major clades exhibit different evolutionary histories and patterns of phenotypic evolution (McMahan *et al*. 2013; López-Fernández *et al*. 2013; Arbour & López-Fernández 2014; Burress 2016).

Methods

Shape analysis

We quantified shape on photographs of specimens representing 97 cichlid species accessioned in the Auburn University Museum of Natural History (AUM) using geometric morphometrics (supplementary file 1, Table S1). These species are well distributed across the Neotropical cichlid phylogeny and are representative of their morphological diversity (López-Fernández *et al*. 2010; 2013). To quantify biologically important shape variation we used principal component analysis of 16 homologous landmarks on the lateral view of the body (supplementary file 1, Fig. S1) and a combination of seven homologous and 10 sliding landmarks on the dorsal view of the lower pharyngeal jaw (LPJ; supplementary file 1, Fig. S2). Sliding landmarks are not associated with a homologous structure, but are used to quantify the curvature between two homologous landmarks. All sliding landmarks were evenly spaced between homologous landmarks (Fig. S2). Photos were consolidated and landmarked using tpsUtil (Rohlf 2004) and tpsDIG2 (2006), respectively. Landmarks were then superimposed, aligned, and (if present) sliding landmarks were slid using tpsRelw (2007). We then extracted these aligned coordinates, calculated species means, and used the means as a data matrix for phylogenetic principal component analysis (pPCA). We used the phyl.pca command in the phytools package (Revell 2009) to generate principal component scores that are free of phylogenetic covariance (Polly *et al*. 2013). To account for phylogenetic uncertainty in estimating pPCA scores, we generated pPCA scores using 1000 trees from the posterior distribution of a BEAST analysis (see below). We then used the mean pPCA scores for each species in the phylogenetic comparative methods (supplementary file 1, Table S2). Although pPCA takes into account the non-independence of samples, there are some limitations. For example, some of the resulting axes can be correlated and interpretation of the axes can be difficult in some cases (Polly *et al*. 2013). Additionally, using only the first few pPC (or PC) scores as univariate data creates some bias towards the major axes of shape variation and thus may exclude additional dimensions of complex multivariate traits such as shape (Uyeda *et al*. 2015). Although the axes of shape variation may be orthogonal, it is unlikely that the aspects of shape that they depict are functionally or evolutionarily independent. To account for this complex multi-dimensional nature of shape, we utilized exclusively multivariate methods (see below for analysis-specific details).

To evaluate the direction and magnitude of shape changes associated with Neotropical cichlid phenotypic diversification, we mapped the phylogeny (see below) onto the phylogenetic principal components (i.e., phylomorphospace; Siddlauskas 2008) using Mesquite v2.75 (Maddison & Maddison 2011). During this procedure, ancestral node values are estimated using weighted squared-change parsimony (Maddison 1991; Revell *et al*. 2007). The resulting phylomorphospace depicts both the magnitude and direction of shape changes along each branch.

Phylogenetic and divergence time analysis

We estimated the phylogeny and divergence times using BEAST (Drummond *et al*. 2012). We modified the BEAST input file originally used by López-Fernández *et al*. (2010) (doi:10.5061/dryad.34621), which was based on the data and phylogeny published in López-Fernández *et al*. (2013). López-Fernández *et al*. (2010) included five loci (cytb, 16S, ND4, RAG2, and S7 intron 1) for 166 species of cichlids, primarily focusing on the diversity of the Neotropical Cichlinae. We made two notable modifications to the input file. First, we added 12 additional species for which we were able to collect morphometric data that were not in the previous López-Fernández *et al*. (2010) analysis, using sequences from other published studies (supplementary file 1, Table S3; Farias *et al*. 2001; Pereya & Garcia 2008; Smith *et al*. 2008; Piálek *et al*. 2012; Říčan *et al*. 2013). We aligned these sequences to the López-Fernández *et al*. alignment using the ClustalW alignment algorithm implemented in Geneious 6.1.8 (Larkin *et al*. 2007; Kearse *et al*. 2012). Second, López-Fernández *et al*. (2010) defined a number of clades as monophyletic, but to better assess the phylogenetic relationships given the additional taxa, we removed clade constraints for monophyly except for four strongly-supported clades, including the ingroup (Cichlinae) and the nodes with fossil constraints (Cichlasomatini, Heroini, and *Gymnogeophagus*); this necessitated increasing chain length to reach convergence, so each chain was run for 200 million generations, logging every 20,000 generations. Other settings were the same as in López-Fernández *et al*. (2010), including speciation priors, models of sequence evolution across gene partitions, divergence time calibrations, and setting of the uncorrelated relaxed clock model (Drummond *et al*. 2006). Analysis was performed using BEAST v1.8.1 on the Auburn University CASIC HPC cluster (Drummond *et al*. 2012). We ran three independent chains. Convergence (ESS for parameters > 200) was assessed with Tracer v1.5, and the maximum clade credibility (MCC) tree (supplementary file 2) was computed from the posterior distribution of trees by combining the results of all three chains and excluding burn-in using LogCombiner v1.7.5 and TreeAnnotator v1.7.5. Because the MCC tree provides only a point estimate of the phylogeny, we also extracted a subset of 1000 equally-spaced trees from the posterior distribution to account for phylogenetic uncertainty in comparative phylogenetic analyses.

Model fitting

We fitted multivariate evolutionary models for body and LPJ shape using the mvmorph package (Clavel *et al*. 2015). Fitted models include Brownian motion (BM), early burst (EB), and Ornstein-Uhlenbeck (OU). We fit these models on the distribution of 1,000 trees to account for phylogenetic uncertainty.

Phenotypic correlations

We calculated phylogenetically independent contrasts using the pic function in the geiger package (Harmon *et al*. 2008). We extended the univariate calculation of contrasts to multivariate characters, using the formulation of multivariate contrasts from McPeek *et al*. (2008). We performed this analysis repeatedly on the distribution of 1,000 trees to account for phylogenetic uncertainty. We then tested for correlations between body and LPJ multivariate phylogenetically independent contrasts using a Pearson’s product-moment correlation with the cor.test function and summarized these results across the distribution of 1000 trees to account for phylogenetic uncertainty.

Stages model of evolution

The stages model of evolution posits that habitat-associated adaptation precedes trophic-associated adaptation during the course of adaptive radiation (Streelman & Danley 2003). To test if this model extends to body and LPJ shape evolution, we used the divergence order test (DOT; Ackerly *et al*. 2006). This method compares the average node age in the phylogeny weighted by the absolute value of contrasts of a trait at each node. This weighted average age provides an indication of whether the divergence in a trait tends to occur relatively early or late in a clade's history. A difference in weighted average ages between traits (D) provides support for a difference in the timing of divergence of the traits. Statistical significance of $D > 0$ is assessed by bootstrapping maximum-likelihood reconstructed ancestral states. We performed this analysis repeatedly on the distribution of 1,000 trees, each with 1,000 bootstrap replicates. We then assessed the number of trees for which the D statistic was significantly greater than zero (at $p < 0.05$). We modified the original scripts of Ackerly *et al*. (2006) to use the reconstruct function (implemented in ape; Paradis et al. 2004) for maximum-likelihood ancestral state reconstruction instead of ANCML (Schluter *et al*. 1997). The reconstruct function uses exact matrix computations to reconstruct ancestral states (Royer-Carenzi & Didier 2016). We also extended the univariate calculation of contrasts originally implemented for the DOT to multivariate characters, using the formulation of multivariate contrasts from McPeek *et al*. (2008). Scripts are available at https://github.com/miltontan/divergence_order_test (Accessed 2016 May 10).

Results

Body shape

pPC1 explained 34.6% of the total shape variation among species and separated species based mainly on body depth. Elongate bodies (e.g., *Crenicichla* and *Cichla*) were associated with negative values, and deep-bodied or discoid shaped bodies (e.g., *Symphysodon* and *Pterophyllum*) were associated with positive values (Fig. 1*a*). pPC2 explained 14.0% of the variation in shape among species and separated species based on jaw orientation, snout length, and relative eye position (e.g., craniofacial configuration). Ventrally positioned jaws and eyes positioned high on the head were associated with positive pPC2 values (e.g., *Geophagus*, *Satanoperca*, *Guianacara*, and *Gymnogeophagus*), and terminally positioned jaws with laterally positioned eyes were associated with negative pPC2 values (e.g., *Crenicichla*, *Cichla*, and *Astronotus*). The Cichlasomatini have limited body shape diversity, occupying only areas of morphospace associated with near-zero values for both pPCs (Fig. 1*a*). The Heroini encompass all morphospace occupied by the Cichlasomatini, plus unique regions of morphospace associated with deep-bodies (i.e., positive pPC1 values). The Geophagini overlap the Cichlasomatini and Heroini, but includes a unique region of morphospace associated with negative pPC1 and pPC2 values. Phylomorphospace of body shape (Fig. 1*b*) depicts the direction and magnitude of shape changes along the branches of the phylogeny. There are many instances of convergence among the major tribes as well as clades that are characterized by major changes in body shape (e.g., elongate *Crenicichla* and discoid *Pterophyllum* and *Symphysodon*; Fig. 1*b*).

Pharyngeal jaw shape

pPC1 explained 48.2% of the shape variation among LPJs (Fig. 1*c*). Positive pPC1 values were associated with widely-spaced lateral processes and long medial process (e.g., *Crenicichla*, *Australoheros*, *Amatitlania*, *Herotilapia*, *Hysophrys*, and *Tomicichla*), and negative values were associated with narrowly spaced lateral processes and short medial process (e.g., *Cichla*, *Chaetobranchus*, *Petenia*, and *Satanoperca*). pPC2 explained 18.6% of the variation in LPJ shape (Fig. 1*c*). Positive pPC2 values were associated with short lateral processes and large dentigerous areas (e.g., *Cichla*, *Crenicichla minuano*, *Thorichthys*, *Herichthys*, and *Hysophrys*), and negative values were associated with long lateral processes and small dentigerous areas (e.g., *Biotoecus*, *Chaetobranchus flavescens*, *Crenicichla reticulata*, and *Apistogramma*). Phylomorphospace of LPJ shape depicts the direction and magnitude of shape changes along branches of the phylogeny. There are many instances of convergence among the major tribes as well as clades that are characterized by major changes in LPJ shape (e.g., reduced LPJ morphologies exhibited by *Cichla*; Fig. 1*d*).

Model fitting

Patterns of body and LPJ shape evolution generally fit different models of evolution – early burst (EB) and OU models, respectively (Table 1). Body shape evolution fit an EB model best across 744 of the 1000 trees, whereas LPJ shape evolution best fit an OU model across all 1000 trees (Table 1). Among the Geophagini, body shape equally fit EB and OU models, whereas LPJ shape evolution best fit an OU model based on AIC; however, only in 458 of the 1000 trees (Table 1). Among the Heroini, body and LPJ shape evolution best fit an OU model of evolution across 801 and 998 of the 1000 trees, respectively (Table 1). Among the Cichlasomatini, body and LPJ shape evolution best fit a BM model of evolution across 776 and 999 of the 1000 trees, respectively (Table 1).

Phenotypic correlations

Multivariate phylogenetically independent contrasts of body and LPJ shape were significantly correlated among cichlines across all 1000 trees (Table 2); however, this pattern was largely due to body and LPJ shape being correlated among the Heroini across 994 of the 1000 trees (Table 2; Fig. 2). In contrast, body and LPJ shape were correlated among the Geophagini across only 274 of the 1000 trees. Body and LPJ shape were not correlated among the Cichlasomatini across any of the 1000 trees (Table 2; Fig. 2).

Stages model of evolution

Across the Cichlinae, LPJ shape diversified significantly after body shape on 160 of the 1000 trees, indicating that the timing of diversification was not statistically different from zero (*P*=0.167; Table 3). Within the Cichlasomatini, the traits diversified at different times across 288 of the 1000 trees, which was not statistically significant (*P*=0.16; Table 3). Within the Geophagini, LPJ shape diversified after body shape across all 1000 trees, which was statistically significant (*P*<0.001; Table 3). Lastly, within the Heroini, LPJ shape diversified after body shape across 18 of the 1000 trees, which was not statistically significant (*P*=0.227; Table 3).

Discussion

Correlated and uncorrelated trait evolution

Body and pharyngeal morphology exhibited different evolutionary patterns among clades such that the Geophagini and Heroini occupy largely different regions of body shape morphospace associated with elongated and discoid body shapes, respectively, whereas LPJ shape exhibited more overlap among the three clades. Indeed, body and LPJ shape evolution were uncorrelated within the Geophagini and Cichlasomatini. Although body and pharyngeal shape evolution were correlated among Neotropical cichlids, this pattern was driven by one major clade, the Heroini, which exhibited correlated body and LPJ shape evolution. The major axes of body shape, body depth and mouth orientation, are likely associated with specialization along the benthic-to-pelagic habitat axis (Winemiller et al. 1995; Burress 2015). The benthic-to-pelagic habitat axis is a major dimension of ecological diversification in lake-dwelling cichlids in Africa (Hulsey et al. 2013; Cooper et al. 2010; Muschick et al. 2012; Machado-Schiaffino et al. 2015) as well as Middle America (Kusche et al. 2014; Elmer et al. 2014), and fishes in general (reviewed in Seehausen & Wagner 2014). This is likely the case in Neotropical river-dwelling cichlids, where major lineages have specialized in benthivory and forage via winnowing prey items from mouthfuls of substrate (López-Fernández et al. 2013; 2014), whereas others (e.g., zooplanktivores and piscivores) inhabit or forage in the pelagic zone (Winemiller et al. 1995; López-Fernández et al. 2012; Burress et al. 2013a).

In contrast, the major axes of LPJ shape, the orientation and length of the medial and lateral processes, are associated with the soft-bodied to hard-shelled prey axis and relative omnivory among Neotropical cichlids (Burress 2016). These are major trophic dimensions along which cichlids have diversified, including throughout African lakes (Muschick et al. 2012; Kidd et al. 2006), Middle American lakes (Elmer et al. 2010), as well as African and Neotropical rivers (Winemiller et al. 1995). Omnivory is a relatively opportunistic trophic role and is often considered an adaptive response to heterogeneous and unpredictable resources (Jepsen & Winemiller 2002 and references therein) and is likely an ancestral characteristic of Neotropica cichlids (Burress 2016). In contrast, durophagy is considered a more specialized, often derived, trophic role among Neotropical cichlids (Burress 2016; Říčan et al. 2016); however, both are similar in that they involve the exploitation of relatively nutrient-poor resources that are physiologically and mechanically demanding for fishes to processes and assimilate (Burress et al. 2016 and references therein).

Body and LPJ shape evolution and their associated ecological roles are uncorrelated among the Geophagini and Cichlasomatini. This pattern may be because the Geophagini diversified early along the benthic-to-pelagic habitat axis when piscivorous *Crenicichla* (Burress et al. 2015; Burress 2016) arose from a clade containing primarily benthic-associated species that forage by sifting prey from mouthfuls of substrate (López-Fernández et al. 2013; 2014). Also, despite some exceptions such as the aforementioned *Crenicichla* (Burress et al. 2013a,b), the Geophagini are also largely omnivorous (Winemiller et al. 1995; López-Fernández et al. 2010) and likely colonized a novel adaptive peak early in their diversification associated with benthivory (López-Fernández et al. 2013; Arbour & López-Fernández 2013) and thus have not explored other adaptive opportunities. *Crenicichla* likely further contributed to the uncorrelated body and LPJ shape evolution with the Geophagini due to their considerable pharyngeal jaw and trophic diversification despite highly conserved body shapes (Burress et al. 2013a). In contrast, the Cichlasomatini exhibit much less body and LPJ shape diversity (López-Fernández et al. 2013; Burress 2016) and have likely been precluded from exploring adaptive opportunities such as diversification along the benthic-to-pelagic and soft-bodied to hard-shelled prey axes by the other diverse clades with which they co-occur throughout South America (López-Fernández et al. 2013).

Body and LPJ shape evolution were correlated among the Heroini, which suggests that either habitat- and trophic-associated adaptations were coupled during their evolutionary history or that body shape reflects primarily trophic-associated adaptation rather than habitat-associated adaptation. The Heroini exhibit parallel diversification into a myriad of ecomorphs during and since their colonization of Middle American from South America (Říčan et al. 2016), which may have provided the ecological opportunity necessary to link habitat- and trophic-associated specialization and the associated phenotypic adaptations. Indeed, Arbour & López-Fernández (2014) found that the Heroini may occupy numerous adaptive peaks that correspond to having large jaw muscles and high suction-force potential. Therefore, much of their body shape diversity may represent adaptation of craniofacial features associated with the generation of crushing force, which is likely necessary for some of the trophic roles they have repeatedly evolved involving the exploitation of molluscs, macrocrustaceans, and fruits (Burress 2016 and references therein).

The stages model of adaptive radiation

Adaptive radiation has been hypothesized to occur in temporal stages such that habitat- and trophic-associated adaptations occur in sequential steps (Streelman & Danley 2003). For example, cichlids in Lake Malawi adapted to rocky habitats, then adapted to foraging on specific items associated with rocky surfaces (Danley & Kocher 2001; Kocher 2004). This model has also been tested in Lake Tanganyika, where the opposite pattern was observed (trophic traits diversified before habitat-associated traits; Muschick et al. 2014).

Here, we tested if the stages model extends to body and pharyngeal jaw evolution among Neotropical cichlids. We found that the major axes of body shape diversified significantly earlier in time than major axes of LPJ shape within only the Geophagini. In contrast, body and LPJ shape evolution was not sequential across the Cichlinae or within the Cichlasomatini or the Heroini. Thus, at these scales we cannot reject the possibility that these traits diversified simultaneously. This discrepancy among clades may be due to a combination of the old age of the Geophagini, coupled with the early evolution of benthivory and its associated body shapes, which later diversified further into sifting and non-sifting clades (López-Fernández et al. 2013; Arbour & López-Fernández 2013). The sifting species, particularly, have highly conserved LPJ morphology, which is likely associated with the facilitation of winnowing minute prey items from mouthfuls of substrate while foraging (Burress 2016). Thus, the early and late diversification of body and LPJ shape, respectively, may coincide with the early evolution of benthivory and more recent evolution of sifting and non-sifting benthivores among the Geophagini.

Body and LPJ shape may represent a mixture of habitat- and trophic-adaptations. For example, the relative width of the lateral processes (e.g., pPC1) may be constrained by body shape such that species with laterally compressed bodies (e.g., *Pterophyllum* and *Geophagus*) tend to have LPJ lateral processes that are narrowly-spaced, whereas species with thick- or tubular-shaped bodies (e.g., *Vieja* and *Crenicichla*) tend to have widely-spaced LPJ lateral processes (Burress 2016). Therefore, identifying traits that represent habitat or trophic adaptations, and not a combination of both, may limit tests of the stages model of adaptive radiation. Nevertheless, the major axes of body shape variation used here have well-established correlations with habitat use among cichlids, particularly associated with the benthic-pelagic habitat axis (Elmer et al. 2010; Burress 2015). Furthermore, the major axes of LPJ shape variation reflect variation in degrees of omnivory, piscivory, and specialization along the soft-bodied to hard-shelled prey axis (Burress 2016). Therefore, Neotropical cichlid diversification may not be consistent with the stages model of adaptive radiation (Streelman & Danley 2003), except within the Geophagini. Neotropical cichlids diversified in their body shapes, potentially reflecting specialization along the benthic-to-pelagic habitat axis simultaneously as they diversified in their LPJ shapes, which represents specialization along the soft-body to hard-shelled prey axis. This largely non-staged diversification process is in contrast with African cichlids of Lake Malawi in which habitat diversification occurs prior to trophic diversification (Danley & Kocher 2001; Kocher 2004) and Lake Tanganyika in which trophic diversification occurs prior to habitat diversification (Muschick et al. 2014).

Conclusions

In this study, we examined body and pharyngeal shape diversification among Neotropical cichlids to test whether patterns are clade-specific, if body and LPJ evolution were correlated, and whether body and LPJ diversification occurred in sequential stages. We found that patterns of phenotypic diversification were clade-specific. Within the Geophagini, body and LPJ evolution were uncorrelated, likely because they occurred sequentially through time. In contrast, within the Heroini, body and LPJ evolution were correlated and occurred simultaneously through time. The Geophagini have likely evolved via an early burst of diversification as they colonized an adaptive peak associated with benthivory (López-Fernández et al. 2013), whereas the Heroini exhibit more recent, parallel diversification into a myriad of ecomorphs (Říčan et al. 2016). Therefore, these clades arose via considerably different patterns of diversification that may depict the different evolutionary opportunities afforded by the subsequent colonization of South and Middle America (Arbour & López-Fernández 2016).

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Table 1. Model fit estimates for multivariate (PC1 and PC2) body and pharyngeal jaw shape evolution among Neotropical cichlid fishes. Values depict the mean (±SD) log-likelihood and AIC summarized across the posterior distribution of 1000 trees. ΔAIC represents the difference in AIC relative to the best fit model. *P* represents the proportion of the 1000 trees in which that model was the best fit based on AIC.

Clade/trait	Model	log-likelihood	AIC	ΔAIC	*P*
Cichlinae					
Body shape	BM	460.0±5.7	-909.3±11.4	6.8	0.001
	EB	464.5±4.3	-916.1±8.4	0	0.744
	OU	464.9±7.8	-912.2±15.7	3.9	0.255
LPJ	BM	342.5±6.5	-674.4±12.9	26.5	0.0
	EB	342.7±6.5	-672.5±13.0	28.4	0.0
	OU	359.2±3.2	-700.9±6.4	0	1.0
Geophagini					
Body shape	BM	161.1±18.1	-310.3±36.2	19.6	0.030
	EB	172.4±25.9	-329.9±51.8	0	0.513
	OU	175.6±10.7	-329.9±21.3	0	0.457
LPJ	BM	115.9±23.8	-219.9±47.6	17.1	0.542
	EB	116.0±23.9	-217.2±47.7	17.0	0.0
	OU	133.0±6.4	-244.7±12.8	0	0.458
Heroini					
Body shape	BM	159.3±6.2	-306.6±12.4	7.9	0.138
	EB	159.6±6.6	-304.2±13.2	10.3	0.061
	OU	168.0±2.8	-314.5±5.5	0	0.801
LPJ	BM	122.6±6.6	-233.2±13.3	20.4	0.002
	EB	122.6±6.6	-230.3±13.3	23.3	0.0
	OU	137.6±2.2	-253.6±4.4	0	0.998
Cichlasomatini					
Body shape	BM	84.9±4.3	-153.8±8.5	0	0.776
	EB	85.9±3.4	-150.6±6.9	3.2	0.023
	OU	93.4±3.3	-150.2±6.5	3.6	0.201
LPJ	BM	78.0±2.3	-140.1±4.6	0	0.999
	EB	78.4±2.4	-135.5±4.8	4.6	0.001
	OU	80.1±1.1	-123.6±2.2	16.5	0.0

Table 2. Relationships between body and lower pharyngeal jaw shape evolution across the Neotropical cichlid phylogeny and major clades. Results (mean±SD) are summarized across 1000 trees from the posterior distribution. *P* depicts the proportion of the 1000 trees in which the relationships were statistically significant.

Clade	R^2	*T*	*P*
Cichlinae	0.172±0.032	4.34±0.50	1.0
Geophagini	0.091±0.044	1.77±0.48	0.274
Cichlasomatini	0.0043±0.0072	-0.02±0.24	0.0
Heroini	0.23±0.051	3.12±0.45	0.994

Table 3. Results from the diversification order test (DOT) for the relative divergence times of body and lower pharyngeal jaw shape evolution among Neotropical cichlid fishes. *D* is the mean difference in age of LPJ (pPC1 and pPC2) relative to the body (pPC1 and pPC2). Non-zero is the proportion of the 1000 trees from the posterior distribution in which *D* is significantly different from zero ($P<0.05$). *P* is the probability of the divergence times of body and LPJ shape evolution being zero summarized across the 1000 trees.

Clade	*D* (±SD)	Non-zero	*P*
Cichlinae	1.75±0.85	0.160	0.167
Geophagini	8.89±1.24	1.0	<0.001
Cichlasomatini	2.66±1.61	0.288	0.160
Heroini	1.05±0.53	0.018	0.227

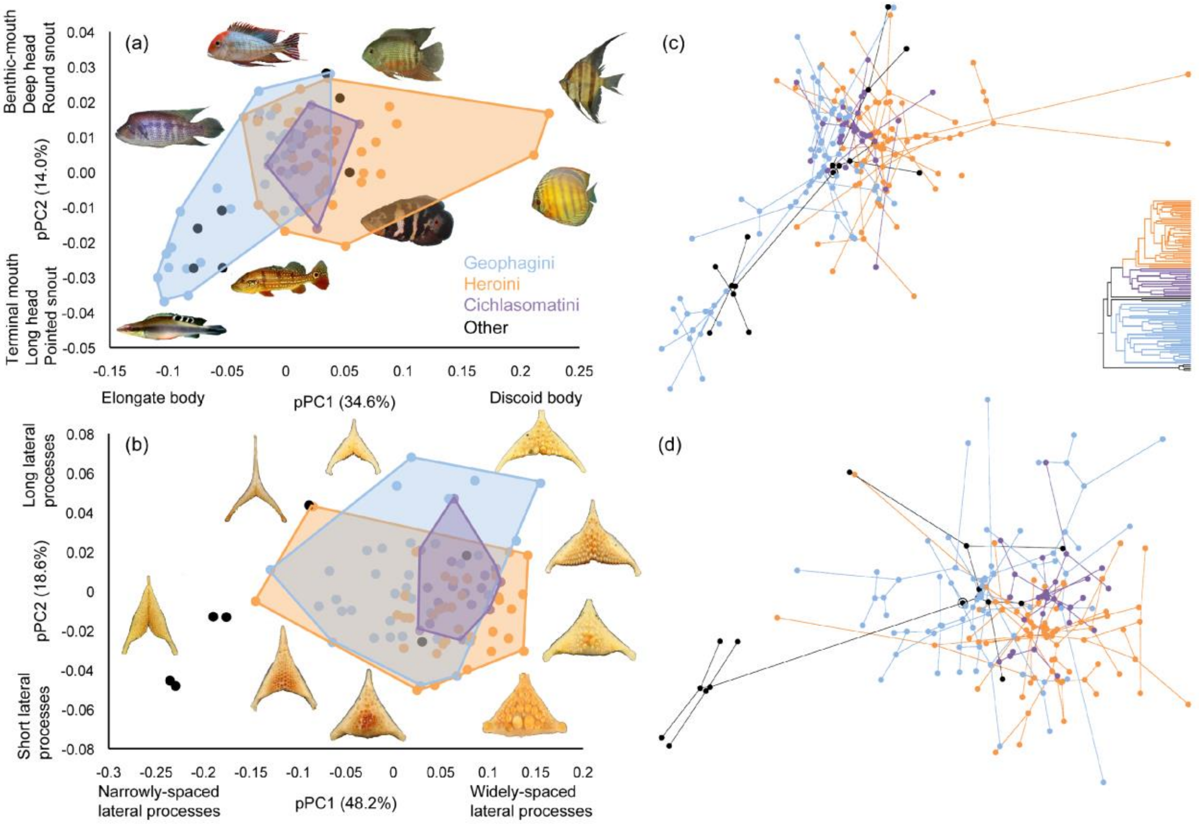

Fig. 1. Major axes of (a) body shape variation and (b) body shape phylomorphospace and (c) major axes of lower pharyngeal jaw (LPJ) shape variation and (d) LPJ phylomorphospace among 97 Neotropical cichlid species. Values are the means from the posterior distribution of 1000 trees. Inset phylogeny depicts the relationships among the major Tribes. Major lineages are colour-coded. Minor lineages (e.g., Chaetobranchini, Astonotini, and Cichlini) are pooled together (Other). Photos depict the shape of species associated with the shape extremes. For references to colour see the online version. Photos by E.D.B. and courtesy of J. Rapps.

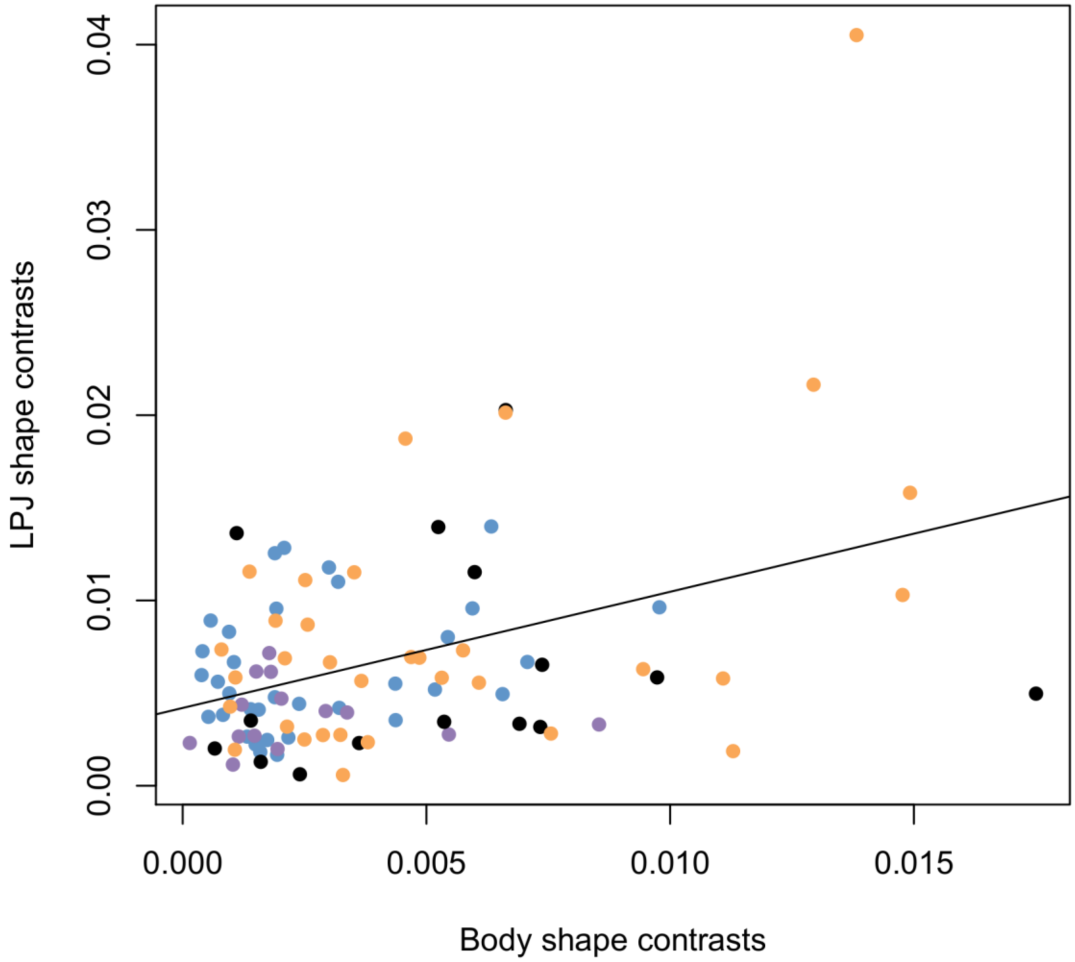

Fig. 2. The relationship between multivariate phylogenetically independent contrasts of body shape (PC1 and PC2) and lower pharyngeal jaw shape (PC1 and PC2) among Neotropical cichlid fishes. Nodes associated with major clades are depicted in colour: Heroini (orange), Geophagini (blue), and Cichlasomatini (purple). Nodes not corresponding to these clades are shown in black. For references to colour see the online version.

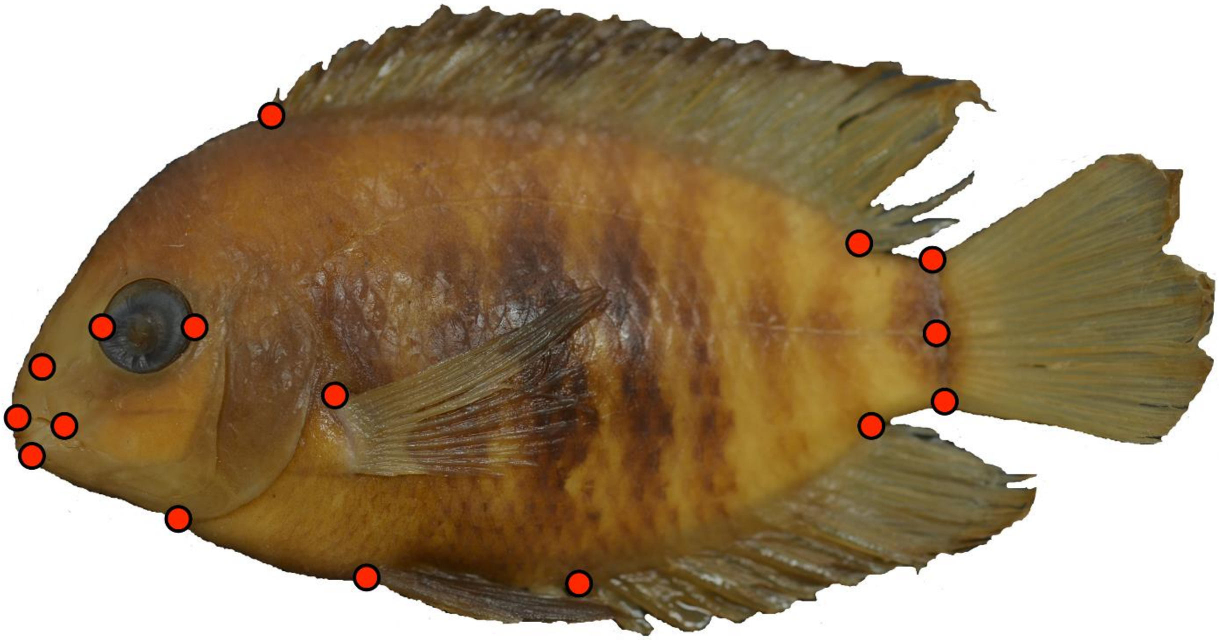

Figure S1. Landmark scheme used for assessment of lateral body shape. All dots represent homologous landmarks.

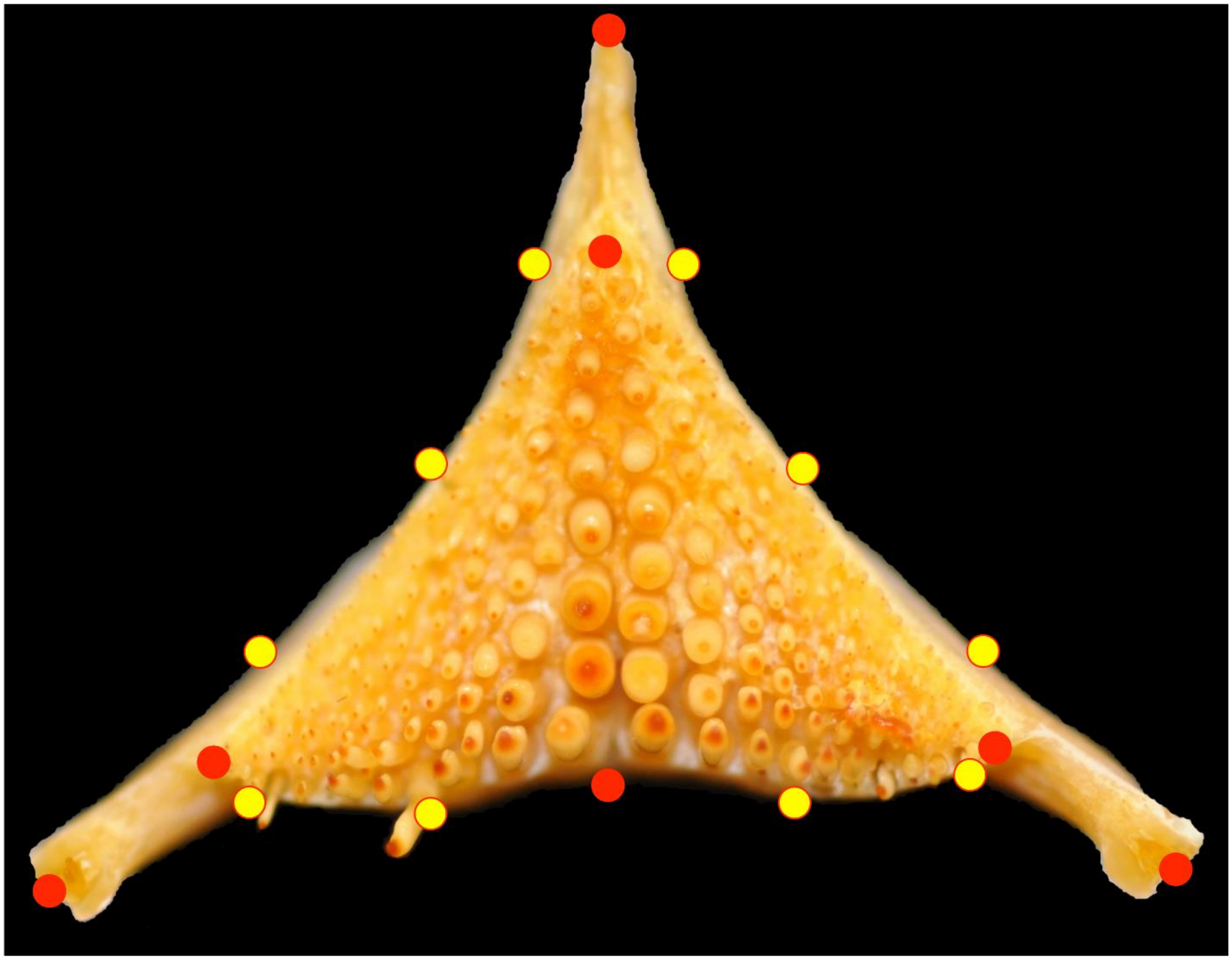

Figure S2. Landmark scheme used for assessment of lower pharyngeal jaw shape. Dots represent homologous (red) and sliding (yellow) landmarks.

Chapter 4: Island- and lake-like parallel adaptive radiations replicated in rivers

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Abstract

Parallel adaptive radiations have arisen following the colonization of islands by lizards and lakes by fishes. In these classic examples, adaptive radiation is a response to the ecological opportunities afforded by the colonization of novel ecosystems and similar adaptive landscapes that favor the evolution of similar suites of ecomorphs despite independent evolutionary histories. Here, we demonstrate that parallel adaptive radiations of cichlid fishes arose in South American rivers. Speciation-assembled communities of pike cichlids (*Crenicichla*) have independently diversified into similar suites of novel ecomorphs in the Uruguay and Paraná Rivers, including crevice feeders, periphyton grazers, and molluscivores. There were bursts in phenotypic evolution associated with the colonization of each river and the subsequent expansion of morphospace following the evolution of the ecomorphs. These riverine clades demonstrate that characteristics emblematic of textbook parallel adaptive radiations of island- and lake-dwelling organisms are feasible evolutionary outcomes even in labile ecosystems such as rivers.

Introduction

Adaptive radiation is an evolutionary response to ecological opportunity such that the rise of adaptations coincides with expansion of ecological diversity within a lineage (Simpson 1953; Schluter 2000; Givnish 2015). These ecological opportunities may include access to novel resources, relaxed selection on ecologically important traits allowing for the exploration of novel phenotypes, or relaxed competition that allows for exploration of novel regions of the adaptive landscape (Schluter 2000). Islands and lakes have served as the foundation for parallel adaptive radiations after their colonization by *Anolis* lizards and cichlid fishes, respectively (Losos 2009; Seehausen 2015). Series of islands and lakes likely provide access to common resources, similar adaptive landscapes, and thereby elicit the evolution of similar suites of ecomorphs despite independent evolutionary histories. The discrete nature of islands and lakes also constrains immigration and emigration such that *in-situ* speciation results in monophyletic groups that are endemic to that ecosystem (i.e., species flocks).

Rapid speciation, phenotypic diversification, and convergence are hallmarks of adaptive radiation (Givnish 2015; Seehausen 2015). Islands and lakes have provided unique examples of parallel adaptive radiation in which diversification on different islands/lakes has produced similar outcomes despite independent evolutionary histories. For example, *Anolis* lizards have diversified into similar suites of habitat specialists on different islands of the Greater Antilles (Losos 2009). These island-specific clades exhibit phenotypic convergence upon inferred adaptive peaks and the onset of diversity dependence as ecological opportunities wane as niches fill towards capacity (Harmon *et al*. 2005; Mahler *et al*. 2010; 2013). Likewise, lakes have provided similar opportunities for cichlid fishes, which have diversified in parallel in lakes throughout Africa and Middle America (Elmer *et al*. 2014; Seehausen 2015). These lake-specific clades exhibit phenotypic convergence, diversification along common environmental gradients such as the benthic-to-pelagic habitat and the hard-shelled to soft-bodied prey axes, as well as diversity dependent evolution (Kocher *et al*. 1993; Schliewen *et al*. 2001; Wagner *et al*. 2014; Elmer *et al*. 2014; Machado-Schiaffino *et al*. 2015). In addition to these prominent examples, parallel adaptive radiations have also occurred in postglacial whitefish and stickleback (Rundle et al. 2000; Østbye et al. 2006), Hawaiian spiders and angiosperms (Blackledge & Gillespie 2004; Gillespie 2004; Givnish et al. 2009), and island land snails (Johnson et al. 2000).

Although, a few riverine cichlid species flocks have been described (Sullivan *et al*. 2002; Schwarzer *et al*. 2011), these lack the dramatic accumulation of ecological roles and their associated adaptations that characterize their lake-dwelling counterparts and have not evolved in parallel (reviewed in Seehausen 2015; Burress 2015). These conflicting patterns of diversification may be because the adaptive landscape is very different in lakes and rivers. This disparity may be explained by a combination of factors (summarized from Seehausen 2015): 1) river communities are immigration-assembled such that co-occurring ecomorphs are often from disparate lineages that were united by unstable and shifting basin configurations (as opposed to speciation-assembled communities that arise in stable lakes), 2) many ecomorphs observed in lakes may be implausible evolutionary results in rivers due to niches that are uncommon or temporally unstable in rivers, and 3) niches are partitioned to minute ecological scales in lakes where stable conditions favor the evolution of accommodating processes such as resource partitioning.

Despite the aforementioned paucity of *in situ* diversification and lack of cases of parallel evolution within rivers (Seehausen 2015; Burress 2105), one promising example has recently been proposed in the Paraná and Uruguay rivers of South America where the resident pike cichlid (*Crenicichla*) communities exhibit similar ecomorphs (Piálek *et al*. 2012). These communities are endemic and consist of species that co-occur throughout their drainage and exhibit dramatic ecomorphological diververgence (Lucena & Kullander 1992; Serra *et al*. 2011; Piálek *et al*. 2012; Burress *et al*. 2013). More recently, this putative example of parallel evolution was expanded following the discovery of additional species in the Paraná River that also have ecomorphological counterparts in the Uruguay River (Piálek *et al*. 2015). Furthermore, these two communities may be monophyletic and distantly related (Lucena & Kullander 1992; Serra *et al*. 2011; Piálek *et al*. 2012; Burress *et al*. 2013).

In this study, we test the hypothesis that the similar ecomorphs observed in the Paraná and Uruguay rivers arose via parallel phenotypic and trophic diversification. To test this hypothesis, we employed a series of statistical tests. Firstly, we establish the evolutionary independence of the two communities using a novel whole genome SNP (ddRADseq) phylogeny. Secondly, we evaluate if the similarities of these ecomorphs is due to selection towards similar adaptive peaks rather than similarity that may have arose via a neutral model of evolution. Thirdly, we evaluate if these ecomorphs arose from similar ancestral states using ancestral state reconstruction of phenotypes and trophic guilds. Lastly, we test for bursts in the rates of speciation and diversification rates throughout the evolutionary history of pike cichlids. We then discuss these riverine clades in the context of other prevalent examples of parallel adaptive radiations such as those of lizards on islands and fishes in lakes (Mahler *et al*. 2013; Seehausen 2015).

Material and methods

Phylogeny construction

We sampled 64 *Crenicichla* (includung *Teleocichla*) species that represent all major lineages and five additional South American cichlid taxa that comprised the outgroup (i.e., *Retroculus* sp., *Satanoperca daemon*, *Apistogramma* sp., *Biotodoma wavrini*, and *Geophagus* sp.). We sampled all eight members of the Uruguay River species flock and all nine members of the Paraná River species flock. The ddRADseq library preparation and bioinformatic processing of the obtained tags were performed as described in Říčan *et al*. (2016). Homologous loci were aligned based on a reference mapping of reads onto the genome of *Oreochromis niloticus* GCA_000188235.1 (http://www.ensembl.org) and SNPs were called in Stacks v1.35 (Catchen *et al*. 2011). Only fixed (homozygotic) SNPs were extracted from loci with a minimum depth of five and present in a minimum of 70% of the samples. The resulting matrix included 25,128 variable sites. The tree was inferred from a concatenated SNP matrix in RAxML v8.2.4 (Stamatakis 2014) under a GTR+Γ model, which was estimated with jModelTest (Posada 2008). To account for potential ascertainment bias, we used a Stamatakis correction (Leaché et al. 2016), which corrected for 326,937 unrepresented constant sites. We used 100 bootstrap replicates to evaluate branch support in RAxML.

To calibrate the ML phylogeny and establish relative node ages within *Crenicichla* for diversification analyses, we used congruification (Eastman *et al*. 2013) to impose secondary calibrations on nodes that were congruent with a time-calibrated phylogeny including cichlids by Friedman *et al*. (2013). Friedman et al. (2013) used 14 fossil calibrations for the ages of 13 outgroup nodes distributed across Percomorpha and the root node using a relaxed-clock analysis in BEAST to calibrate their phylogeny, which included 156 percomoph species, including 91 cichlids. Specifically, three nodes were time-calibrated based on the MCC age estimate from Friedman *et al*. (2013) phylogeny: the MRCA of *Crenicichla* and *Retroculus* (29.2 MYA; 95% C.I. 35.1-25.6 MYA), the MRCA of *Crenicichla* and *Satanoperca* (18.0 MYA; 95% C.I. 22.3-15.0 MYA), and the MRCA of *Crenicichla* and *Geophagus* (20.8 MYA; 95% C.I. 25.2-17.5 MYA). Divergence time estimation was then performed in treePL (Smith & O'Meara 2012) using the input file generated by congruification. Following this procedure, the ML phylogeny was pruned to include only the 57 species for which we have trait data (figure S1).

Morphological analyses

We quantified body and LPJ shape of 220 individuals representing 57 *Crenicichla* species using landmark based geometric morphometrics. We sampled six members of the Uruguay River species flock and nine members of the Parana River species flock. We used 15 homologous and eight sliding landmarks to describe the shape of the body in lateral view (figure S2). We used four homologous and 18 sliding landmarks to describe the shape of the lower pharyngeal jaw (LPJ) in dorsal view (figure S2). Sliding landmarks were evenly spaced between homologous landmarks. All analyses were performed using the tps program suite. Images were consolidated and landmarked using tpsUtil (Rohlf 2004) and tpsDIG2 (Rohlf 2006), respectively. Procrustes fit and principal component (PC) scores were generated using tpsRelw (Rohlf 2007). Scale, rotation, and translation were removed from the analyses during the Procrustes fit. Additionally, we measured two variables with intuitive associations with the benthic-to-pelagic habitat axis and the soft-bodied to hard-shelled prey axis: mouth angle and LPJ mass, respectively. Mouth angle was measured directly from photographs in tpsDIG2 (Rohlf 2006) using the measure function. Measurements were adjusted such that a perfectly horizontal mouth corresponded with 90° (i.e., benthic-oriented mouths were <90° and superior-oriented mouths were >90°). LPJ mass was measured on a digital scale to the nearest 0.00001 g. Magnitude was accounted for by calculating residuals from the regression with standard length. We calculated species means for PCs, angles, and residuals for all subsequent analyses.

Testing for convergence

To assess some key features of the adaptive landscape and quantify convergence, we used SURFACE analysis (Ingram & Mahler 2013), which used stepwise Akaike Information Criterion to locate the number of regime shifts (k) on the phylogeny and then identify whether these shifts are towards convergent regimes (i.e., trait optima). This process involved iteratively adding regime shifts using a Hansen model, then iteratively removing shifts to identify convergent regimes (k'). The reduction in complexity (k-k') corresponds to the number of regimes that are evolving towards common sets of traits (i.e., optima) and thus can be collapsed into a common regime (i.e., convergence; Δk). These evolutionary regimes were projected onto the phylogeny and morphospace to visualize the adaptive landscape. Convergence can result from BM-like processes (Polly 2004; Stayton 2008); therefore, we compared parameters estimated from observed trait data to those generated from data simulated from BM to assess if the observed degree of convergence is more than expected by chance. We generated 100 simulated data sets using the surfaceSimulate function (Ingram & Mahler 2013).

We then estimated the evolutionary history of trophic guild diversification using maximum-likelihood (mk1 model) ancestral state reconstruction and stochastic character mapping in Mesquite version 3.02 (Maddison & Maddison 2015). The mk1 model assumes the probability of all trait changes are equal. Maximum-likelihood analyses find the ancestral states (i.e., internal nodes) that maximize the probability that the observed states (i.e., terminal nodes) would evolve under a stochastic model (Schluter *et al*. 1997; Pagel 1999). Stochastic character mapping (Neilsen 2002; Huelsenbeck et al. 2003) simulates precise histories of character evolution such that they depict the character states at nodes but also at all points along branches between nodes. Character changes along branches are predicted by the rates of character change (Maddison & Maddison 2015). Species were pooled into general trophic guilds based on existing descriptions of *Crenicichla* trophic ecology: piscivore, invertivore, molluscivore, crevice-feeder, and periphyton grazer (see Results). The piscivore category includes only species that feed almost exclusively upon fishes. In contrast, the invertivore category includes species that feed primarily upon invertebrates but may also consume secondary fractions of fishes (i.e., generalists). To simultaneously evaluate the direction and magnitude of shape change and trophic guild evolution along branches of the phylogeny, we overlaid the phylogeny and trophic guild ancestral state reconstruction onto the PC scores (i.e., phylomorphospace; Sidlauskas 2008) in Mesquite version 3.02 (Maddison & Maddison 2015). In this procedure, internal (i.e., ancestral) node values are estimated using squared-change parsimony weighted by the degree of molecular change on the respective branches of the tree (Maddison 1991; Revell *et al*. 2007).

Testing for shifts in diversification rates

We estimated the distribution of discrete shifts in speciation rate as well as mouth angle and LPJ mass diversification rates across the phylogeny and through time using BAMM 2.1.0 (Rabosky 2014; Rabosky *et al*. 2014a). Priors for all BAMM runs were set using the setBAMMpriors command in BAMMtools (Rabosky *et al*. 2014b), with the number of expected shifts set to 1.0. BAMM includes an implementation of an algorithm to account for non-random incomplete taxon sampling in the estimation of diversification rates by allowing for specification of clade-specific sampling fractions (Rabosky et al. 2014a). We specified sampling fractions for each species-group as the proportion of species in our phylogeny out of the total valid species in each species-group. Diversification (speciation-extinction) analyses were run for 25M generations, MCMC output was written every 20,000 generations, and event data were written every 10,000 generations. Trait diversification analyses were run for 5M generations, MCMC output was written every 2,000 generations, and event data were written every 5,000 generations. BAMM output was further analyzed in BAMMtools (Rabosky *et al*. 2014b) to calculate and plot the 95% credible shift set. As recommended in the BAMM documentation, coda in R (Plummer *et al*. 2006) was used to assess whether the MCMC chain included an adequate number of samples of the posterior distribution by determining if the ESS > 200 for the number of shifts and log likelihood. We assessed the number of rate shifts in the posterior distribution (with 10% burnin excluded) relative to the prior distribution, which indicated that the posterior was not sensitive to the prior as can occur in some situations (Moore *et al*. 2016) such as when there is a low probability of rate shifts (Mitchell & Rabosky 2016; Rabosky et al. 2017).

To independently assess rates of morphological evolution through time, we used node-height tests. We calculated the absolute value of phylogenetically independent contrasts and regressed those against the height (i.e., time since root) of the node at which they were calculated. Contrasts are Brownian rate parameters (McPeek 1995); therefore, a significant positive relationship between absolute rate contrasts and node height would indicate that rates of morphological evolution have increased through time (Freckleton & Harvey 2006).

Results

Phenotypic convergence

The Uruguay and Paraná River species flocks exhibited considerable overlap in body shape (figure 1*a*), lower pharyngeal jaw shape (figure 1*b*), LPJ mass and mouth angle (figure 1*c*). Both species flocks also occupy a large volume of morphospace (figure 1). SURFACE identified non-convergent and convergent evolutionary regimes (figure 2*a*). Three convergent regimes were restricted to the Paraná and Uruguay River species flocks (figure 1*a*). The degree of phenotypic convergence (Δk=4) was not sampled using trait data simulated under BM (Δk = 0-3; figure 2*b*), indicating highly non-random evolutionary processes generating similar ecomorphs in these species flocks.

These convergent regimes coincided, in part, with the independent evolution of periphyton grazing, crevice feeding, and molluscivory within the Uruguay and Paraná radiations (figure S3). The first convergent regime (green in figure 2) included *C. tapii* (Paraná) as well as *C. hadrostigma* and *C. empheres* (Uruguay), which was characterized by an optimum defined by curved snouts, small mouths, robust LPJ, and mostly benthic-oriented mouths (figure 2*c*). The second regime (brown in figure 2) included *C. tuca* (Paraná) and *C. tendybaguassu* (Uruguay) and was characterized by tapered bodies, terminal mouths, and hypertrophied lips (figure 2c). The third regime (red in figure 2) included only *C. tesay* (Paraná) and *C. minuano* (Uruguay) and was characterized by an optimum defined by intermediate mouth size, hypertrophied LPJ, and terminal mouths (figure 2*c*). All three of these regimes included optima that fell outside the observed trait space for body and LPJ shape. Outlier optima may indeed represent evolution towards distant optima; however, it may also represent rapid adaptation to a new optimum that results in the optimum being interpreted by the model as distant (Ingram & Kai 2014). The latter is likely the case here considering the rapid ecological diversification within these clades. *Crenicichla taikyra* (Paraná) occupies its own regime (light gray in figure 2), whereas several species within both species flocks are united within an ancestral non-convergent regime (black in figure 2). Outgroups generally fell into two non-convergent regimes. The *C. lugubris*, *C. saxatilis*, *C. wallacii*, *C. reticulata*, and the (non-Paraná and Uruguay) *C. lacustris* group species were united within a non-convergent regime (black in figure 2). Likewise, the *Teleocichla* group was united within a second non-convergent regime (dark gray in figure 2).

Trophic convergence

The ancestral trophic state of both species flocks was well-resolved as invertivore based on our maximum likelihood ancestral state reconstruction and stochastic character mapping (figure S3). Within the Paraná River, this ancestral trophic state is retained in *C. mandelburgeri*, *C. hu*, and *C. ypo*, whereas all other species exhibit a novel trophic state (figure 2*d* and S3). Piscivory evolved independently in *Crenicichla* sp. Urugua-í line and *C. iguassuensis*, molluscivory evolved once, in *C. tesay* and *C. taikyra*, and periphyton grazing and crevice feeding evolved in *C. tapii* and *C. tuca*, respectively (figure 2*d* and S3). Within the Uruguay River, the ancestral trophic state is retained only in *C. empheres*, whereas piscivory evolved once in *C. celidochilus* and *C. missioneira*, crevice feeding evolved in *C. tendybaguassu*, molluscivory evolved in *C. minuano*, and periphyton grazing evolved in *C. hadrostigma* (figures 2*d* and S3). Piscivory evolved in the Uruguay (*C. celidochilus* and *C. missioneira)* and Paraná (*C*. sp. Urugua-í line and *C. iguassuensis*) Rivers; however, this does not represent parallel evolution because these species retain the ancestral body and LPJ morphologies indicating that ancestral morphologies may have been co-opted for novel trophic functions (figure 2*a*,*d* and S3). Character state changes associated with the evolution of crevice feeding and periphyton grazing were recent indicating that they arose rapidly (figure S3). A host of derived trophic states evolved independently in both rivers from similar ancestral states and in association with the evolution of novel trait combinations (figure 2*d*). Namely, these cases involved the evolution of molluscivory, crevice feeding, and periphyton grazing and the corresponding expansion of morphospace associated with novel trophic role-specific craniofacial and LPJ morphologies (figures 2*d*).

Diversification rates

Rates shifts are not independent of one another such that there are many possible shift configurations. The most probable shift configuration for speciation rates includes no rate shifts (figures S4 and S5). Less probable shift configurations include having one rate shift near the base of the clade containing both species flocks or having a shift associated with the origin of the Uruguay River species flock (figures S4 and S5).

For diversification of mouth angle (MA), the most probable shift configuration includes two shifts near the base of each species flocks (figure 3*a*,*b*). Less probable shift configurations vary in the placement of the shift along the branch leading to the Uruguay River species flock and the placement of the shift near the base of the Paraná species flock, sometimes excluding some of the piscivores and invertivores (figure S6). Rate through time plots revealed that evolutionary rates have accelerated through time (figure 3*c*). There was a significant positive relationship between absolute contrasts and the height of the nodes at which they were calculated (R^2=0.161; F=10.37; P=0.002), indicating that rates of evolution have increased over time. Furthermore, node-height tests indicate that the rapid rate increase near present day is driven by the Parana and Uruguay species flocks (figure 3*c*).

For diversification of LPJ mass, the most probable shift configuration included three shifts (figure 3*d*), including an early shift at the base of the clade containing both species flocks, and more recent shifts at the base of the Uruguay River species flock and within the Paraná River species flock (figure 3*e* and S7). Less probable shift configurations varied in their inclusion of a shift near the base of the clade containing both species flocks, but generally had shifts at the base of or within both species flocks (figure S7). Rate through time plots revealed that evolutionary rates have accelerated through time (figure 3*f*). There was a significant positive relationship between absolute contrasts and the height of the nodes at which they were calculated (R^2=0.134; F=8.35; P=0.006), indicating that rates of evolution have increased over time. Furthermore, node-height tests indicate that the rapid rate increase near present day is driven by the Parana and Uruguay species flocks (figure 3*f*).

Discussion

Parallel diversification into similar suites of ecomorphs is a central theme of adaptive radiation within discrete ecosystems such as islands and lakes. Textbook examples of parallel adaptive radiations such as those of anole lizards and cichlid fishes provide predictable evolutionary outcomes such as non-random phenotypic evolution towards inferred adaptive peaks, the evolution of specializations, and the onset of diversity dependence as niches fill towards capacity (Kocher *et al*. 1993; Losos 2011; Mahler *et al*. 2010; 2013; Seehausen 2015). We found that pike cichlids exhibit parallel adaptive radiation in the Paraná and Uruguay Rivers (figure 4) that is consistent with those of island- and lake-dwelling clades and is uncharacteristic for river-dwelling fish communities.

Similar suites of ecomorphs have evolved within the Paraná and Uruguay Rivers from similar ancestral states and despite independent evolutionary histories (figures 2 and S3). The evolution of specialized trophic roles were strictly associated with the evolution of novel phenotypes and therefore contributed to a dramatic expansion of phenotypic diversity during these clades exploration of the adaptive landscape (figure 2*d*). Non-random phenotypic diversification towards inferred adaptive peaks associated with unique morphologies such as hypertrophied and atrophied pharyngeal jaws (i.e., molluscivores and piscivores, respectively), hypertrophied oral lips (i.e., crevice feeders), and benthic-oriented snouts (i.e., periphyton grazers) led to high degrees of convergence between these two clades. These trophic roles and their associated adaptations are rare among river-dwelling cichlids (Burress 2015; 2016); however, they are conspicuously associated with adaptive radiations of lake-dwelling clades. For example, African cichlids have diversified extensively within primary production-associated trophic roles (e.g., algae scrapers) in lakes Tanganyika and Malawi (Burress 2015). Rapid transitions to herbivory are otherwise rare among freshwater fishes (Seehausen & Wagner 2014). Likewise, hypertrophied pharyngeal jaws associated with durophagy (i.e., eating hard-shelled prey) and hypertrophied lips associated with feeding from rocky crevices are also rare among Neotropical cichlids, but have often arose during adaptive radiation in African and Middle American lakes and are common sources of polymorphism between incipient species pairs in lakes and within some extremely polymorphic species (reviewed in Burress 2015).

The hallmark phylogenetic signature of adaptive radiation includes an initial burst in diversification rates following the colonization of a novel environment, followed by slowing after the onset of diversity dependence (Glor 2010). This period of slowing occurs in response to ecological constraints as niches fill towards capacity (Freckleton & Harvey 2006). Pike cichlids likely colonized subtropical South America via stream capture between southern tributaries of the Amazon and the headwaters of the La Plata River basin (Albert & Carvalho 2011; Brea & Zucol 2011; Carvalho & Albert 2011). We found evidence of a burst in speciation rates at the base of the subtropical clade (figure S4 and S5). The paucity of cichlid (and non-cichlid) lineages that occur in subtropical South America (Albert *et al*. 2011) may have resulted in competitive release from the comparatively diverse Amazonian lineages and communities. In contrast, bursts in phenotypic diversification were more closely associated with the Paraná and Uruguay River clades and the rise of the parallel ecomorphs (figure 3). Rates of phenotypic evolution increased through time, include dramatic increases near present day and are thus inconsistent with diversity-dependence associated with niche filling (Freckleton & Harvey 2006). These clades may be too young to exhibit such patterns of morphological evolution.

Diversification along the benthic-to-pelagic habitat axis has been prodigious among adaptive radiations of lake-dwelling cichlids (Seehausen and Wagner 2014; Burress 2015), including within Lake Malawi (Hulsey *et al*. 2013), Lake Tanganyika (Cooper *et al*. 2010), Cameroonian crater lakes (Schliewen et al. 2001), Ugandan crater lakes (Machado-Schiaffino *et al*. 2015), and Nicaraguan crater lakes (Barluenga *et al*. 2006; Elmer *et al*. 2014; Kusche *et al*. 2014). Most of the parallel ecomorphs that arose in the Paraná and Uruguay Rivers are strictly benthic such that they consume substrate-associated primary producers or invertebrates (i.e., periphyton grazers, crevice feeders, and molluscivores); however, some species have specialized upon exploiting schooling fishes from the water column (Burress *et al*. 2013). In contrast, vast depths and steep shoreline reefs that characterize many lakes provides dramatic ecological gradients that may have permitted the evolution of a variety of pelagic trophic roles including open-water piscivores as well as filter-feeders (Burress 2015). Diversification along the benthic-to-pelagic habitat axis may be constrained within the Paraná and Uruguay River clades because the axis is more physically constrained in rivers and permits fewer evolutionary outcomes.

The Paraná and Uruguay River species flocks exhibit many characteristics generally attributed to the textbook examples of parallel adaptive radiation in island- and lake-dwelling organisms. Normally, the adaptive landscape may be very different in lakes and rivers such that river communities are immigration-assembled rather than speciation assembled and some regions of the adaptive landscape may be implausible evolutionary results in rivers due to niches that are uncommon or temporally unstable in such environments (Seehausen 2015). Nevertheless, the

Paraná and Uruguay Rivers have provided suitable circumstances for the rise of lake-like adaptive radiations. Namely, these rivers are mostly shallow, clear, and have rocky substrate, which likely provided the opportunity for the exploitation of primary production-associated trophic roles (i.e., algae grazers) as is provided along the littoral zone of lakes. Second, these basins are home to few cichlid and non-cichlid lineages. Thus, after colonization of subtropical South American streams, pike cichlids may have experienced relaxed competition for mostly vacant niches. Lastly, pike cichlids colonized a novel adaptive zone among Neotropical cichlids associated with elongate bodies adapted for ram feeding (López-Fernández et al. 2013), which may have predisposed the lineage to further trophic-based exploration of the adaptive landscape. This specific set of circumstances may have provided the opportunity for these clades to diversify in a pattern reminiscent of island-dwelling anoles (Mahler *et al*. 2010) and lake-dwelling cichlids (Seehausen 2015) such that similar suites of ecomorphs arose rapidly in parallel.

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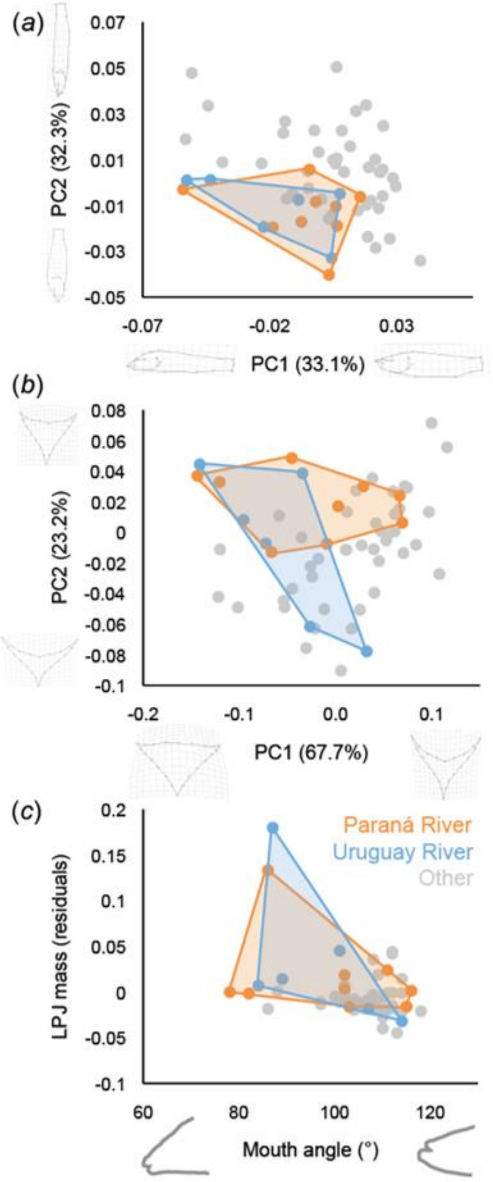

Fig. 1. Major axes of body (*a*) and lower pharyngeal jaw (LPJ; *b*) shape variation, and (*c*) mouth angle and size-relative LPJ mass among 57 *Crenicichla* species. Wire frames depict shapes associated with the extremes of each axis. Each point depicts a species. The Paraná and Uruguay River species flocks are highlighted in color.

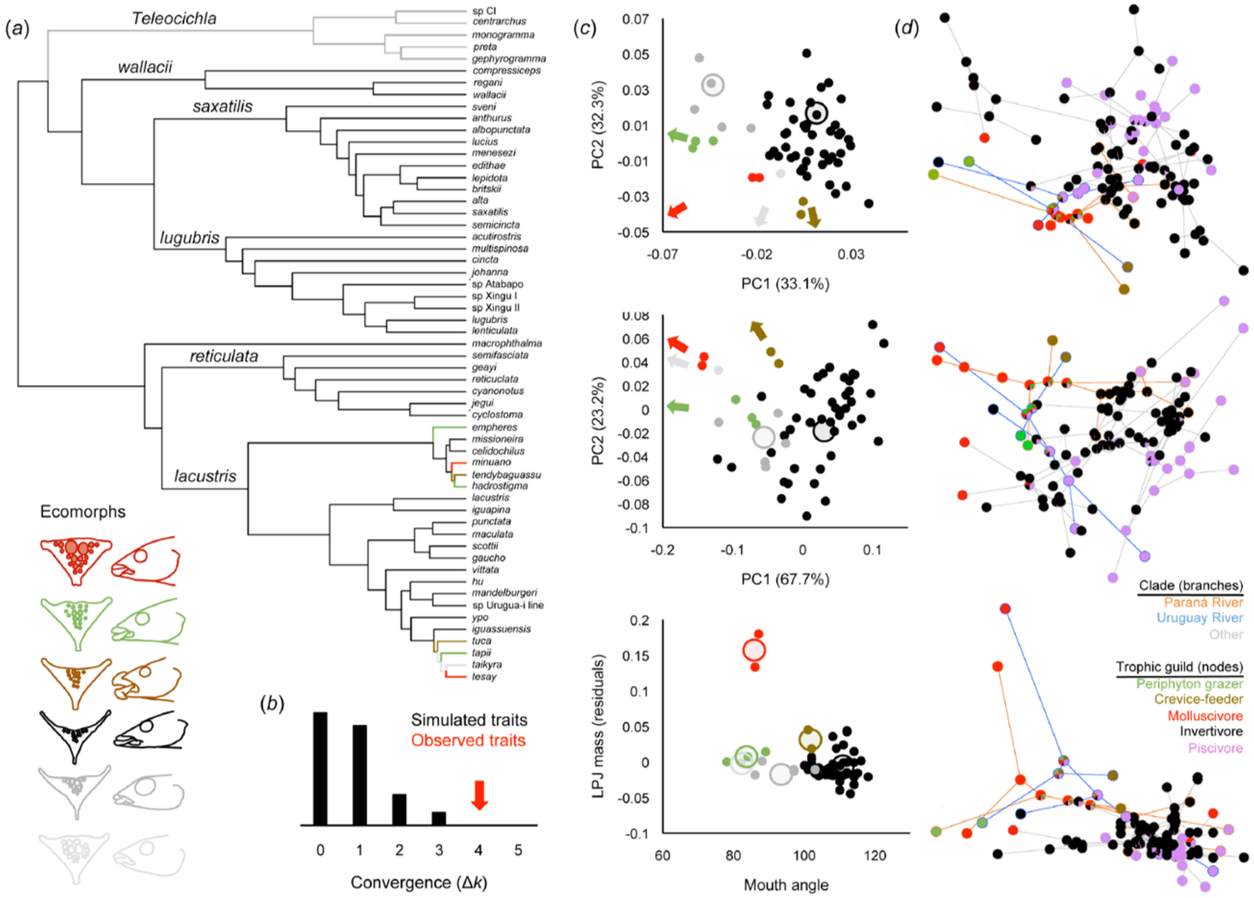

Fig. 2. Phenotypic and trophic convergence between the Paraná (orange) and Uruguay River (blue) species flocks. (*a*) Non-convergent (gray-scale branches) and convergent (like-colored branches) evolutionary regimes across 57 *Crenicichla* species from SURFACE analysis. (*b*) The observed degree of convergence relative to an expected distribution under a Brownian motion model of evolution. (*c*) The distribution of inferred adaptive peaks (large circles) and species (small circles) across morphospace. (*d*) Phylomorphospace depicting the direction and magnitude of shape change (branches) and corresponding trophic guild evolution (nodes) based on ML ancestral state reconstruction.

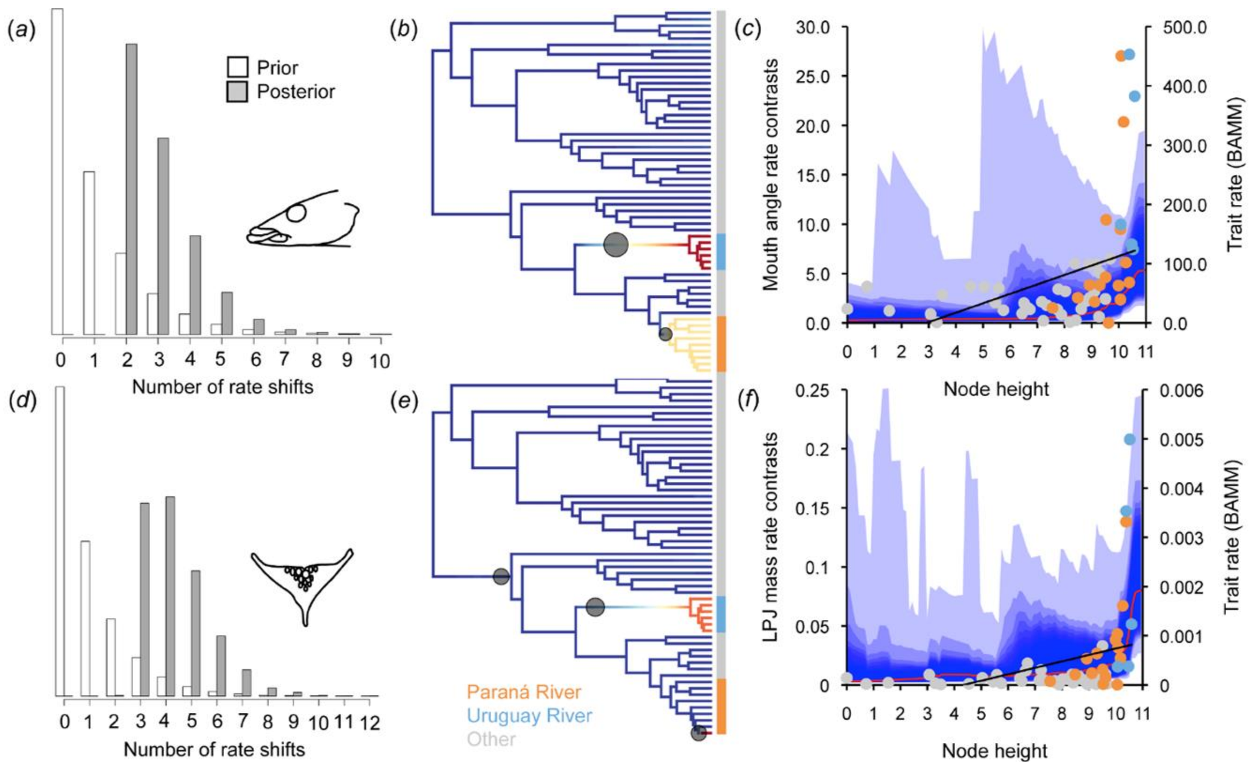

Fig. 3. Rates of phenotypic evolution across the *Crenicichla* phylogeny. Prior and posterior distribution of rate shifts (*a*,*d*), most probable shift configuration from BAMM (*b*,*e*), rate through time (*c*,*f*), and node-height test (*c*,*f*) for mouth angle (*a*-*c*) and size-relative lower pharyngeal jaw (LPJ) mass (*d*-*f*). Less probable shift configurations are shown in the online Supplementary Information. Mean rate through time (red lines) is from BAMM analysis and the best-fit lines (black lines) are fitted to the node-height tests.

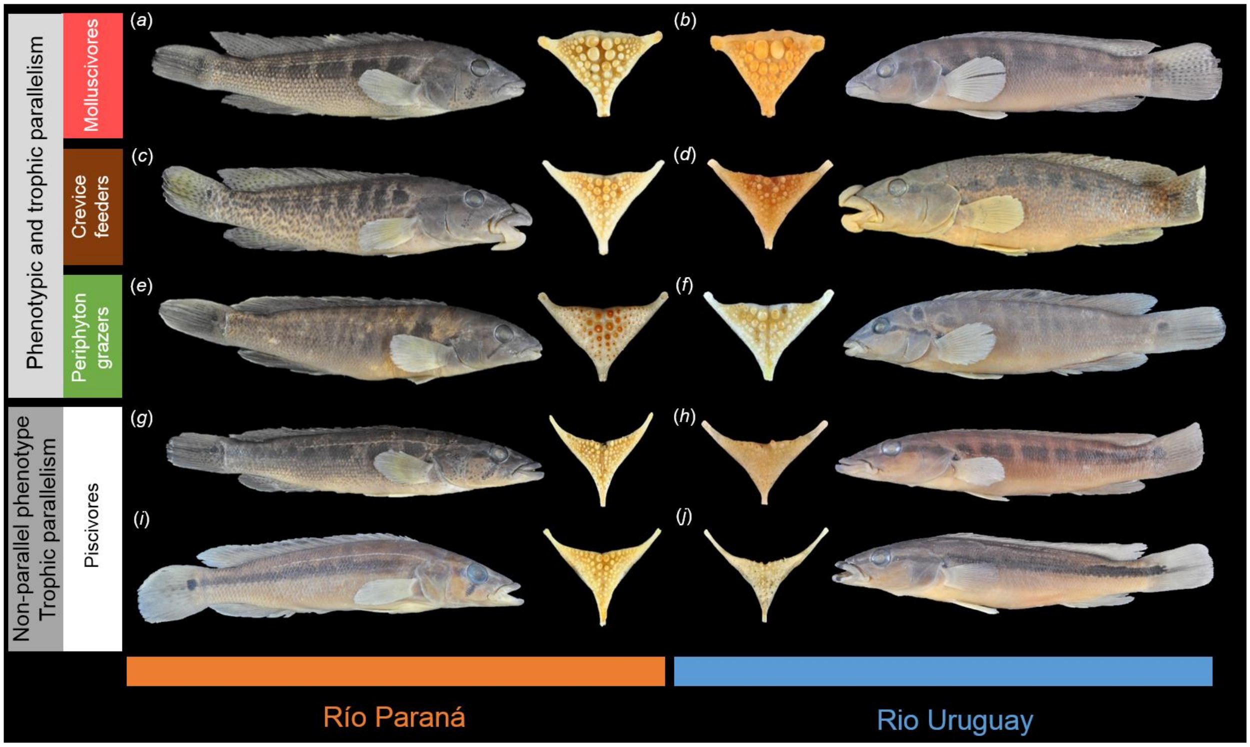

Fig. 4. Parallel and non-parallel evolution of ecomorphs in the Uruguay and Paraná River species flocks. Parallel evolution of molluscivores: *Crenicichla tesay* (*a*) and *C. minuano* (*b*), crevice feeders: *C. tuca* (*c*) and *C. tendybaguassu* (*d*), and periphyton grazers: *C. tapii* (*e*) and *C. hadrostigma* (*f*). Non-parallel phenotypic diversification, but trophic convergence among piscivores: *C. iguassuensis* (*g*), *C.* sp. Urugua-i line (*h*), *C. missioneira* (*i*), and *C. celidochilus* (*j*).

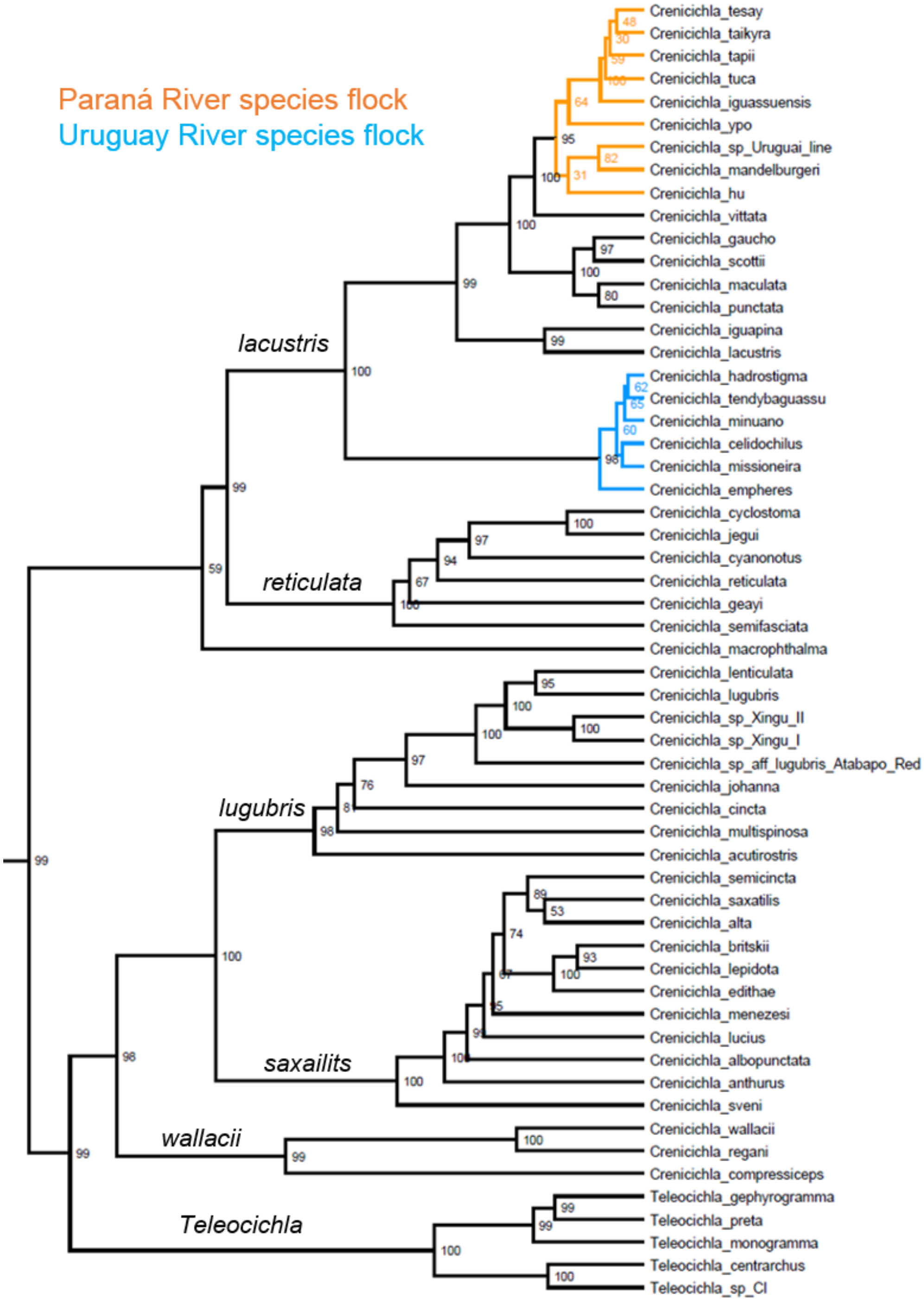

Fig. S1. Phylogeny used in the analyses in the paper. Names on internal branches correspond with the major species-groups. Node values are based on 100 bootstraps.

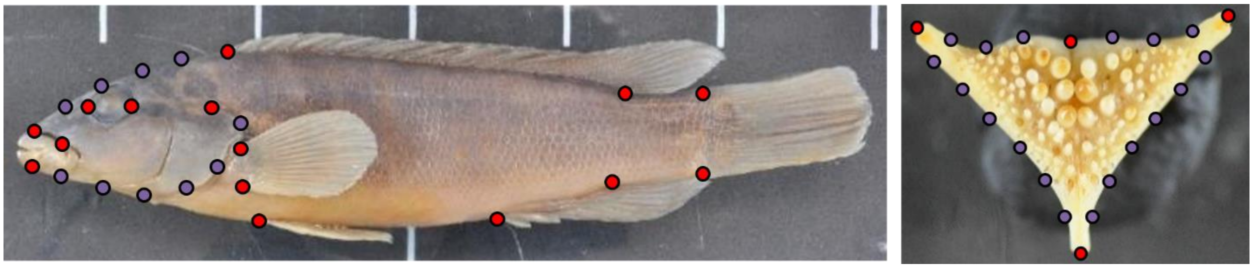

Fig. S2. Landmark scheme used to quantify body (left) and lower pharyngeal jaw (right) shapes: homologous (red) and sliding (purple) landmarks.

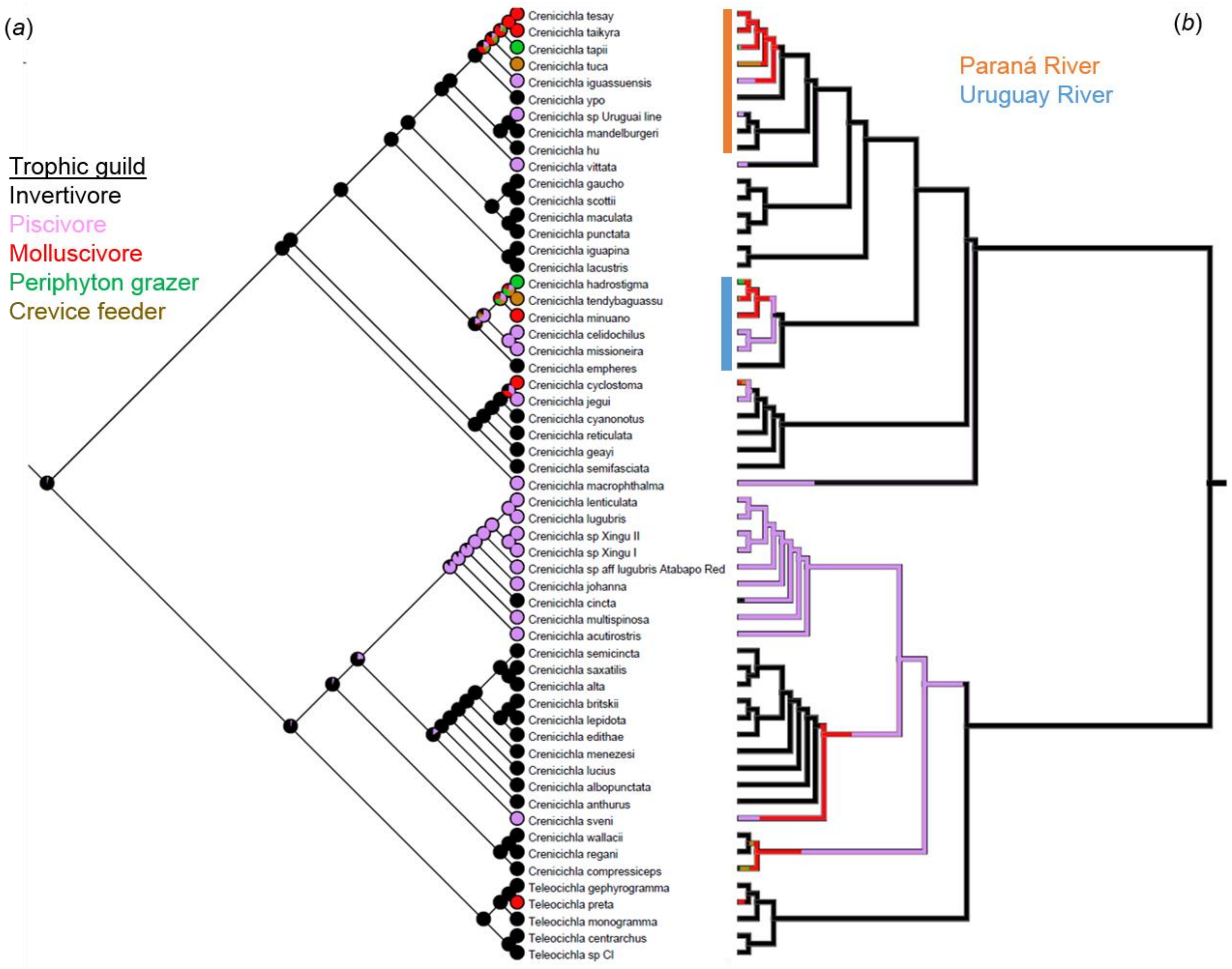

Fig. S3. Ancestral state reconstruction of trophic guild evolution among *Crenicichla* based on (a) maximum-likelihood and (b) stochastic character mapping.

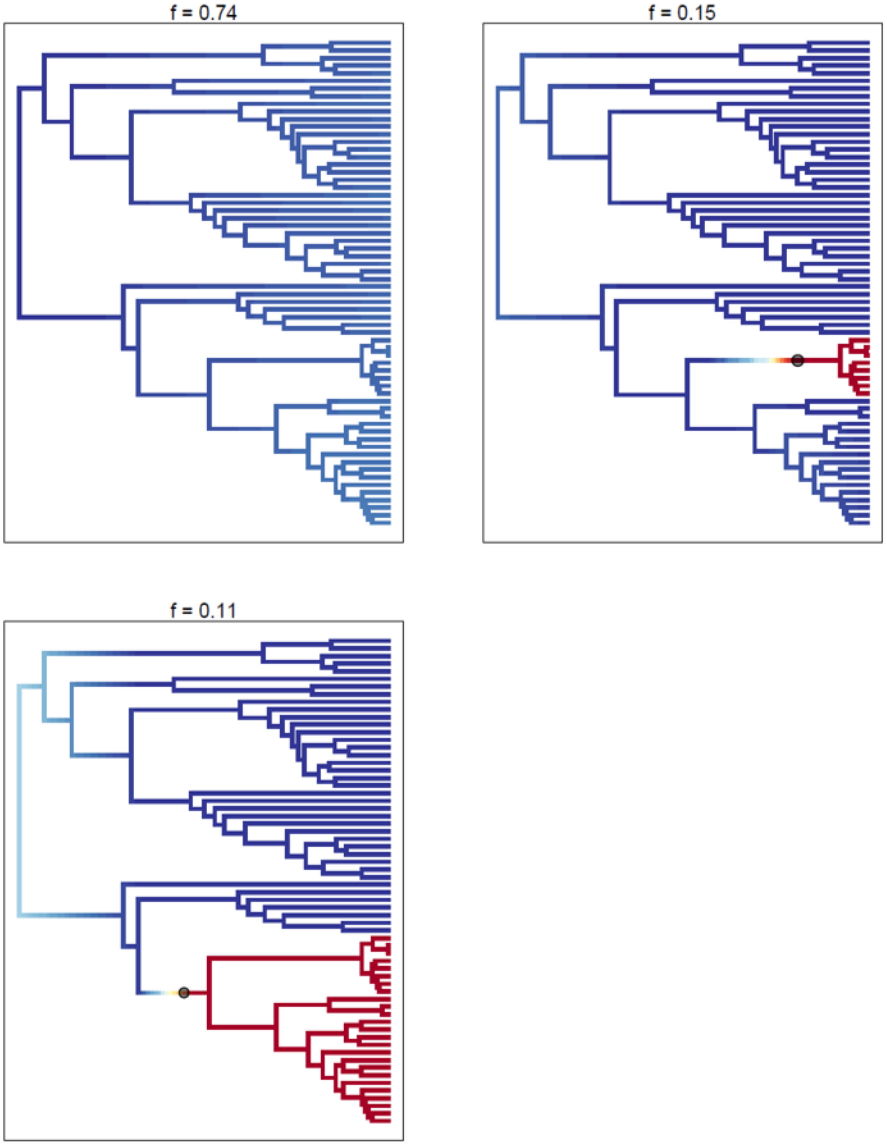

Fig. S4. 95% credible shift set for shifts in speciation rates across the *Crenicichla* phylogeny. Gray dots depict the location of rate shifts. f depicts the frequency of each shift configuration.

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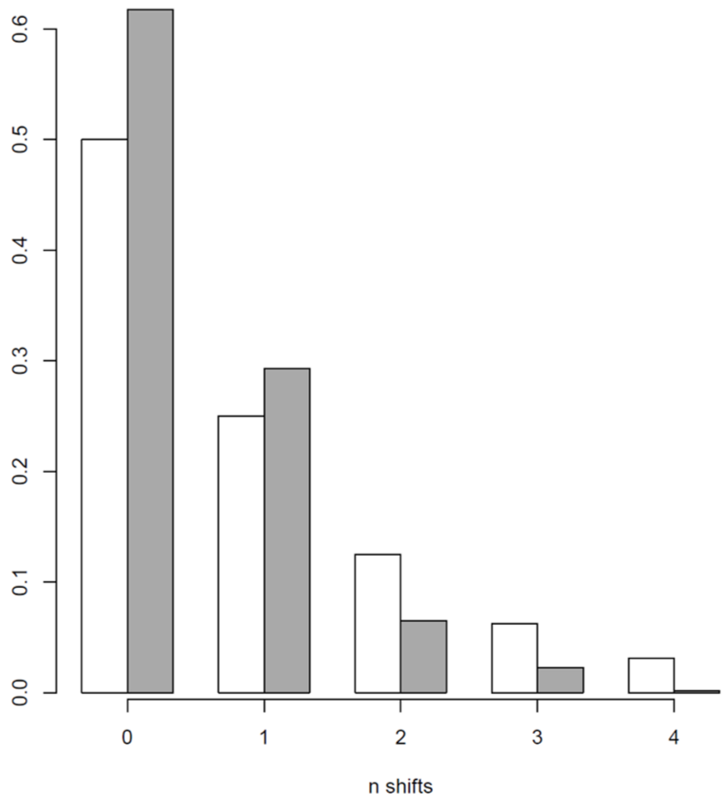

Fig. S5. Prior (white bars) and posterior (gray bars) distributions of the number of rate shifts for speciation among pike cichlids.

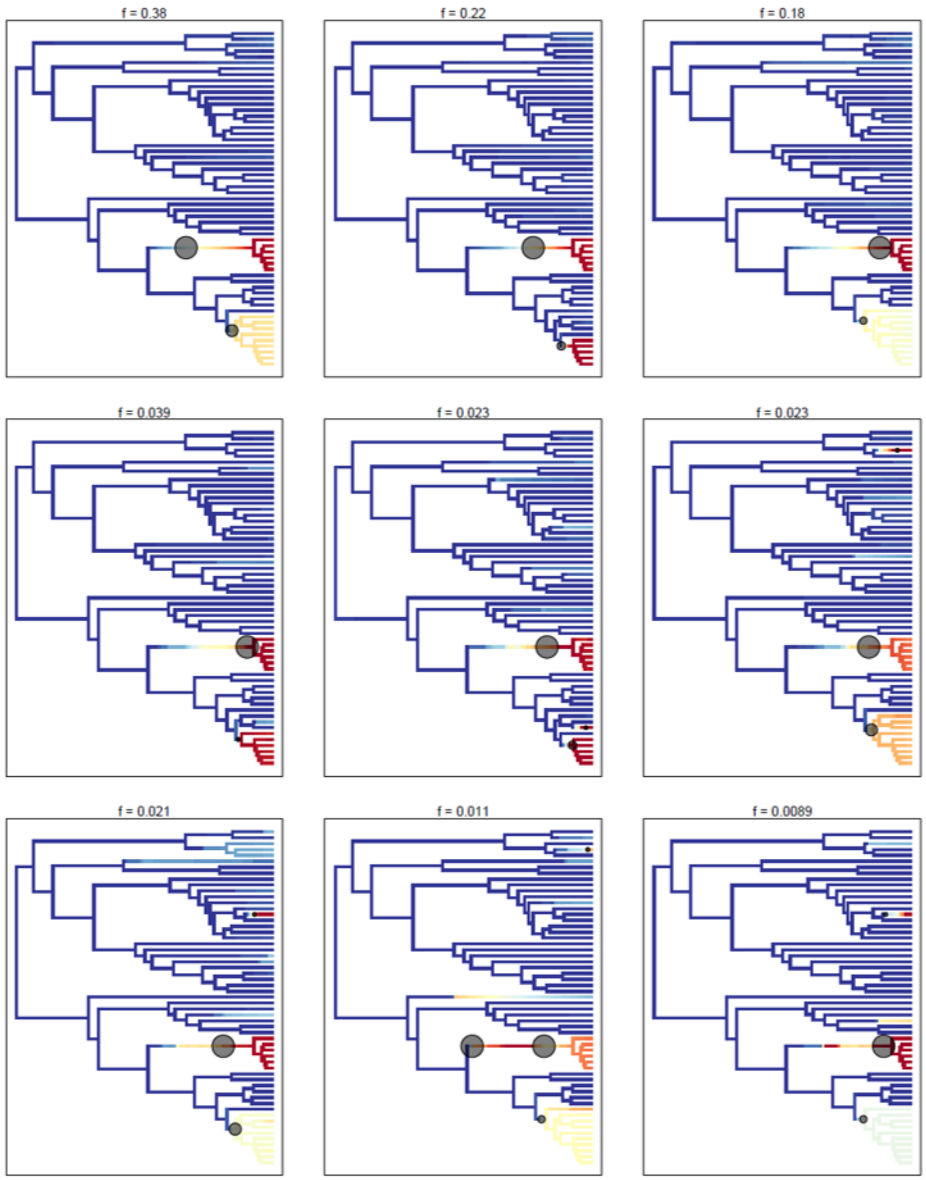

Fig. S6. 95% credible shift set for shifts in the rates of mouth angle evolution across the *Crenicichla* phylogeny. Gray dots depict the location of rate shifts. f depicts the frequency of each shift configuration.

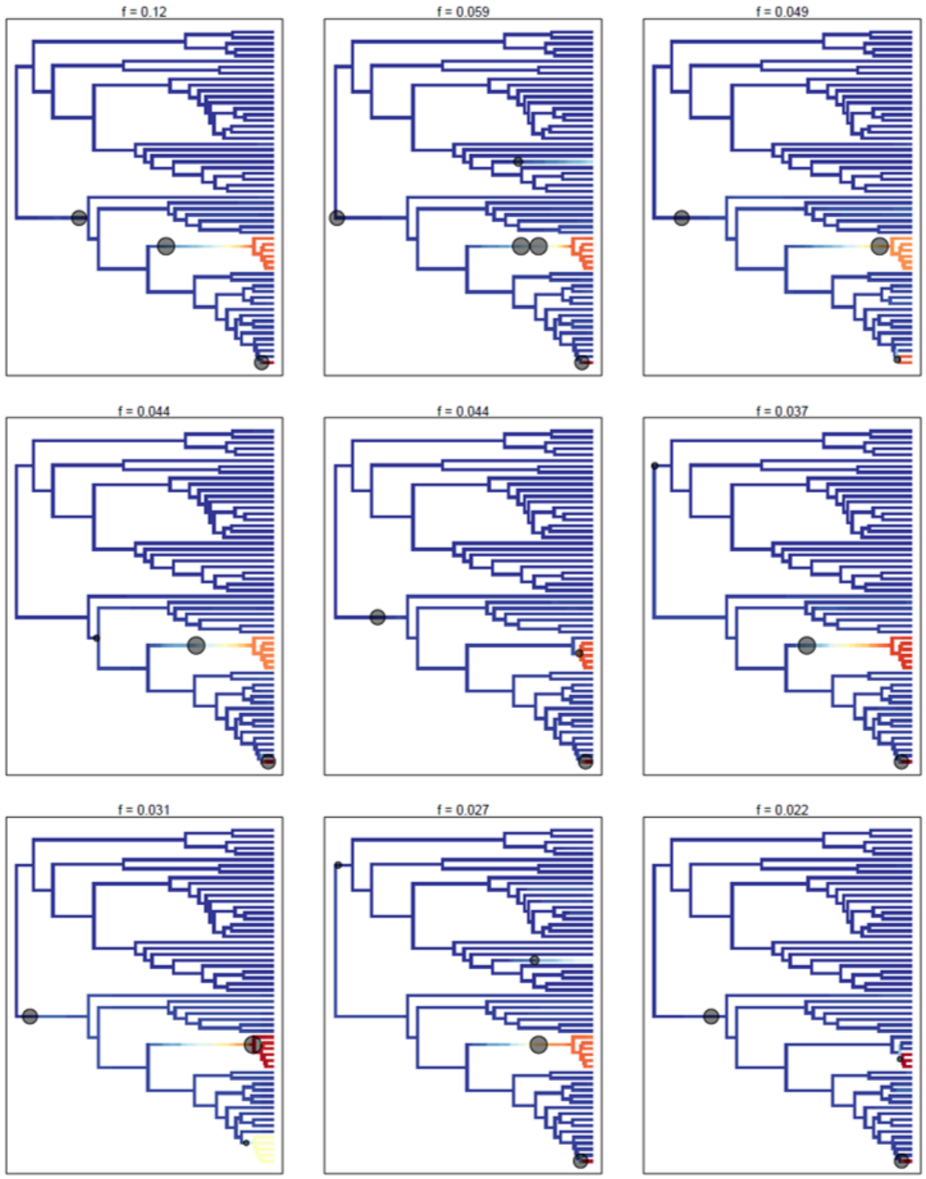

Fig. S7. 95% credible shift set for shifts in the rates of lower pharyngeal jaw evolution across the *Crenicichla* phylogeny. Gray dots depict the location of rate shifts. f depicts the frequency of each shift configuration.