

Head Shape Modulates Diversification of a Classic Cichlid Pharyngeal Jaw Innovation

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Submitted March 24, 2019; Accepted April 25, 2019; Electronically published September 13, 2019

Online enhancements: appendix. Dryad data: <https://doi.org/10.5061/dryad.q5846sq>.

ABSTRACT: Functional innovations are often invoked to explain the uneven distribution of ecological diversity. Innovations may provide access to new adaptive zones by expanding available ecological opportunities and may serve as catalysts of adaptive radiation. However, diversity is often unevenly distributed within clades that share a key innovation, highlighting the possibility that the impact of the innovation is mediated by other traits. Pharyngognathy is a widely recognized innovation of the pharyngeal jaws that enhances the ability to process hard and tough prey in several major radiations of fishes, including marine wrasses and freshwater cichlids. We explored diversification of lower pharyngeal jaw shape, a key feature of pharyngognathy, and the extent to which it is influenced by head shape in Neotropical cichlids. While pharyngeal jaw shape was unaffected by either head length or head depth, its disparity declined dramatically with increasing head width. Head width also predicted the rate of pharyngeal jaw evolution such that higher rates were associated with narrow heads. Wide heads are associated with exploiting prey that require intense processing by pharyngeal jaws that have expanded surfaces for the attachment of enlarged muscles. However, we show that a wide head constrains access to adaptive peaks associated with several trophic roles. A constraint on the independent evolution of pharyngeal jaw and head shape may explain the uneven distribution of ecological diversity within a clade that shares a major functional innovation.

Keywords: adaptive radiation, adaptive landscape, functional constraint, morphology, pharyngognathy.

Introduction

Key innovations are morphological, physiological, or behavioral traits that permit the lineage in which they evolve to interact with the environment in a novel way that underlies a subsequent expansion of ecological diversity. The uneven phylogenetic distribution of diversity is often partly attributed to major innovations that drove bouts of phenotypic di-

versification. Key innovations have been identified along many branches of the tree of life, including the evolution of wings and complete metamorphosis in insects (Nicholson et al. 2014), adhesive silk in spiders (Bond and Opell 1998), and alternative photosynthetic pathways in plants (Quezada and Gianoli 2011; Silvestro et al. 2013). But not all innovations with the potential to enhance diversity realize that potential. There are a number of factors that can limit the macroevolutionary impacts of innovations, including whether origination of the trait is paired with the availability of sufficient resources to sustain an ecological expansion (Vermeij 2001), trade-offs that are incurred by the innovation itself that can have a constraining impact (McGee et al. 2015), and the need for the trait to be synergistically aligned with other parts of the phenotype (Meyer et al. 2012). This last mechanism is especially intriguing because it raises the possibility that the macroevolutionary impact of a key innovation may be mediated by other traits and lineages that possess the potential innovation may consequently vary in their diversification.

The success of ray-finned fishes, which comprise more than half of all vertebrate diversity, is partly attributable to a highly versatile feeding mechanism. All ray-finned fishes have a pharyngeal jaw apparatus that is formed from modified gill-arch elements and used in prey processing (Lauder and Wainwright 1992). This second set of jaws has the potential to decouple prey capture from prey processing, allowing specialization of the oral and pharyngeal jaws for different functions and promoting trophic diversification. Several lineages of ray-finned fishes have evolved a derived condition of the pharyngeal jaws, termed “pharyngognathy” (but often simply “pharyngeal jaws” in the literature; Stroud and Losos 2016), which renders these jaws functionally more potent and represents a classic innovation hypothesized to have been a stimulus to adaptive radiation (Liem and Greenwood 1981). Pharyngognathy involves fusion of the left and right fifth ceratobranchial bones into a single lower pharyngeal jaw element, a synovial joint between the dorsal surface of the upper jaw bones and the underside

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Am. Nat. 2019. Vol. 194, pp. 693–706. © 2019 by The University of Chicago. 0003-0147/2019/19405-59144\$15.00. All rights reserved. DOI: 10.1086/705392

of the neurocranium, and a muscular sling that directly connects the lower pharyngeal jaw to the neurocranium (Liem and Greenwood 1981; Stiassny and Jensen 1987; fig. 1). This configuration provides a strong biting mechanism, which facilitates the exploitation of hard, tough, and chewy prey that would otherwise be difficult to process (Kaufman and Liem 1982; Liem 1986; Burress 2016).

Pharyngognathy has evolved independently at least five times but most famously in wrasses and parrotfishes (Labridae; Kaufman and Liem 1982) that dominate coral reef ecosystems and in cichlids (Liem 1973) that dominate tropical freshwater rivers and lakes throughout Africa and the Americas. In these groups, pharyngognathy is hypothesized to have played a central role in their proliferation and ecological diversity by facilitating the exploitation of hard-shelled prey, such as mollusks and armored crustaceans, as well as nutrient-poor prey, such as algae and detritus (Liem 1973; Yamaoka 1978; Hulsey 2006; Wainwright et al. 2012; Burress 2016). By permitting access to these hard-to-access trophic zones, pharyngognathy has expanded the adaptive landscape available to these clades.

While the trophic diversity of labrids and cichlids has been linked to the pharyngeal jaw innovation (Liem 1973; Hulsey 2006; Wainwright and Price 2016), patterns of uneven diversity are also apparent within these groups (Alfaro et al. 2009; Burress and Tan 2017). This observation raises the possibility that other factors mediate the macroevolutionary impacts of the pharyngeal jaw innovation. Why do some lineages exhibit elevated rates of functional and ecological diversification while others do not? One possibility is that secondary traits interact with pharyngognathy such that some trait combinations are more synergistic and associated with high diversity while others limit diversification, producing a pattern of ecological diversity that is mediated by the secondary trait.

In this study, we focus on the relationship between pharyngeal jaw shape and head shape and explore the possibility that head shape may mediate diversification of the lower pharyngeal jaw. Our study group is the monophyletic radiation of about 500 cichlid species found in the Americas. Pharyngeal jaw shape and head shape are known to evolve adaptively in cichlids, as both are related to feeding behavior and habitat use (López-Fernández et al. 2013; Seehausen 2015; Burress et al. 2018a, 2018b). The relative depth, length, and width of the head may relate to habitat use, locomotion, and mode of prey acquisition (Webb 1984). There is some indirect evidence of correlations between aspects of the lower pharyngeal jaw and other traits in cichlids. For example, the evolution of lower pharyngeal jaw mass and oral jaw protrusion are correlated in Middle American cichlids (Hulsey et al. 2006). In other studies, some traits have been linked insofar as they load together on a common principal component, such as the depth of the lower pharyngeal jaw,

head length, and head depth (López-Fernández et al. 2012); the mass of the lower pharyngeal jaw and the masses of some oral jaw muscles (Arbour and López-Fernández 2013); and oral jaw protrusion (Arbour and López-Fernández 2014). These relationships indicate the possibility of an interaction between the evolution of traits pertaining to the head and lower pharyngeal jaw.

To evaluate the relationship between head and pharyngeal jaw shape, we look for three potential patterns that would imply different dynamics in their evolution. First, lower pharyngeal jaw shape is unaffected by head shape, and similar levels of diversity of the pharyngeal jaws occur across all head shapes (fig. 2A). We would interpret this pattern as indicating no limitations imposed on pharyngeal jaw evolution by head dimensions. This pattern should also maximize the overall combined diversity of cichlid head and pharyngeal jaw shapes. Second, pharyngeal jaw shape is strongly correlated with head shape such that one is a strong predictor of the other. This pattern could come about if, for example, length and width of the pharyngeal jaw must match the respective head dimensions to achieve proper functioning (fig. 2B). Third, the diversity of pharyngeal jaw shape changes with head shape, suggesting that an extreme head shape imposes constraints on the pharyngeal jaws (fig. 2C). Such a pattern might occur if, for example, strongly laterally compressed fish are limited to pharyngeal jaws with a high aspect ratio (i.e., limited along the side-to-side axis), while pharyngeal jaw diversity is high for other head shapes.

Methods

Shape Measurements

We quantified head and lower pharyngeal jaw shape in 287 individuals representing 96 of the roughly 500 species of Neotropical cichlids and representing 54 of the 77 genera. We sampled species to capture the extensive morphological and ecological diversity found across South and Middle American cichlids (López-Fernández et al. 2010, 2013; Burress 2016). The lower pharyngeal jaw was dissected from preserved specimens and photographed in dorsal view. To quantify shape variation, we used principal component analysis of seven homologous and 10 sliding landmarks (fig. A1; figs. A1 and A2 are available online), adapted from other landmark schemes used to assess lower pharyngeal jaw shape in cichlids and other pharyngognathous fishes (Burress 2016; Aguilar-Medrano 2017; Burress et al. 2018a). Sliding landmarks were not associated with a homologous structure but were evenly spaced between homologous landmarks (fig. A1). Photographs were landmarked using tpsDIG2 (Rohlf 2006). We then superimposed images, aligned and slid landmarks, and generated principal components using tpsRelw (Rohlf 2007). Following recent recommendations to avoid conducting

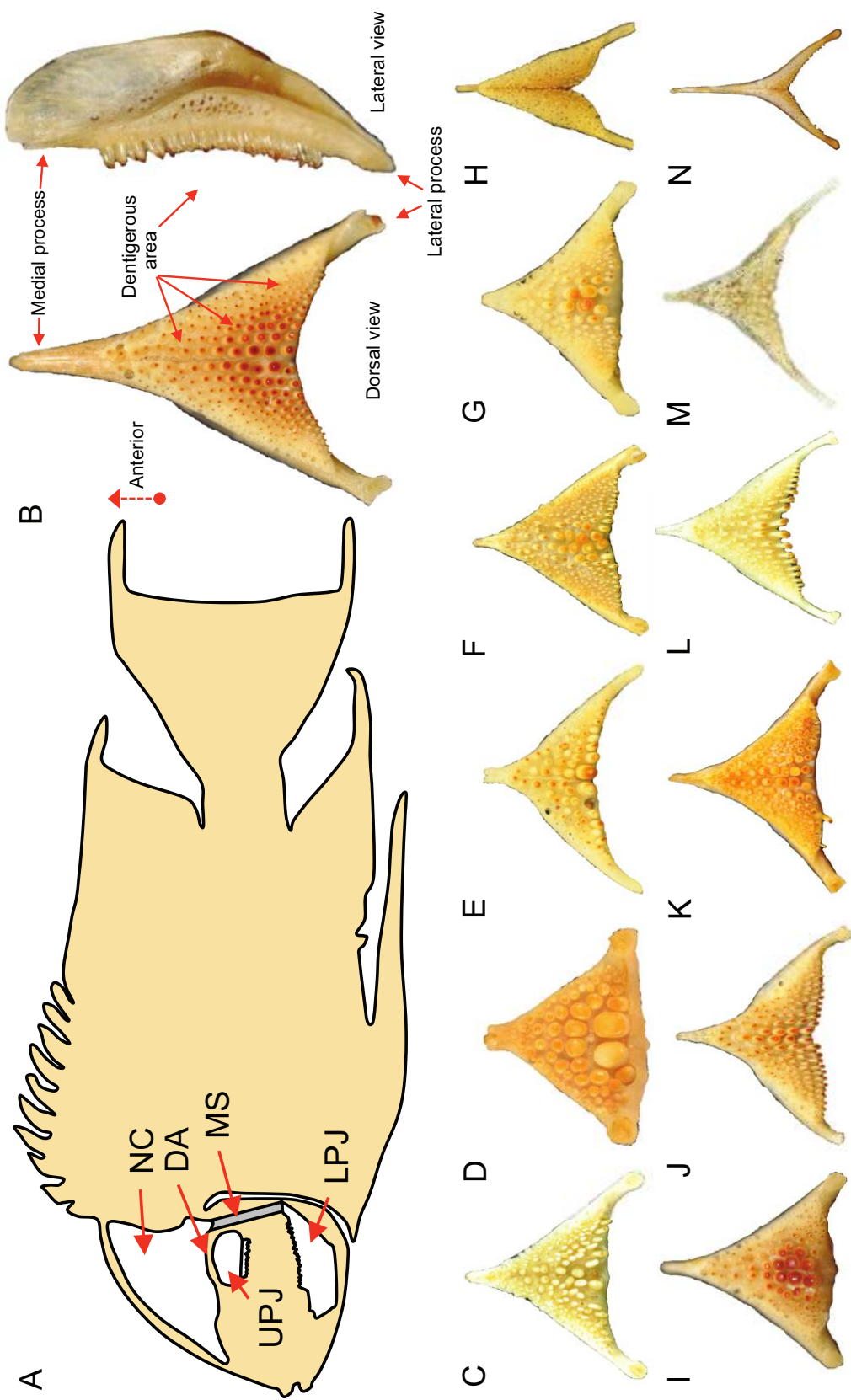


Figure 1: A, Diagram depicting the major pharyngognathous elements of a general cichlid: neurocranium (NC), upper pharyngeal jaw (UPJ), diarthrotic articulation (DA), lower pharyngeal jaw (LPJ), and muscular sling (MS). B, Diagram depicting the major features of the LPJ in dorsal and lateral view. Representative cichlid LPJ diversity: *Astronotus ocellatus* (C), *Crenicichla minuano* (D), *Crenicichla reticulata* (E), *Mayaheros urophthalmus* (F), *Australoheros scitulus* (G), *Cichla intermedia* (H), *Thorichthys aureum* (I), *Talamanchaeros sieboldii* (J), *Vieja maculicauda* (K), *Parachromis dovii* (L), *Crenicichla celidochilus* (M), and *Chaetobranchius flavescens* (N).

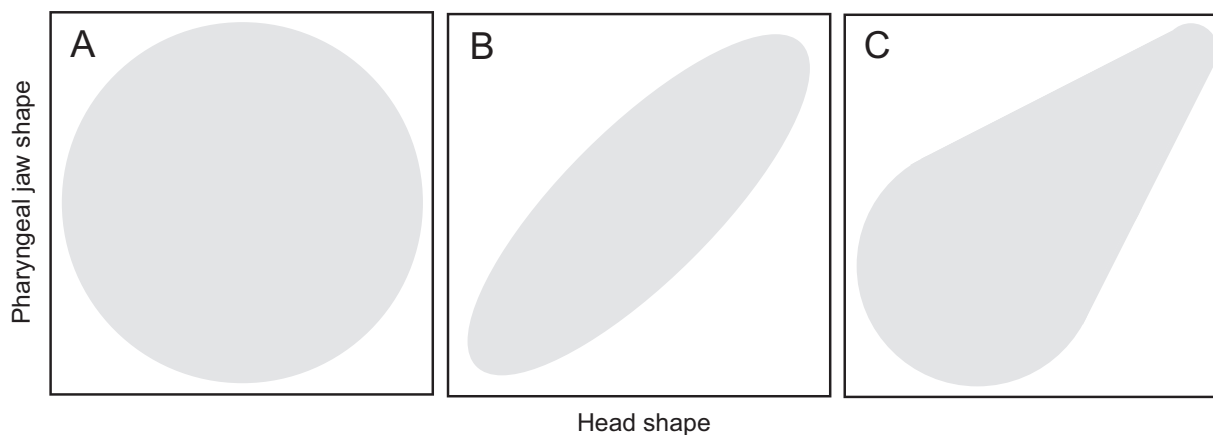


Figure 2: Hypothetical relationships between lower pharyngeal jaw (LPJ) shape and head shape. A, LPJ shape diversity is unconstrained by head shape. B, LPJ shape is strongly correlated with head shape dimensions. C, LPJ shape diversity is unevenly distributed across head shapes because extreme head shapes impose constructional constraints.

comparative analyses with isolated principal components for shape analyses (Uyeda et al. 2015; Adams and Collyer 2018), we first interpreted the major axis of pharyngeal jaw shape variation (i.e., PC1), which described approximately 62% of the shape variation in the dorsal view (fig. 3A). We then quantified this aspect of shape directly using linear measurements of the relative length and width of the lower pharyngeal jaw and calculated the aspect ratio (length/width) to represent jaw shape during subsequent comparative analyses (fig. 3B).

This ratio was highly correlated ($r^2 = 0.92$) with the first principal component from the morphometric analysis (fig. 3B). Linear distances were measured to the nearest 0.01 cm from photographs using the measure tool in tpsDIG2 (Rohlf 2006).

In addition, we measured the three major dimensions of head shape: head width, head depth, and head length. Head width and depth were measured at the posterior edge of the operculum (i.e., roughly at the position of the posterior

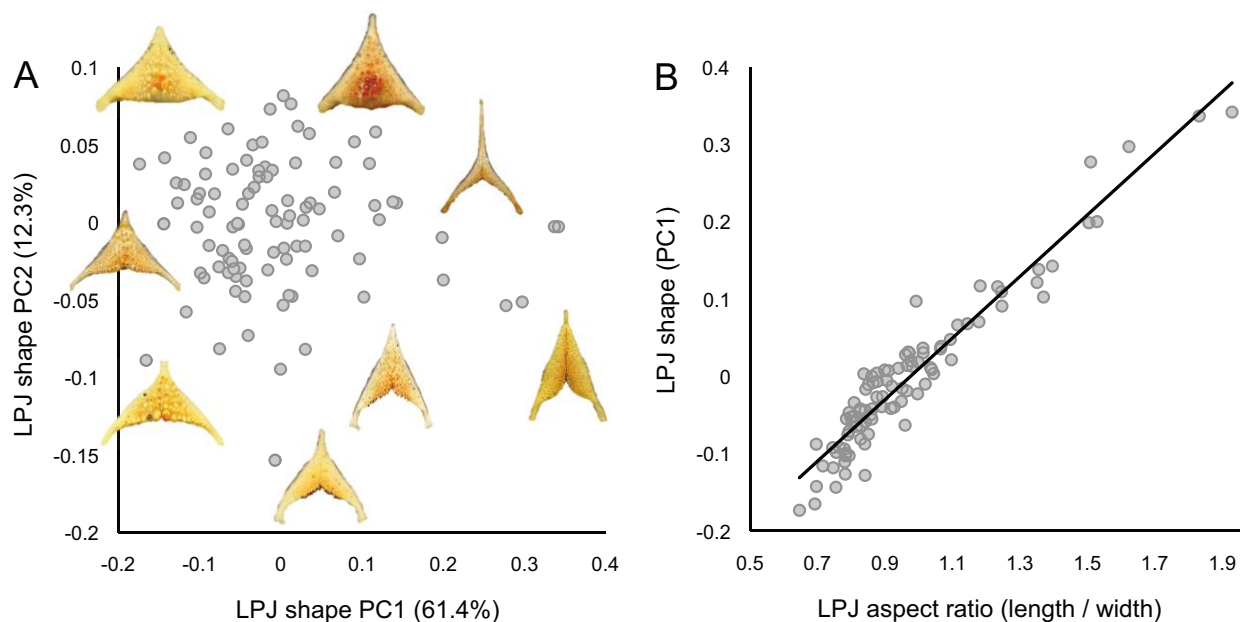


Figure 3: A, Major axes of lower pharyngeal jaw (LPJ) shape variation among 96 species of Neotropical cichlid based on landmark-based principal component analysis. Each point depicts a species mean. Inset images depict the LPJ shapes associated with the adjacent data. B, Relationship between the major axis of LPJ shape variation (PC1) and the aspect ratio (length/width) of the LPJ used in the comparative methods within the study.

margin of the pharyngeal jaw apparatus). Head length was measured from the posterior edge of the operculum to the anterior extent of the premaxilla. Measurements were log transformed, and residuals were calculated from regressions with log fish standard length. These size-corrected measures of head width, depth, and length were used in all subsequent analyses.

Phylogeny

For phylogenetic comparative analyses, we generated a distribution of time-calibrated phylogenetic trees that was densely sampled for cichlids. Briefly, we leveraged congruification to allow for the time calibration of densely sampled, untimed phylogenetic trees (target trees) based on reference trees (Eastman et al. 2013). We previously generated a distribution of densely sampled trees (originally from Burress and Tan 2017). First, to generate the densely sampled phylogenies for cichlids (target trees), we compiled phylogenetic data using the PHLAWD pipeline to query GenBank for the mitochondrial genes 12S, 16S, COI, cyt b, ND2, and ND4, as well as the mitochondrial control region, and the nuclear genes TMO4c4, enc1, RAG1, RAG2, S7 intron 1, SH3PX3, glyt, myh6, plagl2, ptr, sreb2, and tbr1. We concatenated sequences and converted alignment formats using AMAS (Borowiec 2016) and determined the best model of evolution and partitioning scheme available for RAxML using PartitionFinder 2 (Lanfear et al. 2016), and we then inferred a maximum likelihood tree and a distribution of 100 bootstrap replicates using RAxML version 8 (Stamatakis 2014). Next, we used the MCC file made available by Matschiner et al. (2017a, 2017b) as a reference tree for congruification. In their time-calibrated phylogenetic analysis of fishes, they used 147 fossil calibrations for the ages of 10 nodes within Cichlinae plus the crown node, as well as 136 outgroup nodes distributed across other teleost fishes. We time calibrated the 100 bootstrap target trees with the MCC reference time tree using the congruification method (Eastman et al. 2013) implemented in geiger version 2.0 (Pennell et al. 2014), in which shared nodes between the reference and the target are identified, and the ages of these nodes in the reference tree are used as calibrations for the target tree. The congruify and write.treePL functions were used to perform the congruification and generate input files for analysis in treePL (Smith and O'Meara 2012). After congruification, the resulting 100 time trees were used in all subsequent analyses to incorporate phylogenetic uncertainty.

Comparative Analyses

All statistical calculations were performed in R. To determine whether pharyngeal jaw and head shape evolution

were correlated across the phylogeny, we calculated phylogenetic independent contrasts (Felsenstein 1985) using the pic function in the APE package (Paradis et al. 2004). To test whether lower pharyngeal jaw shape diversity is affected by head shape, we employed the rate-by-state test (modified from Reynolds et al. 2016). In this procedure, the absolute values of standardized contrasts of pharyngeal jaw shape were regressed against the ancestral states of head width, depth, and length. The absolute values of standardized contrasts represent direction-indifferent point estimates of the Brownian rate (BM) parameter (Felsenstein 1985). Ancestral states of head width, depth, and length were calculated using the fastAnc function in phytools (Revell 2012), which estimates the ancestral states at nodes of the phylogeny using maximum likelihood (Felsenstein 1985). To account for phylogenetic uncertainty, we repeated the standardized contrast correlations and the rate-by-state tests across a distribution of 100 time trees. Furthermore, to determine whether the effect sizes (correlation coefficients) of the observed traits were beyond those expected from a null model of evolution such as BM, we constructed a null distribution by simulating variables from BM for each time tree using the fastBM function in phytools (Revell 2012) and repeating the phylogenetic independent contrast correlations and the rate-by-state tests for these 100 simulated data sets across the 100 time trees. We then compared the effect sizes of the observed traits directly to those of traits simulated under BM. We assessed the statistical significance of these relationships using robust regression, which weights data points according to their residual such that outliers are down weighted and has been shown to be useful when there are outliers but there is little rationale for their exclusion (Slater and Pennell 2013).

To determine possible evolutionary implications of the relationships between head and pharyngeal jaw shape, we categorized species in two ways. First, we classified species based on their trophic guild. Second, we classified species based on whether their diet included prey that require intense processing by the pharyngeal jaws. For guild delineation, we adapted and expanded existing trophic classifications to place species into broad trophic guilds based on the prey they feed on and the method of prey acquisition (adapted from Burress 2016 and references therein): specialist piscivores, generalist predators, invertivores, sifting and nonsifting detritivores, omnivores, algivores, molluscivores, and planktivores. When possible, these guild delineations were made based on detailed stomach content analyses (46 species; Burress 2016 and references therein) but when absent were based on general descriptions of diets and ecology (e.g., Lowe-McConnell 1969; Bussing 1976). Specialist piscivores consume almost exclusively fishes and switch to piscivory when young (e.g., *Cichla*). General predators are species that consume a mixture of fishes and soft-bodied invertebrates (e.g., *Mayaheros*). Invertivores consume

exclusively small soft-bodied insects and crustaceans and are usually diminutive in size (e.g., *Mikrogeophagus*). Omnivores consume mixtures of animal and plant material and are pickers that do not feed by sifting (e.g., *Aequidens*). Detritivores consume mixtures of decaying algae, woody debris, and leaves and were divided into two groups based on their foraging mode. Sifting detritivores feed principally by plunging their snout into the substrate, taking a mouthful of material, and then sifting inedible items through the gill openings or expelling them from the mouth (e.g., *Geophagus*). Nonsifting detritivores are pickers rather than sifters (e.g., *Heros*). Algivores consume principally algae that is scraped from the surface of rocks or the substrate (e.g., *Neotroplus*). Molluscivores consume large fractions of hard-shelled prey including snails and/or bivalves (e.g., *Thorichthys*). Last, planktivores filter zooplankton (e.g., *Chaetobranchius*) with elongate, densely packed gill rakers. Additionally, we categorized species as having processing-intensive or non-processing-intensive diets. Mollusks and algae were considered processing-intensive prey items due to their hard-to-crush shells and hard-to-digest cells, respectively, and for which pharyngeal jaws play central roles during processing (Xie 2001; Hulsey et al. 2005; Hulsey 2006; Burrell 2016). This scheme allows us to emphasize those specific processing-intensive prey items and highlight instances in which they were secondary or tertiary prey items, which were often overlooked in the trophic guild classifications that emphasized major prey types.

To determine whether these guilds have different trait optima, we employed an evolutionary model-fitting framework. We specified trophic guilds as a priori selective regimes and fitted four models of trait evolution using the OUwie function employed in the OUwie R package (Beaulieu et al. 2012; Beaulieu and O'Meara 2015). The evolutionary histories of these selective regimes along the internal nodes and branches of the phylogenetic tree were estimated prior to model fitting using Bayesian stochastic character mapping (Huelsenbeck et al. 2003) with the make.simmap function in the phytools package (Revell 2012). Fitted models include (1) single-rate BM that permits a single regime and trait evolution that proceeds as a random walk and trait variance that increases proportional to time (Felsenstein 1985), (2) single-optimum Ornstein-Uhlenbeck (OU1) model that constrains trait evolution toward a single value (θ) and allows a single α and σ^2 across all selective regimes, and (3) multi-peak Ornstein-Uhlenbeck (OUM) model that permits different state means (θ) and a single α and σ^2 across all selective regimes. Model fit was evaluated using a modified Akaike information criterion (AICc) that incorporates a correction for small sample size (Burnham and Anderson 2002; Burnham et al. 2011). These analyses were repeated across 100 trees from the posterior distribution to account for phylogenetic uncertainty. To ensure that we could properly distinguish

among these models, we simulated data sets under BM, OU1, and OUM processes and then fitted those same three models to the simulated data sets (table A1; tables A1–A3 are available online). Multi-peak OU models were fitted separately using selective regimes defined by (1) trophic guilds and (2) processing-intensive nature of prey items.

Results

Morphological Traits and Comparative Analyses

Standardized independent contrasts of head width, depth, and length were uncorrelated (table A2), indicating that these variables were independent characterizations of head shape. Variation in pharyngeal jaw shape markedly declined with head width (fig. 4A), suggesting a possible constraint on pharyngeal jaw form in species with wide heads. In contrast, pharyngeal jaw diversity was unaffected by head depth (fig. 4B) or head length (fig. 4C). Head width and pharyngeal jaw shape were significantly correlated (fig. 4D) across 97 of the 100 time trees (table 1). In contrast, head depth and length were not correlated with pharyngeal jaw shape (fig. 4E, 4F). These nonsignificant relationships were consistent across all 100 time trees (table 1).

The rate of pharyngeal jaw shape evolution was significantly predicted by the ancestral state of head width (fig. 4G) such that narrower-bodied nodes exhibited faster rates of pharyngeal jaw evolution. This result was consistent across all 100 time trees (table 2). In contrast, the rate of pharyngeal jaw shape evolution was not correlated with the ancestral state of head depth (fig. 4H) or head length (fig. 4I), and these nonsignificant relationships were consistent across all 100 time trees (table 2). To demonstrate that these findings are robust to the decision to use a univariate characterization of the pharyngeal jaw, we demonstrated that the same relationships between pharyngeal jaw diversity and head dimensions are recovered if the pharyngeal jaw aspect ratio is exchanged for morphological disparity calculated with Procrustes distances from the matrix of the aligned specimens (table A3; fig. A2).

Traits simulated with BM were significantly correlated across only seven of the 100 time trees (table 1). The effect size estimates for observed head width were essentially non-overlapping with the null distribution, whereas observed head depth and length effect size estimates were largely overlapping with the null distribution (table 1; fig. 5). Similarly, using traits simulated with BM, rates of evolution were correlated with ancestral states across only six of the 100 time trees (table 2). The effect size estimates for observed head width were essentially nonoverlapping with this null distribution, whereas observed head depth and length effect size estimates were largely overlapping with the null distribution (table 2; fig. 5).

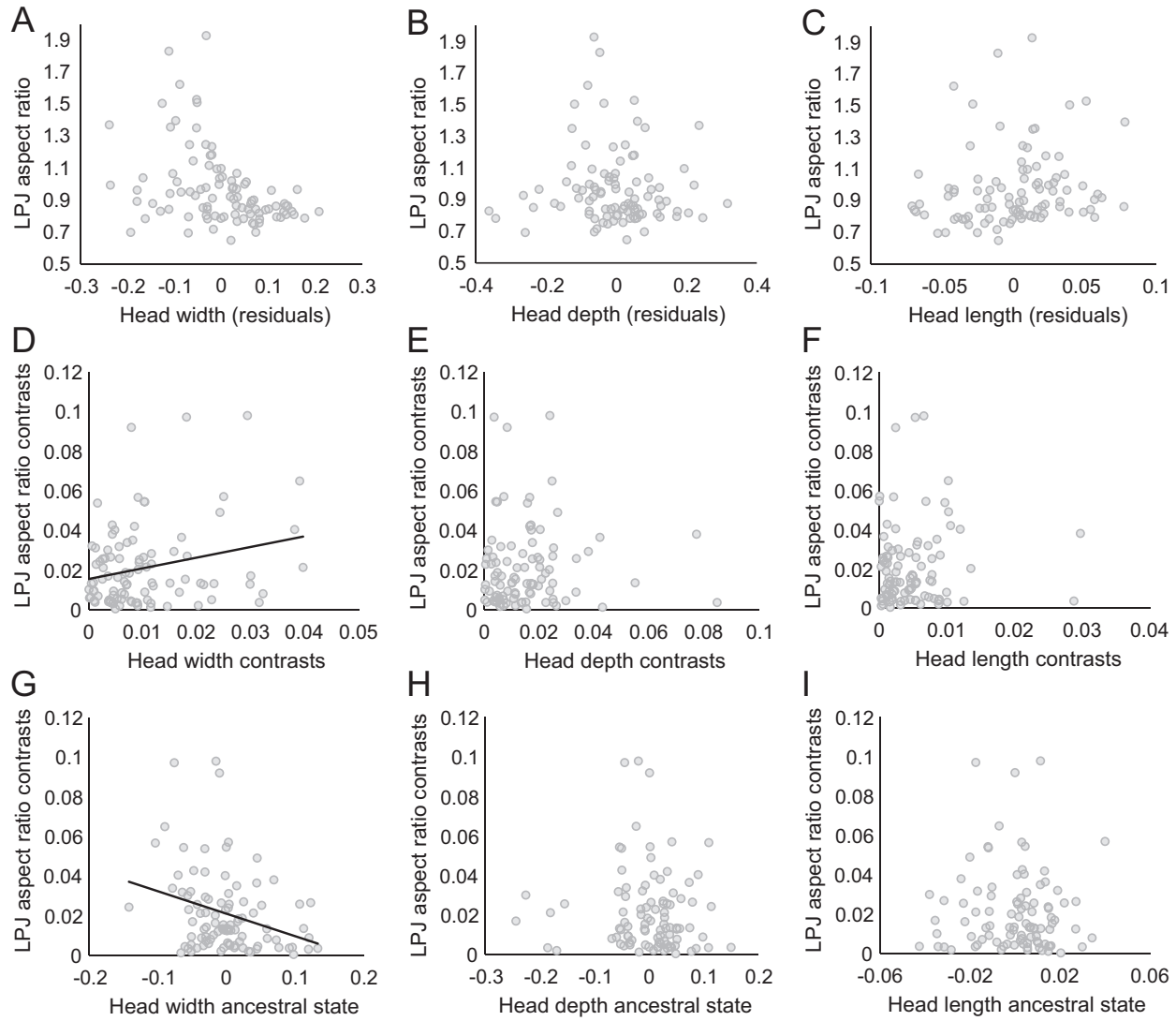


Figure 4: Relationships between pharyngeal jaw aspect ratio and size-relative aspects of head shape, the rates of pharyngeal jaw and head shape evolution, and rate-by-state analyses depicting the rate of pharyngeal jaw shape evolution as a function of ancestral states of head width (A–C), head depth (D–F), and head length (G–I). Data are based on the MCC tree, but see table 2 for statistics summarized from 100 timed trees from a posterior distribution.

Functional Implications

Trophic guilds were well distributed across pharyngeal jaw shapes, including several that are associated with specific pharyngeal jaw shapes such as piscivory, substrate sifting, and algivory (fig. 6). However, several trophic guilds were absent from the top quartile of relative head width (fig. 6), either because their associated pharyngeal jaw shapes were absent (i.e., specialist piscivores, planktivores, and sifting detritivory) or because they were absent despite having suitable pharyngeal jaw shapes (i.e., nonsifting detritivory, invertivory; fig. 6). Species with narrow and intermediate head width exhibited a wider array of trophic guilds, whereas rel-

atively wide-headed species were principally general predators or omnivorous (fig. 6). Processing-intensive diets were largely associated with having a wide head (fig. 6).

Evolutionary Model Fitting

Each fitted model best fit data simulated under the corresponding model (table A1). Head length best fit the single-peak OU model across all 100 trees (table 3). Head depth best fit the multi-peak OU model with processing intensity selective regimes across 71 of the 100 trees (table 3). Head width similarly fit the multi-peak OU models with processing

Table 1: Relationships between the rates of head and lower pharyngeal jaw (LPJ) evolution based on absolute values of phylogenetically independent contrasts

Rate _x	Rate _y	<i>r</i> (range)	<i>P</i> (range)	Prop. <i>P</i> <.05
Head width	LPJ shape	.26 (.19–.36)	.067 (.003–.18)	.43
Head depth	LPJ shape	.05 (.00–.18)	.43 (.006–.94)	.01
Head length	LPJ shape	.09 (.02–.18)	.36 (.03–.82)	.03
Simulated	Simulated	.10 (.00–.30)	.48 (.01–.99)	.07

Note: Statistics are summarized from analyses calculated across 100 timed trees from a posterior distribution. Correlation coefficients (*r*) test the hypothesis that the rates of head and LPJ morphological evolution are correlated. *P* denotes mean (range) statistical significance based on robust regression. Prop. *P* = proportion of the 100 timed trees in which the estimate of *r* is significant.

intensity and trophic guild selective regimes, which best fit over 52 and 23 of the 100 trees, respectively (table 3). The aspect ratio of the lower pharyngeal jaw best fit the multi-peak OU model with trophic guilds as selective regimes across all 100 trees (table 3). Because there were more than two selective regimes in this model, we examined the θ estimates for head width and the aspect ratio of the lower pharyngeal jaw to determine which trophic guilds have different optima. Specialist piscivory, planktivory, sifting detritivory, and nonsifting detritivory have distinct trait optima in one or both traits, whereas the remaining guilds had similar optima (fig. 6). Notably, there were no optima located in the upper quartile of head width (fig. 6). The nonsifting detritivory optima was estimated along the periphery of the shape space occupied by members of that guild, possibly indicating rapid adaptation to that optima (Ingram and Kai 2014), a phenomenon that has been reported in other fishes (Friedman et al. 2016; Burress et al. 2018b).

Discussion

The diversification of cichlid pharyngeal jaws is modulated by head width. While pharyngeal jaw shape evolves independently of head length and depth, we found that species of Neotropical cichlid with wide heads have reduced pharyngeal jaw diversity, being restricted to lower pharyngeal jaws with low aspect ratio and laterally oriented processes (fig. 6). This head shape constraint on pharyngeal jaw evo-

lution is further indicated by the negative relationship between head width and the rate of lower pharyngeal jaw shape evolution. Wide heads were found in species that exploit prey that require extensive pharyngeal processing, such as mollusks and algae; however, trophic diversity is low among these fish, and several trophic guilds are absent, including piscivory, planktivory, and sifting and nonsifting detritivory. A wide head appears to interact with the lower pharyngeal jaw in a way that limits the combinations of shapes that are functionally viable, effectively restricting access to some feeding niches and mediating diversification of the pharyngeal jaws.

There is ample evidence that the pharyngeal jaws underlie much of the functional and ecological diversity of several extraordinarily diverse lineages of fishes, including marine wrasses and freshwater cichlids. Wrasse and parrotfishes (Labridae) often dominate coral reef ecosystems where pharyngeal jaws facilitate feeding on hard and tough items, including coral, mollusks, crustaceans, and algae (Price et al. 2011). Cichlids also exhibit considerable trophic diversity, including molluscivory (Hulsey 2006), as well as lineages that scrape algae from rocky surfaces in Lake Malawi (Reintal 1990; Genner et al. 1999; Hulsey et al. 2013), Lake Tanganyika (Rüber et al. 1999; Rüber and Adams 2001; Wagner et al. 2009; Tada et al. 2017), and Middle America (Řičan et al. 2016). Pharyngeal jaws play an important role in processing cellulose-rich food, such as algae, apparently by rupturing cells during mastication, which facilitates digestion

Table 2: Rate-by-state analyses of lower pharyngeal jaw (LPJ) evolutionary rates as a function of ancestral states of head shape

Trait/model	Rate	<i>r</i> (range)	<i>P</i> (range)	Prop. <i>P</i> <.05
Head width	LPJ shape	.30 (.24–.34)	.005 (.0004–.04)	1.00
Head depth	LPJ shape	.07 (.02–.12)	.50 (.17–.80)	.00
Head length	LPJ shape	.02 (.00–.10)	.83 (.53–.99)	.00
Simulated	Simulated	.10 (.00–.29)	.48 (.03–.99)	.06

Note: Statistics are summarized from analyses calculated across 100 timed trees from a posterior distribution. Correlation coefficients (*r*) test the hypothesis that ancestral head shapes can predict the rates of LPJ morphological evolution. *P* denotes mean (range) statistical significance based on robust regression. Prop. *P* = proportion of the 100 timed trees in which the estimate of *r* is significant.

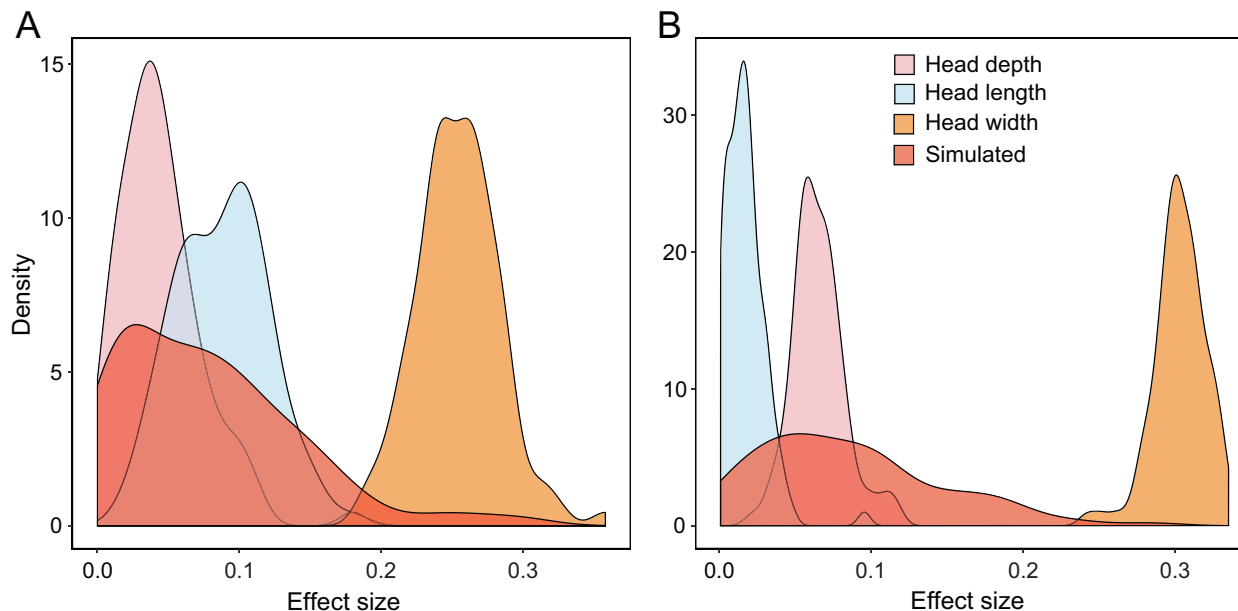


Figure 5: Distribution of effect sizes (correlation coefficients) of the relationship between the rates of lower pharyngeal jaw (LPJ) and head shape evolution (A) as well as the relationship between the rate of LPJ shape evolution and the ancestral state of head shape (B) across 100 time trees. Simulated traits were simulated across the phylogenetic trees using Brownian motion. See tables 1 and 2 for summary statistics of each test.

(Xie 2001). Likewise, the evolution of benthivory, particularly substrate sifting, was associated with an early burst of morphological diversification in Neotropical cichlids (Arbour and López-Fernández 2013; López-Fernández et al. 2013). The lower pharyngeal jaw appears to play an important role during substrate sifting, with rapid cyclic adduction and abduction appearing to characterize the winnowing behavior that helps these fish separate minute edible particles from mouthfuls of substrate (López-Fernández et al. 2014). Additionally, many sifting species exhibit a pharyngeal jaw shape that is found only in association with that trophic function (fig. 6). The pharyngeal jaw innovation has facilitated access to many trophic niches that are otherwise poorly accessible to fishes (McGee et al. 2015).

Our results indicate that the diversity of Neotropical cichlid pharyngeal jaws depends on head shape. Therefore, factors that drive head shape evolution may indirectly impact the capacity of cichlids to colonize available trophic niches. A wide head may be conducive to exploiting processing-intensive prey such as mollusks and algae (fig. 6) by accommodating a stout pharyngeal jaw with laterally oriented processes and expanded surfaces on both the jaw and the neurocranium to attach robust pharyngeal muscles. These characteristics are especially favorable when the generation of biting force is paramount, as is the case for crushing shelled organisms and rupturing algae cells (Xie 2001; Hulsey et al. 2005; Hulsey 2006; Burrell 2016). These features

also characterize the central functional gain provided by the pharyngeal jaw innovation by providing access to prey that require extensive pharyngeal processing, evidenced by the elevated rate in which processing-intensive diets have evolved in pharyngognathous fishes (McGee et al. 2015). However, an apparent implication of having a wide head is limited subsequent access to alternative trophic niches. We found that species with wide heads exhibit restricted trophic diversity because the pharyngeal jaw shapes associated with several guilds do not occur in combination with this head shape.

No wide-headed species in our data set has a lower pharyngeal jaw with high aspect ratio. What functional considerations might make a wide head and pharyngeal jaw with narrowly spaced lateral processes incompatible? There may be spatial considerations at play concerning the proper functioning of the pharyngeal jaws and integration with surrounding structures (Barel 1982; Smits et al. 1996). The cichlid pharyngeal jaw has large levator posterior and fourth levator externus muscles, which originate on the ventral-lateral margin of the neurocranium and insert on the lateral processes of the lower pharyngeal jaw, providing the strong bite characteristic of pharyngognathous fishes (Stiassny and Jensen 1987; Hulsey et al. 2006). For the lateral processes to be positioned directly ventral to the position of these muscles, the lower pharyngeal jaw must be about as wide as the skull. Thus, if a pharyngeal jaw with high aspect ratio were

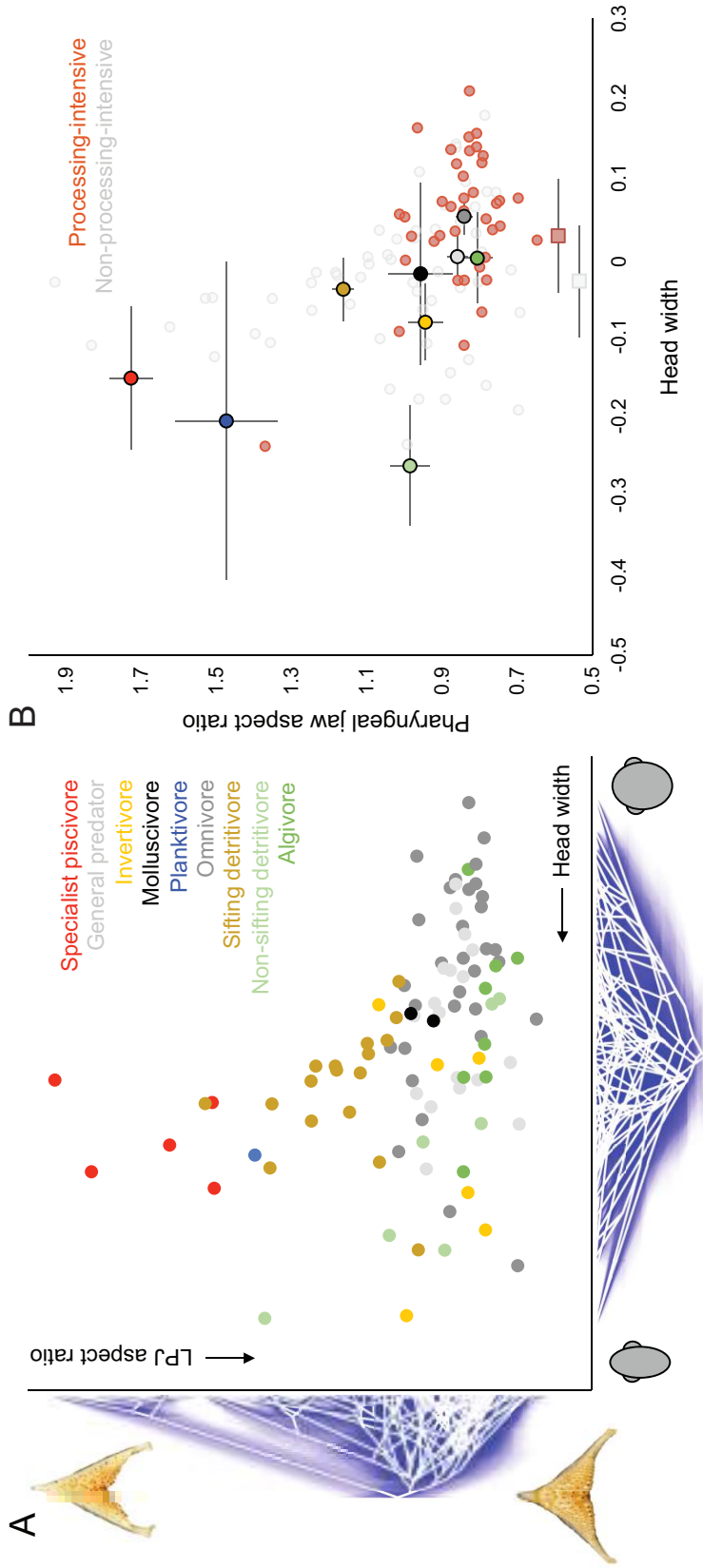


Figure 6: Relationship between lower pharyngeal jaw (LPJ) shape and head width and the distribution of cichlid diets within morphospace. *A*, Points depict species means and their corresponding trophic guild. Maximum-likelihood ancestral state reconstructions are shown beside each axis to demonstrate that multiple lineages occupy the extremes of each axis. *B*, Large points depict the mean (\pm SE) estimated trait optima associated with trophic guild selective regimes. Small points in *B* depict species means and the degree to which the species' diet requires extensive processing. Squares in *B* depict the mean (\pm SE) estimated head width optima associated with processing intensity selective regimes. Note that the scale of the X-axis is slightly different in each panel to accommodate the error bars in *B*.

Table 3: Macroevolutionary model fitting of the aspect ratio of the lower pharyngeal jaw (LPJ) and head dimensions using trophic guild (OUM_g) and processing intensity (OUM_p) as a priori selective regimes

Trait and model	ln L	AICc	ΔAICc	Prop.
LPJ aspect ratio:				
BM	32.8 (29.5–35.0)	−61.5 (−65.8 to −54.8)	64.3	.00
OU1	33.5 (30.8–35.3)	−60.8 (−64.6 to −55.3)	64.9	.00
OUM _g	75.5 (50.5–81.3)	−125.8 (−137.4 to −75.8)	.0	1.00
OUM _p	34.7 (32.4–37.2)	−60.9 (−66.0 to −56.4)	64.9	.00
Head width:				
BM	104.0 (97.3–106.5)	−203.9 (−208.9 to −190.4)	3.7	.00
OU1	105.7 (101.4–107.4)	−205.2 (−208.5 to −196.5)	2.7	.25
OUM _g	116.3 (108.0–127.3)	−207.5 (−229.5 to −190.9)	5.3	.23
OUM _p	108.0 (104.3–113.4)	−207.5 (−218.4 to −200.2)	.0	.52
Head length:				
BM	170.8 (161.9–176.9)	−337.4 (−349.8 to −319.7)	32.9	.00
OU1	188.3 (187.9–188.9)	−370.3 (−371.6 to −369.6)	.0	1.00
OUM _g	194.1 (191.6–196.7)	−363.0 (−368.3 to −358.1)	7.3	.00
OUM _p	188.6 (188.0–189.7)	−368.9 (−370.9 to −367.6)	1.5	.00
Head depth:				
BM	63.5 (53.21–63.6)	−122.9 (−132.1 to −102.2)	19.1	.00
OU1	73.1 (69.1–75.3)	−139.9 (−144.4 to −132.0)	2.4	.12
OUM _g	81.7 (79.4–86.5)	−138.3 (−147.7 to −133.5)	4.7	.17
OUM _p	75.2 (71.6–80.7)	−142.0 (−153.0 to −134.8)	.0	.71

Note: Values depict the log likelihood (ln L), the Akaike information criterion adjusted for sample size (AICc), the mean difference in AICc relative to the best-fit model across 100 trees (ΔAICc), and the proportion of the 100 trees in which each model was the best fit based on AICc (Prop.). BM = Brownian motion; OU1 = single-peak Ornstein-Uhlenbeck; OUM = multi-peak Ornstein-Uhlenbeck.

to fit into a wide-headed fish, it would have to be large in overall size, with a relatively long anterior-posterior axis, possibly forcing a poor match with other structures in this region of the buccal cavity. Furthermore, unusual enlargement of the lower pharyngeal jaw might reduce available space in the buccal cavity that may be necessary for some processing tasks (i.e., winnowing; Hoogerhoud 1987) as well as require reallocation of space dedicated to respiratory structures (i.e., the size and shape of the gills; Witte et al. 1990; Smits et al. 1996). These trade-offs may deter the evolution of pharyngeal jaws with narrowly spaced lateral processes in wide-headed species.

While we found significant effects of head width on pharyngeal jaw diversity, we did not find similar effects with head depth or length. Species that exploit processing-intensive prey tended to have deeper heads (table 3); however, this dimension of head shape, unlike head width, does not have optima associated with trophic guilds and therefore may be unlikely to influence further trophic diversification or pharyngeal jaw diversity. Head length does not have optima associated with trophic guilds or processing intensity. This trait may be associated with different aspects of these species' ecology, such as adaptation to different habitats, locomotion modes, and/or flow regimes (Webb 1984), for which there is some evidence in Neotropical cichlids (López-Fernández et al. 2013). Alternatively, head length may reflect functioning of the oral jaws and prey acquisition, rather than the pharyngeal jaws and

prey processing, which is also a notable axis of diversification in Neotropical cichlids (Arbour and López-Fernández 2014).

The relationship we find between head width and pharyngeal jaw shape in New World cichlids can be thought of as a constraint on pharyngeal jaw evolution and apparently also on trophic niche diversification. It will be interesting in future work to explore this system in the major radiations of cichlids found in East African rift lakes. These radiations are renowned for their extensive ecological diversity, and one might ask whether they show a similar constraint or have found a way to mitigate the effect of head width on pharyngeal jaw diversification.

For an innovation to dramatically impact diversification, it must be paired with an environment in which sufficient resources are available to accommodate ecological expansion (Vermeij 2001, 2015). The repeated colonization of coral reefs by wrasses and lakes and rivers by cichlids likely set the stage for innovations to facilitate adaptive radiation in both of these groups (Salzburger et al. 2005; Price et al. 2011; Burrell and Wainwright 2018). Innovations must also synergistically align with other traits, such as adjacent anatomical systems (Hoogerhoud 1987; Witte et al. 1990; Smits et al. 1996), and contend with any functional or ecological trade-offs (McGee et al. 2015). We demonstrate that head width modulates evolution of pharyngeal jaw shape, so that most of the trophic diversity found in New World cichlids is

achieved only in species with narrow or moderately wide heads. There appears to be a clear trade-off between adaptation of the pharyngeal jaws to generating a strong bite, the corresponding evolution of a wide head to accommodate a robust pharyngeal jaw and expanded surfaces for muscle attachment, and diversification into other trophic niches for which this morphology is poorly suited. Thus, the adaptive potential of pharyngognathy, a morphological innovation that provided access to processing-intensive prey and underlies much of the exuberant ecological diversity in this group, depends on head width. A constraint on the independent evolution of pharyngeal jaw and head shape mediates diversification of a major innovation and leads to differential access to regions of the adaptive landscape.

While it is attractive to expect that major innovations have pervasive impacts on the lineages in which they evolve, the present study highlights the likelihood that a more nuanced framework is warranted when considering the macroevolutionary impacts of many functional innovations. It seems likely that the capacity of secondary traits to interact with the stimulating or constraining effects of novelties on ecological and morphological diversification may be a general macroevolutionary principal. In this case, the search for key innovations across the tree of life might be expanded to include the search for key trait interactions that have helped shape life's diversification.

Acknowledgments

We are grateful to Jennifer Hodge and Sarah Friedman for feedback on an early version of the manuscript. The distribution of time trees and the morphological data set are available in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.q5846sq>; Burress et al. 2019). Support for this research was provided by National Science Foundation grant DEB-1556953. Thanks to the Auburn University Museum of Natural History for providing access to the specimens used in this study. Feedback from an editor and two anonymous reviewers greatly improved the manuscript.

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Editor: Russell Bonduriansky



Gymnogeophagus terrapurpura from a stream in southern Uruguay. This species is an example of a head shape that is associated with high pharyngeal jaw shape disparity in Neotropical cichlid fishes. Photo credit: Edward Burress.