

The cichlid pharyngeal jaw novelty enhances evolutionary integration in the feeding apparatus

Alexus S. Roberts-Hughes¹, Edward D. Burress², Brian Lam¹, Peter C. Wainwright¹

¹Department of Evolution and Ecology, University of California–Davis, Davis, CA, United States

²Department of Biological Sciences, University of Alabama, Tuscaloosa, AL, United States

Corresponding author: Department of Evolution and Ecology, University of California–Davis, 1 Shields Avenue, Davis, CA 95616, United States. Email: asroberts@ucdavis.edu

Author note: This study was funded in part by the Society for the Study of Evolution's Graduate Excellence Research Grant–Rosemary Grant Advanced Award.

Abstract

The modified pharyngeal jaw system of cichlid fishes is widely viewed as a key innovation that substantially facilitated the evolutionary exuberance of this iconic evolutionary radiation. We conduct comparative phylogenetic analyses of integration, disparity, and rate of evolution among feeding-related, skeletal structures in Neotropical cichlids and North American centrarchids, which lack the specialized pharyngeal jaw. Contrasting evolutionary patterns in these two continental radiations, we test a classic decoupling hypothesis. Specifically, we ask whether the modified pharyngeal jaw in cichlids resulted in enhanced evolutionary independence of the oral and pharyngeal jaws, leading to increased diversity of trophic structures. Contrary to this prediction, we find significantly stronger evolutionary integration between the oral and pharyngeal jaws in cichlids compared to centrarchids, although the two groups do not differ in patterns of integration within each jaw system. Further, though we find no significant differences in disparity, centrarchids show faster rates of morphological evolution. Our results suggest that the modified pharyngeal jaw resulted in less evolutionary independence and slower rates of evolution within the feeding system. Thus, we raise the possibility that the cichlid novelty enhances feeding performance, but does not prompt increased morphological diversification within the feeding apparatus, as has long been thought.

Keywords: pharyngognathy, Cichlidae, Centrarchidae, evolutionary decoupling, morphological innovation

The mechanisms whereby evolutionary novelties influence the nature and pace of diversification are a central issue in our understanding of the history of life. Functional innovations, including skeletal and physiological modifications that enhance an organism's performance, are often cited as key drivers of ecological diversification due to increased accessibility to new niche space (Heard & Hauser, 1995; Hunter, 1998; Mayr, 1963; Schluter, 2000; Simpson, 1944; Vermeij, 2001). However, additional mechanisms may also influence intrinsic evolvability within a functional system (Payne & Wagner, 2019; Wagner et al., 2007; Wake & Roth, 1989). Operating at the anatomical level, a prime example is the concept of functional decoupling. Here, one or more functions of a structural system are transferred to another system, leaving the original structure to respond to natural selection on its primary function without the constraints imposed by performing two or more functions (Liem, 1990; Walker, 2007). Functional decoupling has the potential to significantly impact organismal diversification (Labandeira, 1997; Lauder, 1990) as a release of constraint permits a system to evolve adaptively in ways that may have been prevented previously by the demands of being multifunctional (Schaefer & Lauder, 1986). While functional decoupling is widely recognized as playing a key role in gene (Ohno, 2013; Zhang, 2003) and morphological (Schaefer & Lauder, 1996) evolution, the effect of decoupling at a macroevolutionary level remains controversial (Corn et

al., 2021; Farina et al., 2019a; Gatesy & Middleton, 1997; Holzman et al., 2012; Muñoz et al., 2018).

The feeding apparatus in ray-finned fishes presents a model system for the study of functional decoupling due to the development of the pharyngeal jaw apparatus. Located posterior to the mouth cavity, this is a second jaw system that functions in manipulating and processing prey, thus relieving the oral jaws from a major role in these functions (Lauder, 1983; Wainwright, 2005). Although pharyngeal jaws are found throughout ray-finned fishes (Actinopterygii), pharyngognathy—a complex modification of the pharyngeal jaws—has evolved independently in several highly successful lineages within spiny-rayed fishes (Acanthomorpha; Mabuchi et al., 2007; Wainwright et al., 2012), including cichlids, wrasses, and damselfishes among a few other clades (Kaufman & Liem, 1982; Stiassny & Jensen, 1987). The components of pharyngognathy—including the fusion of the paired lower pharyngeal jaw bones into a singular skeletal structure; a mobile joint between the base of the neurocranium and the dorsal surface of each paired upper pharyngeal jaw bone; and a muscular sling that suspends the lower pharyngeal jaw directly from the neurocranium (Figure 1a)—have been shown to enhance the strength of the prey processing system (Galis & Drucker, 1996; Hulsey & García De León, 2005; Kaufman & Liem, 1982; Lauder, 1983; Liem & Sanderson, 1986; McGeer et al., 2015). Further, because this novel pharyngeal

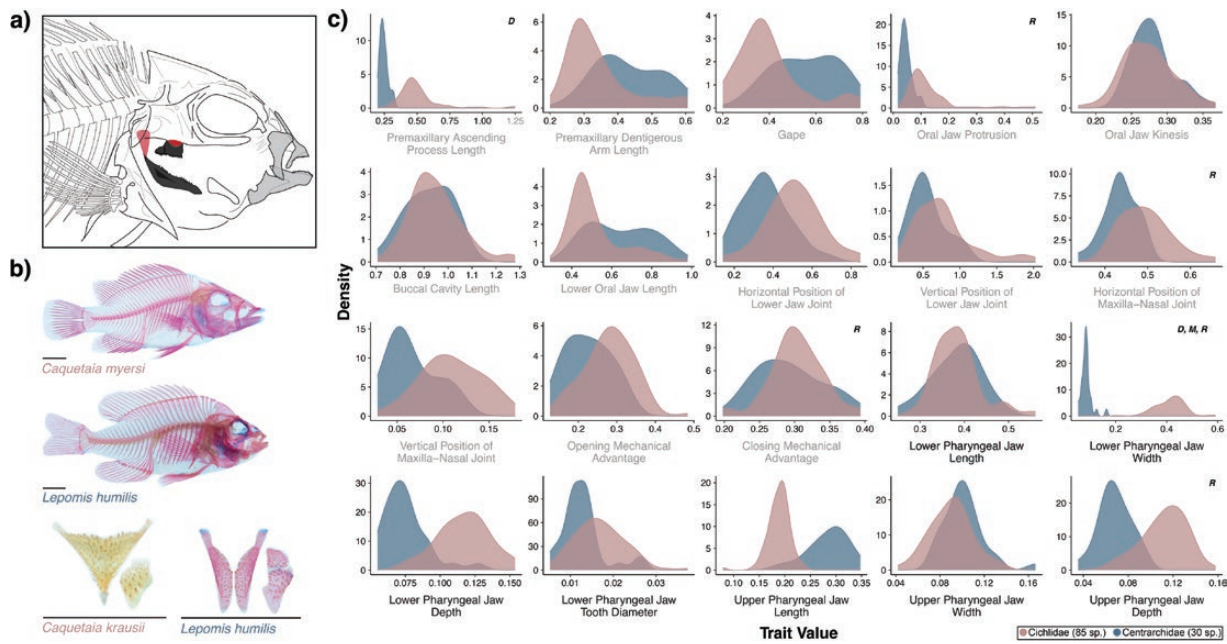


Figure 1. (a) Illustration depicting the oral (gray) and pharyngeal (black) jaw structures involved in prey capture and processing functions, respectively. Though the prey processing apparatus is very similar across ray-finned fishes, pharyngognathous lineages have several modifications, including a fused lower pharyngeal jaw bone (see *C. krausii* in b), a mobile joint between each of the paired upper pharyngeal jaw bones and the neurocranium (red ellipse), and a muscular sling suspending the lower pharyngeal jaw from the neurocranium (red lined triangle). (b) Cleared and stained specimens of *Caquetaia myersi* (top) and *Lepomis humilis* (bottom) exemplify cichlids and centrarchids, respectively. Pharyngeal jaws dissected from *Caquetaia krausii* (left—cichlid) and *Lepomis humilis* (right—centrarchid) display skeletal differences between fishes with and without modified pharyngeal jaws, respectively. Black scale bars represent 10 mm. (c) Density plots show the interspecific variation in shape ratios for the 13 oral (gray titles) and 7 pharyngeal (black titles) jaw traits in 85 cichlids (mauve) and 30 centrarchids (blue), averaged by species. Though two traits show a significant difference in the magnitude of morphological disparity ($p \leq 0.05$ —*D*; Table 1), lower pharyngeal jaw width is the only trait where cichlids and centrarchids show a significantly different mean trait value ($p = 2.90^{03}$ —*M*). Univariate estimates of the Brownian rate parameter reveal that centrarchids show significantly faster evolution for five traits spanning both jaw units (1.90 to 4.60-fold difference; $p < 0.05$ —*R*). Trait measurements and definitions can be found in Supplementary Tables 1 and 2.

jaw configuration (hereafter, “modified pharyngeal jaw” or MPJ) results in the ability to break down harder and tougher prey, it has played a key role in trophic diversification within prominent lineages of tropical freshwater and coral reef fishes (Alfaro et al., 2009; Burress, 2016; Evans et al., 2019b; Hulsey et al., 2008; Kaufman & Liem, 1982; Mcgee et al., 2015). It was famously postulated that the MPJ prompted increased trophic diversification, particularly in cichlids, as the musculoskeletal modifications permitted increased evolutionary specialization of the oral and pharyngeal jaws (Liem, 1973). Liem proposed that the development of the MPJ significantly reduced the coevolutionary constraints on the anatomically independent prey capture and processing systems, predicting that these functionally decoupled systems had also become evolutionarily decoupled. For decades, the modified pharyngeal jaw system has been regarded as a model key innovation that further decoupled the evolution of the oral and pharyngeal jaws, enabling extensive trophic diversification of cichlids (Alfaro, 2014; Heard & Hauser, 1995; Salzburger, 2009; Stroud & Losos, 2016).

Liem’s hypothesis postulated that the MPJ led to increased evolutionary decoupling of the oral and pharyngeal jaw systems, resulting in enhanced morphological diversity of each jaw unit (Liem, 1973). Aspects of this hypothesis have received considerable attention in recent years, as several studies have measured the evolutionary integration between the oral and pharyngeal jaws in various groups of cichlids (Burress & Muñoz, 2021; Burress et al., 2020; Conith & Albertson, 2021; Hulsey et al., 2006; Ronco & Salzburger, 2021). While the

methods and interpretations in these studies vary, this extensive body of work has repeatedly demonstrated that cichlids show weak but significant integration between the oral and pharyngeal jaws. Although the finding of weak integration might appear to provide strong support for Liem’s decoupling hypothesis, what is missing is any insight into whether cichlids show weaker or stronger integration than fishes that lack the modified pharyngeal jaw system. Is the weak but significant evolutionary integration between cichlid jaw systems greater than found in lineages without the modified pharyngeal jaw, less than those groups, or is it unchanged? This issue is key to assessing whether the novel configuration of the cichlid pharyngeal jaw changed the evolutionary dynamics of their feeding apparatus. Furthermore, we must assess whether cichlids, equipped with the MPJ, show greater variation and faster rates of evolution among feeding structures compared to fishes with an unmodified pharyngeal jaw to test Liem’s hypothesis.

In the present study, we explore the evolutionary impact of the modified pharyngeal jaw system by comparing evolutionary patterns of the feeding apparatus in Neotropical cichlids to those in North American centrarchids, which lack the MPJ. Centrarchids are not close relatives of cichlids, although both are members of Acanthomorpha and have been the subject of previous comparisons that noted strong parallels in their diversity, including convergent functional morphology in species with similar diets (Montaña & Winemiller, 2013; Norton & Brainerd, 1993). Although Centrarchidae includes just 40 species, they exhibit many ecomorphological equivalents of

Neotropical cichlids (with over 500 species), including specialized piscivores, benthic insectivores, midwater plankton feeders, and molluscivores (Keast, 1978). Additionally, previous work comparing the ecological communities of the two groups shows similar assemblage patterns and habitat occupation (Montaña et al., 2014). Both lineages inhabit streams, swamps, and lakes, with centrarchids distributed throughout temperate North America and Neotropical cichlids found throughout tropical Central and South America. Thus, we note that our study is not a survey of lineages that lack the MPJ, nor do we focus on a lineage that is closely related to cichlids. Instead, this is a comparison of Neotropical cichlids to one of the only other radiations of freshwater acanthomorphs in the Americas that shows comparable ecological diversity. Because the decoupling hypothesis is framed as an explanation for this diversity in cichlids, comparing these two lineages allows us to disentangle whether the presence of the modified pharyngeal jaw does, in fact, impose differential evolutionary patterns on the feeding system that substantially contribute to cichlid diversification. To conduct this analysis, we measure a set of 13 oral jaw and seven pharyngeal jaw traits in species of Neotropical cichlids and North American centrarchids (Figure 1b and c). Using a phylogenetic approach, we compute and compare patterns of evolutionary integration, disparity, and rate of evolution within the oral and pharyngeal jaws of cichlids and centrarchids. Here, we put forth the first comparative test of Liem's hypothesis where we ask whether fishes with the functionally novel pharyngeal jaw modifications show (a) increased evolutionary decoupling and (b) increased diversification among trophic structures. If Liem's hypothesis holds true, we should find greater morphological diversity, higher rates of morphological evolution, and greater evolutionary decoupling (i.e., decreased integration) of the oral and pharyngeal jaw structures in Neotropical cichlids.

Materials and methods

Study specimens and anatomical measurements

We examined 85 Neotropical cichlid species (Family Cichlidae) and 30 North American sunfish species (Family Centrarchidae) with an average of 5 specimens per species. Cichlidae, being one of seven families where modified pharyngeal jaws are an independently derived synapomorphy for all known species, represents fishes with MPJs. Centrarchids were used to represent fishes lacking the MPJ, as all species exhibit a generalized pharyngeal jaw system that lacks all traits defining an MPJ (Figure 1b). We used published morphological data for 218 cichlid specimens (Burruss et al., 2020; Supplementary Table 1) and obtained an additional 170 and 129 adult cichlid and centrarchid specimens, respectively, from museum collections, collection trips, and the pet trade (Supplementary Table 1) in an effort to maximize the morphological diversity and generic coverage in both families. Within our dataset, we have 50% and 100% sampling of genera for Neotropical cichlids and centrarchids, respectively. Though Neotropical and African cichlids both exhibit trophic morphologies and functions unique to their radiations, these two cichlid sister groups show extensive convergent evolution and shared ecomorphs (Burruss, 2014; Burruss et al., 2017). Thus, we restricted our study to the continental radiation of Central and South American river and lake-dwelling cichlid species, and we aimed to include species that would

showcase the full breadth of ecomorphological variation exhibited by this spectacularly diverse clade (Arbour et al., 2020; Burruss, 2014). By excluding African cichlids, we also reduce the number of confounding factors that contribute to this group's ecomorphological diversity, including lake effects (Seehausen, 2006) and hybridization (Joyce et al., 2011; Meier et al., 2017, 2019). All specimens were fixed in formalin and stored in 70% ethanol prior to this study. Each specimen was then cleared and stained using procedures adapted from Dingerkus and Uhler (1977) and Taylor and Van Dyke (1985), and stained specimens were stored in 90% glycerin. Upper and lower pharyngeal jaws were dissected from each stained specimen and photographed. The left side of each stained specimen was also photographed in a relaxed posture where oral jaws were mostly protracted, according to supplemental procedures outlined in Roberts et al., 2021.

Using digital calipers and NIH ImageJ software (Schneider et al., 2012), we measured anatomical traits to characterize the size, shape, and functionality of the oral jaw and pharyngeal jaw systems. Traits were separated into oral and pharyngeal jaw systems based on their contributions to prey capture or processing functions, respectively (Alexander, 1967; Lauder, 1983; Wainwright, 2005). We measured and computed 13 oral jaw traits including premaxillary dentigerous arm length, premaxillary ascending process length, gape, oral jaw protrusion, buccal cavity length, the horizontal and vertical positions of the lower jaw joint, the horizontal and vertical positions of the maxilla-nasal joint, lower oral jaw length, opening and closing mechanical advantage, and kinesis (Figures 1c and 3b; Supplementary Tables 1 and 2). Kinesis, calculated according to procedures in Burruss et al., 2020, describes the amount of shape change in the oral, four-bar linkage following a fixed rotation of the mandible (Martinez et al., 2018). Finally, we measured seven pharyngeal jaw traits including lower pharyngeal jaw length, width, depth, and tooth width (averaged across the three largest teeth), as well as upper pharyngeal jaw length, width, and depth (Figures 1c and 3a; Supplementary Tables 1 and 2). Pharyngeal jaws from especially small species (e.g., *Apistogramma* and *Erneacanthus*) were measured with calipers under a dissecting microscope. All pharyngeal jaw measurements for the 218 cichlid specimens from Burruss et al. (2020), excluding average tooth width, were retaken, as original trait data collected were not comparable to fishes with generalized pharyngeal jaws.

Morphological measurements for each specimen were converted to shape ratios where each trait value was divided by the cubed root of (head length \times head width \times head depth). By size correcting each specimen using the geometric mean of these three main size dimensions, we account for the complexity of body size and our data capture shape variation due to allometry (Claude, 2013; Klingenberg, 2016; Price et al., 2019). Shape ratios were averaged across specimens, log transformed to achieve normal distributions, and used in all subsequent analyses in R v. 4.2.0 (R Core Team, 2022). For select multivariate analyses, each species' log shape ratios were converted to standard normal deviates (i.e., z-scores) using the scale function (Becker et al., 1988) to ensure that all 20 measurements were in commensurate units and scales (Adams & Collyer, 2019b; Huttegger & Mitteroecker, 2011). These standardized values were used for analyses where noted.

Statistical analyses

To conduct our analyses in a phylogenetic context, we used a time-calibrated molecular phylogeny of ray-finned fishes (Rabosky et al., 2018). This phylogeny, which includes a dense sampling of species in both focal groups, was trimmed to include fishes represented by molecular sequence data and then to match the species included our study. For species that were not present on the phylogeny, we used the closest related species within the same genus as proxy. Four substitutions were made, including *Apistogramma hoignei* for *Apistogramma cacatuoides*, *Cichlasoma dimerus* for *Cichlasoma orientale*, *Pterophyllum leopardi* for *Pterophyllum scalare*, and *Amatitlania nigrofasciata* for *Amatitlania siquia*.

To visualize the multivariate morphological data in shape space, we performed principal component analyses (PCAs) on the correlation matrix of unstandardized, log shape ratios using the `prcomp` function (Becker et al., 1988; Mardia et al., 1979; Venables & Ripley, 2002) in STATS v. 4.2.0 (R Core Team, 2022). Analyses were run on all 20 morphological traits for cichlids and centrarchids separately, as well as in one combined analysis. We ran additional PCAs on the correlation matrices of 13 oral jaw traits and the seven pharyngeal jaw traits separately to visualize how cichlids and centrarchids fall in shape space based on each jaw unit. To test whether the cichlids and centrarchids have statistically different oral and pharyngeal jaw morphology, we performed a phylogenetic multivariate analysis of variance (phylogenetic MANOVA) on the z -scores of all 20 morphological traits over

10,000 iterations under a Brownian Motion model. This same method, implemented with the `procD.pgls` function (Adams, 2014a; Adams & Collyer, 2015, 2016, 2018; Collyer et al., 2015) in GEOMORPH v. 4.0.4 (Adams & Collyer, 2022; Adams et al., 2022; Baken et al., 2021; Collyer & Adams, 2018), was used to perform individual phylogenetic ANOVAs on each of the 20 traits in our data set.

To quantify and compare the magnitude of interspecific variation among cichlid and centrarchid morphologies, we used `morphol.disparity` (Collyer & Adams, 2021; Zelditch et al., 2012) in GEOMORPH. Using unstandardized, log shape ratios, we estimated overall disparity for all 20 morphological traits, two subsets of traits representing each jaw unit, and for each individual trait. We also estimated and contrasted the Brownian multivariate rate parameter for all 20 traits, two subsets of traits representing each jaw unit, and for each individual trait using `compare.evol.rates` (Adams 2014b; Denton and Adams 2015; Adams and Collyer 2018, 2019a) in GEOMORPH.

We used the `phylo.integration` function (Adams & Collyer, 2016, 2018, 2019a; Adams & Felice, 2014; Collyer et al., 2015) in GEOMORPH to quantify the degree of morphological covariance between oral jaw and pharyngeal jaw traits in an evolutionary context over 10,000 iterations. As this was done separately on the z -scores from cichlids and centrarchids, we then compared the strength of between-jaw-unit integration between families with the `compare.pls` function (Adams & Collyer, 2016; Collyer et al., 2015) in

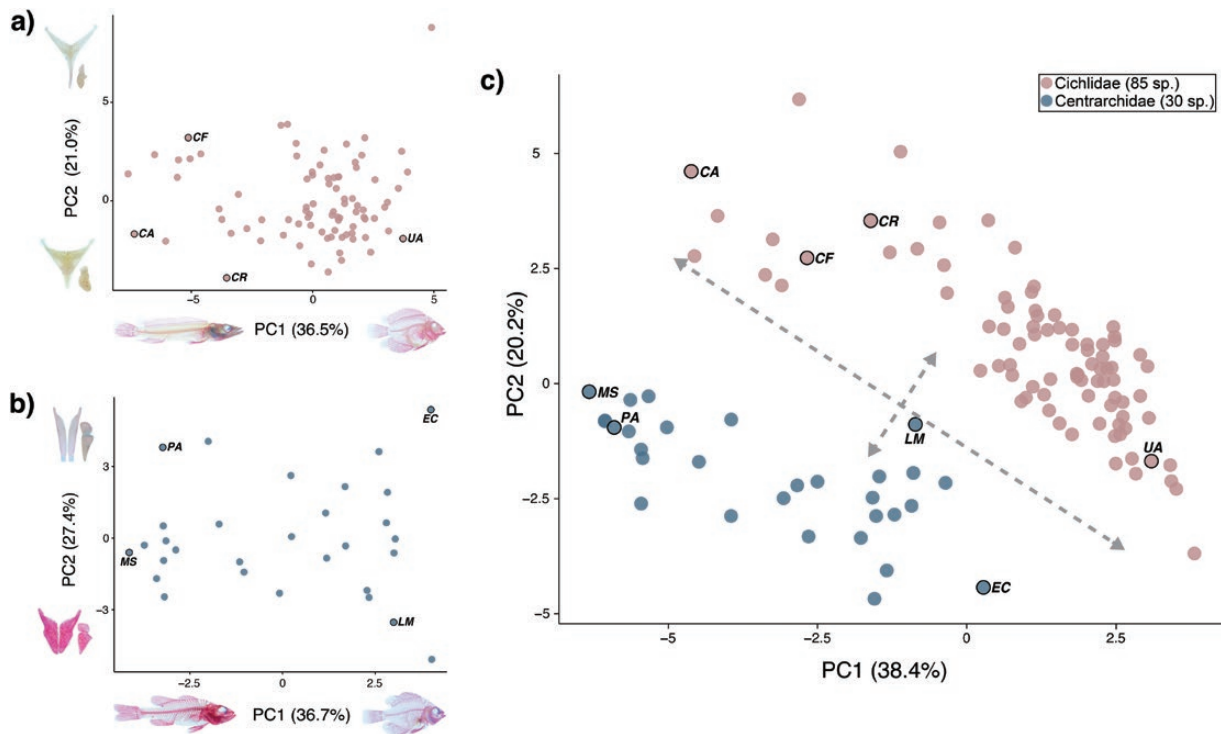


Figure 2. Principal component analyses (PCAs) of all 20 oral and pharyngeal jaw traits for cichlids (a) and centrarchids (b) where PC1 primarily captures variation in oral jaw and craniofacial shapes and PC2 primarily captures variation in pharyngeal jaw shapes. Images exemplify extreme body and jaw shapes on each axis including *Crenicichla alta* (cichlid; left), *Uaru amphiacanthoides* (cichlid; right), *Micropterus salmoides* (centrarchid; left), and *Enneacanthus chaetodon* (centrarchid; right) on PC1, and pharyngeal jaws from *Chaetobranchius flavescens* (cichlid; top), *Crenicichla reticulata* (cichlid; bottom), *Pomoxis annularis* (centrarchid; top), and *Lepomis microlophus* (centrarchid; bottom) on PC2. (c) Combined PCA of cichlids (mauve) and centrarchids (blue) shows that the two groups occupy distinct areas of morphospace. Dotted gray lines roughly align with principal components 1 and 2 from the individual PCAs of cichlids and centrarchids, showing that variation in pharyngeal jaw anatomy—primarily captured by PC2 in (a) and (b)—strongly contributes to the distinction of these two clades. Principal component loadings can be found in [Supplementary Table 3](#).

GEOMORPH. For each family, we used standard normal deviates to calculate the degree of evolutionary integration within each jaw system using the integration.Vrel function (Conaway & Adams, 2022; Pavlicev et al., 2009) and compared the results using the compare.ZVrel (Conaway & Adams, 2022) in GEOMORPH. Further, using standard normal deviates and the phylo.modularity and compare.CR functions (Adams, 2016; Adams & Collyer, 2019a; Adams & Felice, 2014) in GEOMORPH, we quantified and contrasted the degree of evolutionary modularity in each family. Finally, we produced the evolutionary correlation matrices for each family using code modified from integration.Vrel and the cor function (Becker et al., 1988; Kendall, 1938, 1945) in STATS. We then used the corrplot function (Friendly, 2002; Murdoch & Chow, 1996) in CORRPLOT v. 0.92 (Wei & Simko, 2021) to visualize these matrices.

Results

Morphological diversity and rates of evolution

Based on our analysis of morphological data from 85 species of Neotropical cichlids and 30 species of North American sunfishes, we find that the two groups contain similar amounts of interspecific morphological variation in the feeding apparatus, but occupy distinct areas of morphospace (Figures 1–3; Supplementary Table 3). When cichlids (Figure 2a) and centrarchids (Figure 2b) are examined in separate PCAs, PC1 in both groups primarily captures variation in oral jaw morphology and head shape. Along PC1, we find small-mouthed, deep-bodied fishes, such as *Symphysodon discus* (cich.), *Uaru amphiacanthoides* (cich.), *Lepomis megalotis* (cent.), and *Enneacanthus chaetodon* (cent.) at one extreme of the axis. In contrast, more elongate fishes with larger mouths, such as *Crenicichla alta* (cich.), *Petenia splendida* (cich.), *Micropterus henshalli* (cent.), and *Micropterus salmoides* (cent.), fall on the opposite extreme of this axis. The second principal component primarily highlights diversity in pharyngeal jaw morphology, where species with wider, more robust pharyngeal jaws, such as *Crenicichla reticulata* (cich.), *Herichthys labridens* (cich.), *Lepomis microlophus* (cent.), and *Lepomis gibbosus* (cent.), lie at one extreme on PC2. Fishes with

thinner, more elongate jaws, as seen in *Symphysodon discus* (cich.), *Chaetobranchius flavescens* (cich.), *Enneacanthus chaetodon* (cent.), and *Pomoxis annularis* (cen.), lie at the other extreme of PC2.

When combined into a single PCA, cichlids and centrarchids display no overlap in a plot of PC1 versus PC2 (Figure 2c). The primary and secondary axes of variation capture similar patterns of morphological diversity in the two families when plotted separately. Thus, cichlids and centrarchids lie almost parallel in shape space, with substantial separation between the two groups along the axis that delineates differences primarily in pharyngeal jaw morphology. Principal component analyses of oral and pharyngeal jaw traits separately show that the two groups differ more strongly in pharyngeal jaw morphology (Figure 3; Supplementary Table 3). However, a phylogenetic multivariate analysis of variance (MANOVA) performed on the standard normal deviates of all 20 traits shows no significant difference between cichlid and centrarchid morphology ($p = 0.97$), with phylogenetic ANOVAs on individual traits showing a significant difference for lower pharyngeal jaw width only ($p = 2.90 \times 10^{-3}$; Figure 1c). We note that this difference is apparent despite measuring the distance from the posterior midline to the lateral edge of the ceratobranchial in cichlids (i.e., half of the lower pharyngeal jaw as this is comparable to the unmodified lower pharyngeal jaw in centrarchids; Supplementary Table 2). These results highlight that pharyngeal jaw morphology, specifically lower pharyngeal jaw width, is a key anatomical difference between cichlids and centrarchids.

Counter to our prediction based on the decoupling hypothesis (Liem, 1973), total morphological disparity of the feeding apparatus is similar in the two groups. While cichlids show significantly more disparity in premaxillary ascending process length (4.41-fold difference, $p = 0.05$) and centrarchids show significantly more disparity in lower pharyngeal jaw width (2.25-fold difference, $p = 0.05$), cichlids are slightly, but not significantly, more morphologically diverse than centrarchids overall ($p = 0.68$, Table 1; Figure 1c). Although the differences are non-significant, oral jaw disparity is higher in cichlids, while pharyngeal jaw disparity is slightly higher in centrarchids. Also counter to our expectation, the overall

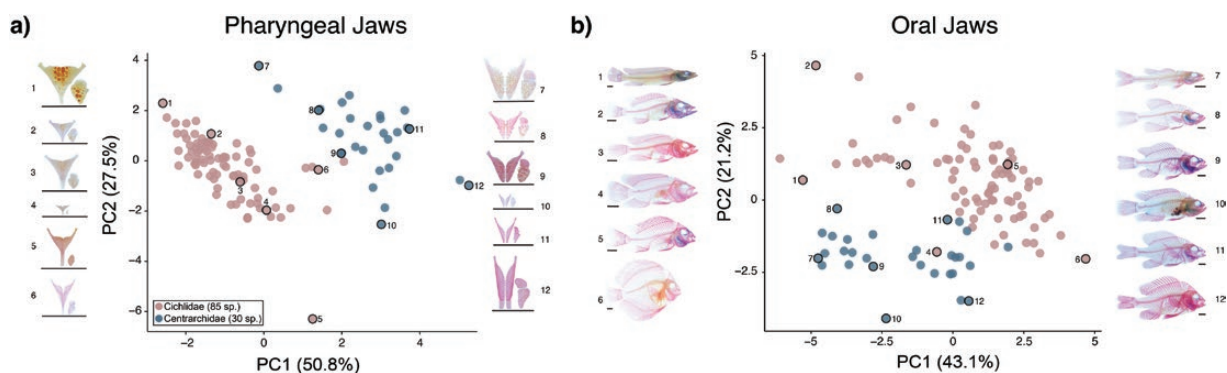


Figure 3. Principal component analyses show that cichlids (mauve; 1–6) and centrarchids (blue; 7–12) occupy different areas of morphospace in both pharyngeal and oral jaw anatomy. (a) Exemplifying the diversity of pharyngeal jaw shapes, we show jaws from (1) *Herichthys steindachneri*, (2) *Thorichthys meeki*, (3) *Andinoacara rivulatus*, (4) *Apistogramma bitaeniata*, (5) *Symphysodon discus*, and (6) *Cichla monoculus* to represent cichlids; and (7) *Lepomis gibbosus*, (8) *Lepomis miniatus*, (9) *Lepomis auritus*, (10) *Enneacanthus chaetodon*, (11) *Micropterus punctulatus*, and (12) *Pomoxis nigromaculatus* to represent centrarchids. (b) Exemplifying the diversity of oral jaw and craniofacial shapes, we show (1) *Crenicichla lugubris*, (2) *Petenia splendida*, (3) *Parachromis managuensis*, (4) *Astronotus ocellatus*, (5) *Biotodoma cupido*, and (6) *Symphysodon discus* to represent cichlids; and (7) *Micropterus henshalli*, (8) *Parachromis managuensis*, (9) *Lepomis gulosus*, (10) *Ancantharcus pomotis*, (11) *Lepomis microlophus*, and (12) *Lepomis megalotis* to represent centrarchids. Black scale bars represent 10 mm. Principal component loadings can be found in Supplementary Table 3.

Table 1. Estimates of morphological disparity (Procrustes variance) and the Brownian rate parameter for multivariate trait evolution in cichlids and centrarchids. The two groups show no significant differences in morphological disparity, while centrarchids show significantly faster rates of evolution within the feeding apparatus overall (1.98-fold difference). Separate rate analyses on each jaw unit reveal 2.16-fold faster oral jaw evolution in centrarchids, but no significant difference in rates of pharyngeal jaw evolution. Significant *p*-values and effect sizes are bolded.

Analysis	Cichlidae	Centrarchidae	<i>p</i> -value (effect size) of pairwise difference
Disparity (overall)	1.38	1.27	0.68
Oral jaw disparity	1.09	0.95	0.54
Pharyngeal jaw disparity	0.29	0.32	0.74
Rate of evolution (overall)	3.25E-03	6.43E-03	1.00E-4 (3.85)
Oral jaw rate	4.04E-3	8.73E-03	1.00E-4 (3.37)
Pharyngeal jaw rate	1.78E-3	2.16E-03	0.24 (0.81)

multivariate rate of morphological evolution is nearly 2-fold faster in centrarchids compared to cichlids ($p = 1.00^{-04}$, [Table 1](#)). Centrarchids also show faster rates of evolution in both jaw units, though the difference is only significant within the oral jaw system ($p = 1.00^{-04}$, [Table 1](#)). Univariate analyses reveal that centrarchids have increased diversification rates for traits in each jaw unit, including premaxillary protrusion (4.60-fold difference, $p = 1.00^{-04}$), the horizontal position of the maxilla-nasal joint (1.85-fold difference, $p = 0.04$), closing mechanical advantage of the lower jaw (1.90-fold difference, $p = 0.04$), lower pharyngeal jaw width (2.82-fold difference, $p = 7.00^{-04}$), and upper pharyngeal jaw depth (2.30-fold difference, $p = 9.10^{-03}$; [Figure 1c](#))

Patterns of evolutionary integration

The coevolutionary patterns among traits within each jaw system are broadly similar in cichlids and centrarchids ([Figure 4a](#)). Evolutionary integration within the oral jaw unit is moderate and nearly identical in the two groups, whereas centrarchids show slightly more evolutionary integration among pharyngeal jaw traits than cichlids. Though there are some differences in which specific pairwise trait relationships are stronger in cichlids versus centrarchids ([Figure 4b](#)), there is no significant difference in the strength of evolutionary integration within the oral ($p = 0.70$) or pharyngeal ($p = 0.37$) jaw units in cichlids compared to centrarchids ([Table 2](#)). When we compare the strength of integration between the two jaw units to that within each jaw unit, we find no significant difference between the groups ($p = 0.34$), with cichlids and centrarchids both showing significant, moderate levels of evolutionary modularity ([Table 2](#)).

The key prediction of Liem's hypothesis ([Liem, 1973](#)) was that cichlids should display greater evolutionary decoupling (i.e., less integration) of the oral and pharyngeal jaws due to the development of the modified pharyngeal jaw. However, we find the opposite pattern, as between-jaw-unit evolutionary integration is significantly stronger in cichlids compared to centrarchids ($p = 3.42^{-3}$, [Table 2](#); [Figure 5](#)). Among the pairwise relationships between oral jaw and pharyngeal jaw traits, cichlids have consistently stronger relationships between lower pharyngeal jaw length and oral jaw traits, whereas centrarchids show consistently stronger relationships between upper pharyngeal jaw depth and oral jaw traits ([Figure 4b](#)).

Discussion

Our findings run counter to a classic prediction about the macroevolutionary consequences of the modified pharyngeal jaw apparatus in cichlid fishes. Rather than the predicted pattern

of increased evolutionary decoupling of cichlid oral and pharyngeal jaws ([Liem, 1973, 1990](#)), we instead found that evolutionary integration between the two jaw systems is stronger in Neotropical cichlids when compared to North American centrarchids. Also counter to predictions about cichlid jaw evolution based on a functional analysis of the modified prey processing system ([Galis & Drucker, 1996](#)), the MPJ does not appear to have impacted the integration pattern within each jaw system, as both groups show similar levels of integration within the oral and pharyngeal jaws. Further, though standing morphological diversity of the oral and pharyngeal jaws is indistinguishable between the two groups, centrarchids show faster rates of morphological evolution. This suggests that cichlids' reputation for possessing extreme ecomorphological diversity of the feeding apparatus may be exaggerated, at least for Neotropical cichlids. Though we note that additional work should be done to examine these patterns more broadly in other groups of cichlids and lineages without the MPJ, our results have major implications for our understanding of cichlid diversity and the role of the novel modified pharyngeal jaw in shaping ecomorphological diversification.

Our finding of stronger integration between jaw systems in cichlids suggests that the well-documented, weak but significant integration seen in cichlids ([Burruss & Muñoz, 2021](#); [Burruss et al., 2020](#); [Conith & Albertson, 2021](#); [Hulsey et al., 2006](#); [Ronco & Salzburger, 2021](#)) does not reflect a decrease in evolutionary integration relative to fishes with the unspecialized pharyngeal jaw system, but rather an increase. This finding is counter to Liem's prediction that the MPJ resulted in increased evolutionary decoupling of the oral and pharyngeal jaws. Additional work is needed on other groups to determine the extent to which the higher integration observed here reflects a condition specific to cichlids as opposed to one that is characteristic of all lineages with the MPJ. The absence of significant integration between the oral and pharyngeal jaw systems in centrarchids is notable because it suggests that the presence of the pharyngeal jaw can result in the anticipated evolutionary decoupling of the two jaw systems, at least as manifested in this group.

Although functional, developmental, and evolutionary decoupling have been identified as mechanisms that might facilitate greater overall diversity of functional systems ([Breuker et al., 2006](#); [Collar et al., 2014](#); [Evans et al., 2017, 2019a](#); [Felice et al., 2018](#); [Roberts et al., 2018](#); [Schaefer & Lauder, 1996](#); [Vermeij, 1973](#)), these and other authors have pointed out ways in which stronger integration (the opposite of decoupling) between functional traits can positively affect diversification by enabling efficient, genetically aligned

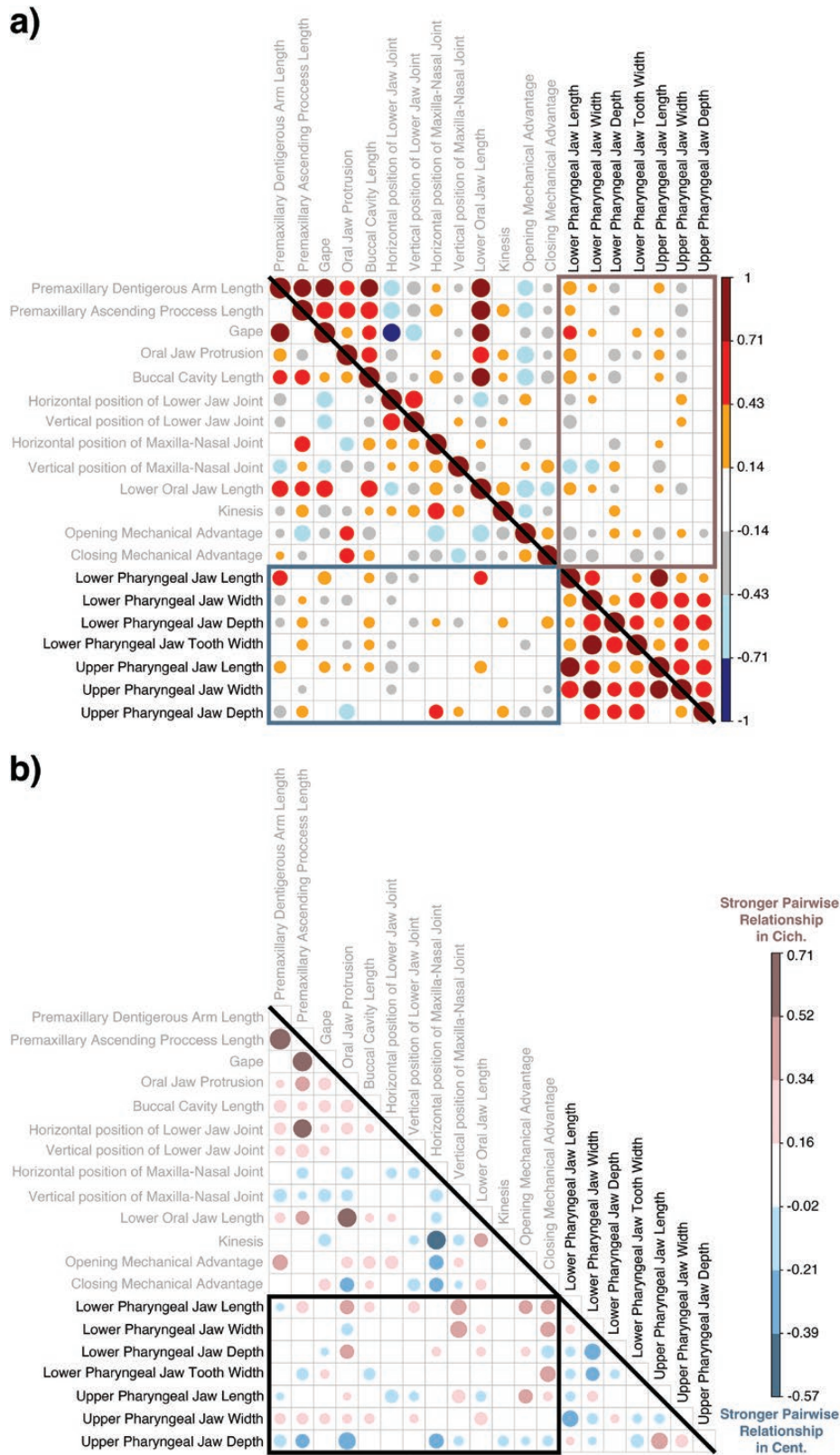


Figure 4. (a) Evolutionary correlation matrices showing the relationships between all 20 oral (gray text) and pharyngeal (black text) jaw traits for cichlids (top triangle) and centrarchids (bottom triangle). (b) Absolute value difference between cichlid and centrarchid pairwise correlations, where brown/pink circles indicate a stronger evolutionary correlation in cichlids and blue circles indicate a stronger evolutionary correlation in centrarchids. Boxes indicate correlations between oral and pharyngeal jaw traits (i.e., between-jaw-unit relationships).

Table 2. Evolutionary integration of oral and pharyngeal jaw systems (r-PLS) is stronger in cichlids compared to centrarchids. However, there is no significant difference in the magnitude of integration within each jaw unit (Vrel) or evolutionary modularity (covariance ratio coefficient—between-jaw-unit integration/within-jaw-unit integration). Significant *p*-values and effect sizes are bolded.

Analysis	Cichlidae		Centrarchidae		<i>p</i> -value (effect size) of pairwise difference
	Value	<i>p</i> -value (effect size)	Value	<i>p</i> -value (effect size)	
Between-jaw-system evolutionary integration	0.68	1.00E-4 (4.00)	0.53	0.17 (0.97)	3.42E-3 (2.93)
Oral jaw system evolutionary integration	0.17	(1.34)	0.19	(0.88)	0.70 (0.38)
Pharyngeal jaw system evolutionary integration	0.23	(1.46)	0.30	(1.13)	0.37 (0.90)
Evolutionary modularity	0.64	5.00E-5 (−3.84)	0.54	1.50E-4 (−3.49)	0.34 (0.95)

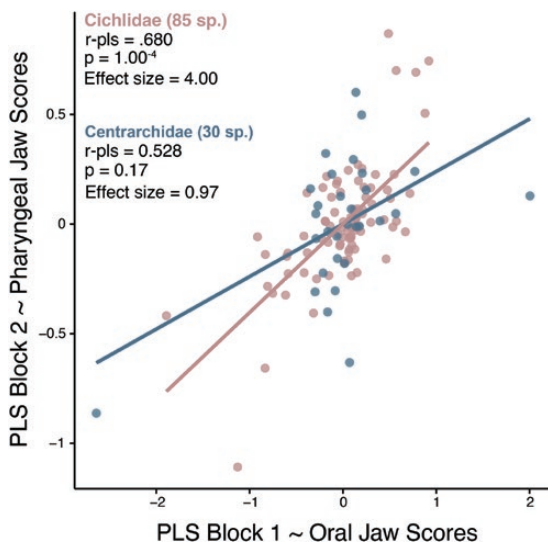


Figure 5. Evolutionary integration between the two jaw systems is stronger in cichlids than in centrarchids (pairwise effect size = 2.93; $p = 3.42 \times 10^{-3}$), based on a phylogenetic, two-block partial least squares analysis of oral against pharyngeal jaw traits (Table 2).

change (Burns et al., 2023; Cheverud, 1996; Goswami et al., 2014; Marroig & Cheverud, 2005; Miller & Olson, 1958; Schluter, 1996; Schwenk & Wagner, 2001). Cichlids appear to have capitalized on stronger integration to some extent, as Neotropical lineages show that trophic ecology is the major factor underlying the integrated evolution of oral and pharyngeal jaw structures (Burruss & Muñoz, 2021; Burruss et al., 2020). Additionally, work with African Rift Lake cichlids has identified *smad7* as a candidate gene expressed in both jaw systems that has the potential to induce correlated trait evolution (Conith & Albertson, 2021). Beyond cichlids, numerous studies show that increased covariance among traits may be associated with the evolution of greater trait variance, more rapid evolution, or more extreme traits (Evans et al., 2021; Farina et al., 2019b; Hernandez & Cohen, 2019; Holzman et al., 2012; Muñoz et al., 2017, 2018; Pos et al., 2019). Thus, there appear to be two potential consequences of evolutionary integration that would result in opposite effects on phenotypic diversification. On one hand, weak trait covariance implies fewer constraints on the extent and rate of morphospace exploration. On the other hand, strong trait covariance may reflect shared underlying genetic control—a

phenomenon that would allow manifold, correlated responses to strong directional selection on a single trait. It seems clear that the diversification of oral and pharyngeal jaws in cichlids and centrarchids does not reflect either extreme, but rather some middle ground, where components are generally weakly integrated and able to evolve with considerable independence, while also potentially sharing some developmental genetic pathways (Conith et al., 2020; Felice et al., 2018).

Our findings raise doubts about the impact of functional decoupling on the diversification of oral and pharyngeal jaws in cichlids. Thus, our results suggest that a re-evaluation is needed of the idea that high phenotypic diversity in cichlids is linked to macroevolutionary consequences of their specialized pharyngeal jaw system. We note that additional quantitative studies are needed to examine the impact of decoupling on African Rift Lake cichlid radiations. However, the young age of these and other lake radiations in Africa strongly suggests that they have experienced high rates of morphological evolution (Hulsey et al., 2010; Matschiner et al., 2020; Ronco et al., 2020). As others have pointed out (Seehausen, 2006), high rates of diversification in the young lake lineages argue against a prominent role of the modified pharyngeal jaw because the novelty is found throughout all cichlids, including older, non-lake lineages that do not show such high rates of evolution. Our finding that the extensive adaptive radiation of Neotropical cichlids (López-Fernández et al., 2013) does not appear to involve exceptional rates of phenotypic evolution, on average, or the accumulation of exceptional morphological disparity within the jaw systems suggests that careful evaluation of the phenotypic diversity of cichlid fishes, relative to other fish groups, is urgently needed. If a pattern is ultimately confirmed where rates of ecomorphological diversification are only exceptional in some lake radiations (Seehausen, 2006, 2015) and/or a limited number of sub-radiations of Neotropical cichlids (Burruss et al., 2022), this will cast further doubt on the impact of the modified pharyngeal jaw on cichlid diversification (Larouche et al., 2020) and strengthen the focus on other factors that characterize adaptive diversification in African and Neotropical cichlids. These include the rich ecological opportunities offered by large, deep tropical lakes (Ronco et al., 2020; Seehausen, 2006; Wagner et al., 2012) as well as the geographically expansive Central and South American river systems (Arbour & López-Fernández, 2016; Burruss et al., 2022); strong patterns of sexual selection (Seehausen et al., 2008; Wagner et al., 2012); a sometimes complex history of multiple transitions between river and

lake habitats (Joyce et al., 2011; Martin et al., 2015; Poelstra et al., 2018; Verheyen et al., 2003); and the existence of ongoing hybridization that generates novel genetic variation for selection to act upon (Irisarri et al., 2018; Meier et al., 2017; Seehausen, 2004, 2006; Svardal et al., 2020).

Conclusion

The hypothesis that functionally novel modifications to the pharyngeal jaw apparatus in cichlids led to evolutionary decoupling and increased diversification of the oral and pharyngeal jaws was first proposed 50 years ago (Liem, 1973). Finding that Neotropical cichlids actually display stronger evolutionary integration between the two jaw systems, slower rates of morphological evolution, and no difference in morphological disparity compared to centrarchid fishes, we provide the first comparative test of this classic hypothesis. Our findings that variation in jaw anatomy does not differ between the two groups and that rates of morphological evolution are actually slower in Neotropical cichlids raise questions about the processes that underlie ecological and functional diversification in cichlids. It is tempting to point to elevated integration in the feeding apparatus as a possible force in cichlid diversification. However, the finding that Neotropical cichlids do not show exceptionally high rates of evolution in the trophic apparatus, but instead evolve at a slower rate than centrarchids, suggest that the phenotypic variation in this continental radiation largely followed patterns of diversification found in other freshwater acanthomorph groups and has accumulated over the long history of the group. Our study also highlights the conflicting theoretical expectations surrounding the concepts of decoupling, which may be expected to both enhance the potential for diversity or constrain it, relative to systems that show stronger integration. Given that multifunctionality is a major hallmark of organismal design, issues of decoupling and integration should continue to lie at the forefront of research in evolutionary morphology.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

All data, code, and supplemental tables are available on Data Dryad at <https://doi.org/10.25338/B8ZP83>.

Author contributions

A.S.RH. and P.C.W. conceptualized the study. A.S.RH., E.D.B., and P.C.W. designed the study. A.S.RH., E.D.B., and B.L. collected all morphological data. A.S.RH. conducted all statistical analyses. A.S.RH. wrote the manuscript with input from P.C.W. All authors reviewed and approved the final version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgments

We thank S. Combes, J. Schmitt, K. Corn, S. Friedman, C. Martinez, A. Barley, D. Satterfield, K. Russell, N. Peoples,

D. Wainwright, M. Burns, and M. Mihalitsis for critical input; K. Cohen for advice on specimen staining; and D. Adams for helpful guidance on statistical analyses. We extend sincere thanks to D. Werneke of the Auburn University Museum of Natural History, D. Catania of the California Academy of Sciences, M. Arce H. and M. Sabaj of the Academy of Natural Sciences of Philadelphia, C. D. McMahan of the Field Museum of Natural History, R. H. Robins of the Florida Museum of Natural History, C. Taylor and A. Dickinson of the Illinois Natural History Survey, S. Parker and P. Chakrabarty of the Louisiana State University Museum of Natural Science, J. Williams of the Smithsonian National Museum of Natural History, K. Conway and H. Prestidge of the Biodiversity and Research Teaching Collections at Texas A&M, and J. G. Mann and H. L. Bart of the Tulane University Museum of Natural History for generously facilitating specimen loans. This research was conducted in accordance with the University of California, Davis' Institutional Animal Care and Use Committee (protocol #20475).

Funding

This research was supported by the Center for Population Biology at UC Davis, the Rosemary Grant Advanced Award from the Society for the Study of Evolution, the Graduate Research Fellowship under Grant No. 1650042 from the National Science Foundation, and a Dissertation Fellowship from the Ford Foundation to A.S.RH; DEB-1556953 from the National Science Foundation to P.C.W.

References

- Adams, D. C. (2014a). A method for assessing phylogenetic least squares models for shape and other high-dimensional multivariate data. *Evolution*, 68(9), 2675–2688. <https://doi.org/10.1111/evo.12463>
- Adams, D. C. (2014b). Quantifying and comparing phylogenetic evolutionary rates for shape and other high-dimensional phenotypic data. *Systematic Biology*, 63(2), 166–177. <https://doi.org/10.1093/sysbio/syt105>
- Adams, D. C. (2016). Evaluating modularity in morphometric data: Challenges with the RV coefficient and a new test measure. *Methods in Ecology and Evolution*, 7(5), 565–572. <https://doi.org/10.1111/2041-210x.12511>
- Adams, D. C., & Collyer, M. L. (2015). Permutation tests for phylogenetic comparative analyses of high-dimensional shape data: What you shuffle matters. *Evolution*, 69(3), 823–829. <https://doi.org/10.1111/evo.12596>
- Adams, D. C., & Collyer, M. L. (2016). On the comparison of the strength of morphological integration across morphometric datasets. *Evolution*, 70(11), 2623–2631. <https://doi.org/10.1111/evo.13045>
- Adams, D. C., & Collyer, M. L. (2018). Multivariate phylogenetic comparative methods: Evaluations, comparisons, and recommendations. *Systematic Biology*, 67(1), 14–31. <https://doi.org/10.1093/sysbio/syx055>
- Adams, D. C., & Collyer, M. L. (2019a). Comparing the strength of modular signal, and evaluating alternative modular hypotheses, using covariance ratio effect sizes with morphometric data. *Evolution*, 73(12), 2352–2367. <https://doi.org/10.1111/evo.13867>
- Adams, D. C., & Collyer, M. L. (2019b). Phylogenetic comparative methods and the evolution of multivariate phenotypes. *Annual Review of Ecology, Evolution, and Systematics*, 50(1), 405–425. <https://doi.org/10.1146/annurev-ecolsys-110218-024555>

- Adams, D. C., & Collyer, M. L. (2022). Consilience of methods for phylogenetic analysis of variance. *Evolution*, 76(7), 1406–1419. <https://doi.org/10.1111/evo.14512>
- Adams, D. C., Collyer, M. L., Kaliontzopoulou, A., & Balken, E. K. (2022). Geomorph: Software for geometric morphometric analyses. R package version 4.0.4.
- Adams, D. C., & Felice, R. N. (2014). Assessing trait covariation and morphological integration on phylogenies using evolutionary covariance matrices. *PLoS One*, 9(4), e94335. <https://doi.org/10.1371/journal.pone.0094335>
- Alexander, R. M. (1967). The functions and mechanisms of the protrusible upper jaws of some acanthopterygian fish. *Journal of Zoology*, 151(1), 43–64. <https://doi.org/10.1111/j.1469-7998.1967.tb02865.x>
- Alfaro, M. E. (2014). Key evolutionary innovations. In: J. B. Losos, D. J. Futuyma, H. E. Hoekstra, R. E. Lenski, A. J. Moore, C. L. Peichel, D. Schluter, & M. C. Whitlock (Eds.), *The Princeton guide to evolution* (pp. 592–598). Princeton University Press.
- Alfaro, M. E., Brock, C. D., Banbury, B. L., & Wainwright, P. C. (2009). Does evolutionary innovation in pharyngeal jaws lead to rapid lineage diversification in labrid fishes? *BMC Evolutionary Biology*, 9, 255. <https://doi.org/10.1186/1471-2148-9-255>
- Arbour, J. H., & López-Fernández, H. (2016). Continental cichlid radiations: Functional diversity reveals the role of changing ecological opportunity in the neotropics. *Proceedings of the Royal Society B: Biological Sciences*, 283, 20160556.
- Arbour, J. H., Montaña, C. G., Winemiller, K. O., Pease, A. A., Soria-Barreto, M., Cochran-Biederman, J. L., & López-Fernández, H. (2020). Macroevolutionary analyses indicate that repeated adaptive shifts towards predatory diets affect functional diversity in Neotropical cichlids. *Biological Journal of the Linnean Society*, 129(4), 844–861. <https://doi.org/10.1093/biolinnean/blaa001>
- Baken, E. K., Collyer, M. L., Kaliontzopoulou, A., & Adams, D. C. (2021). geomorph v4.0 and gmShiny: Enhanced analytics and a new graphical interface for a comprehensive morphometric experience. *Methods in Ecology and Evolution*, 12(12), 2355–2363. <https://doi.org/10.1111/2041-210x.13723>
- Becker, R. A., Chambers, J. M., & Wilks, A. R. (1988). *The new S Language: A programming environment for data analysis and graphics*. Wadsworth & Brooks/Cole.
- Breuker, C. J., Debat, V., & Klingenberg, C. P. (2006). Functional evo-devo. *Trends in Ecology and Evolution*, 21(9), 488–492. <https://doi.org/10.1016/j.tree.2006.06.003>
- Burns, M. D., Collyer, M. L., & Sidlauskas, B. L. (2023). Simultaneous integration and modularity underlie the exceptional body shape diversification of characiform fishes. *Evolution*, 77(3), 746–762. <https://doi.org/10.1093/evolut/qpac070>
- Burruss, E. D. (2014). Cichlid fishes as models of ecological diversification: Patterns, mechanisms, and consequences. *Hydrobiologia*, 748(1), 7–27. <https://doi.org/10.1007/s10750-014-1960-z>
- Burruss, E. D. (2016). Ecological diversification associated with the pharyngeal jaw diversity of Neotropical cichlid fishes. *Journal of Animal Ecology*, 85(1), 302–313. <https://doi.org/10.1111/1365-2656.12457>
- Burruss, E. D., Martinez, C. M., & Wainwright, P. C. (2020). Decoupled jaws promote trophic diversity in cichlid fishes. *Evolution*, 74(5), 950–961. <https://doi.org/10.1111/evo.13971>
- Burruss, E. D., & Muñoz, M. M. (2021). Ecological limits on the decoupling of prey capture and processing in fishes. *Integrative and Comparative Biology*, 61(3), 773–782. <https://doi.org/10.1093/icb/icab148>
- Burruss, E. D., Piálek, L., Casciotta, J., Almirón, A., & Řičan, O. (2022). Rapid parallel morphological and mechanical diversification of South American pike cichlids (Crenicichla). *Systematic Biology*, 72(1), 1–14. <https://doi.org/10.1093/sysbio/syaa018>
- Burruss, E. D., Piálek, L., Casciotta, J. R., Almirón, A., Tan, M., Armbruster, J. W., & Řičan, O. (2017). Island- and lake-like parallel adaptive radiations replicated in rivers. *Proceedings of the Royal Society B*, 285, 20171762.
- Cheverud, J. M. (1996). Developmental integration and the evolution of pleiotropy. *American Zoologist*, 36(1), 44–50. <https://doi.org/10.1093/icb/36.1.44>
- Claude, M. (2013). Log-shape ratios, Procrustes superimposition, elliptic Fourier analysis: Three worked examples in R. *Hystrix*, 24, 94–102.
- Collar, D. C., Wainwright, P. C., Alfaro, M. E., Revell, L. J., & Mehta, R. S. (2014). Biting disrupts integration to spur skull evolution in eels. *Nature Communications*, 5, 5505. <https://doi.org/10.1038/ncomms6505>
- Collyer, M. L., & Adams, D. C. (2018). RRPP: An R package for fitting linear models to high-dimensional data using residual randomization. *Methods in Ecology and Evolution*, 9(7), 1772–1779. <https://doi.org/10.1111/2041-210x.13029>
- Collyer, M. L., & Adams, D. C. (2021). Phylogenetically aligned component analysis. *Methods in Ecology and Evolution*, 12, 359–372.
- Collyer, M. L., Sekora, D. J., & Adams, D. C. (2015). A method for analysis of phenotypic change for phenotypes described by high-dimensional data. *Heredity*, 115(4), 357–365. <https://doi.org/10.1038/hdy.2014.75>
- Conaway, M. A., & Adams, D. C. (2022). An effect size for comparing the strength of morphological integration across studies. *Evolution*, 76(10), 2244–2259. <https://doi.org/10.1111/evo.14595>
- Conith, A. J., & Albertson, R. C. (2021). The cichlid oral and pharyngeal jaws are evolutionarily and genetically coupled. *Nature Communications*, 12(1), 1–11.
- Conith, A. J., Kidd, M. R., Kocher, T. D., & Albertson, R. C. (2020). Ecomorphological divergence and habitat lability in the context of robust patterns of modularity in the cichlid feeding apparatus. *BMC Evolutionary Biology*, 20, 1–20.
- Corn, K. A., Martinez, C. M., Burruss, E. D., & Wainwright, P. C. (2021). A multifunction trade-off has contrasting effects on the evolution of form and function. *Systematic Biology*, 70(4), 681–693. <https://doi.org/10.1093/sysbio/syaa091>
- Denton, J. S. S., & Adams, D. C. (2015). A new phylogenetic test for comparing multiple high-dimensional evolutionary rates suggests interplay of evolutionary rates and modularity in lanternfishes (Myctophiformes; Myctophidae). *Evolution*, 69(9), 2425–2440. <https://doi.org/10.1111/evo.12743>
- Dingerkus, G., & Uhler, L. D. (1977). Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technology*, 52(4), 229–232. <https://doi.org/10.3109/10520297709116780>
- Evans, K. M., Larouche, O., Watson, S. J., Farina, S., Habegger, M. L., & Friedman, M. (2021). Integration drives rapid phenotypic evolution in flatfishes. *Proceedings of the National Academy of Sciences*, 118(18), e2101330118.
- Evans, K. M., Vidal-García, M., Tagliacollo, V. A., Taylor, S. J., & Fenolio, D. B. (2019a). Bony patchwork: Mosaic patterns of evolution in the skull of electric fishes (Apterontidae: Gymnotiformes). *Integrative and Comparative Biology*, 59(2), 420–431. <https://doi.org/10.1093/icb/icz026>
- Evans, K. M., Waltz, B., Tagliacollo, V., Chakrabarty, P., & Albert, J. S. (2017). Why the short face? Developmental disintegration of the neurocranium drives convergent evolution in neotropical electric fishes. *Ecology and Evolution*, 7(6), 1783–1801. <https://doi.org/10.1002/ece3.2704>
- Evans, K. M., Williams, K. L., & Westneat, M. W. (2019b). Do coral reefs promote morphological diversification? Exploration of habitat effects on labrid pharyngeal jaw evolution in the era of big data. *Integrative and Comparative Biology*, 59(3), 696–704. <https://doi.org/10.1093/icb/icz103>
- Farina, S. C., Kane, E. A., & Hernandez, L. P. (2019a). Multifunctional structures and multistructural functions: Integration in the evolution of biomechanical systems. *Integrative and Comparative Biology*, 59(2), 338–345. <https://doi.org/10.1093/icb/icz095>
- Farina, S. C., Knope, M. L., Corn, K. A., Summers, A. P., & Bemis, W. E. (2019b). Functional coupling in the evolution of suction feeding and gill ventilation of sculpins (Perciformes: Cottoidei).

- Integrative and Comparative Biology*, 59(2), 394–409. <https://doi.org/10.1093/icb/icz022>
- Felice, R. N., Randau, M., & Goswami, A. (2018). A fly in a tube: Macroevolutionary expectations for integrated phenotypes. *Evolution*, 72(12), 2580–2594. <https://doi.org/10.1111/evo.13608>
- Friendly, M. (2002). Corrgrams: Exploratory displays for correlation matrices. *American Statistician*, 56(4), 316–324. <https://doi.org/10.1198/000313002533>
- Galis, F., & Drucker, E. G. (1996). Pharyngeal biting mechanics in centrarchid and cichlid fishes: Insights into a key evolutionary innovation. *Journal of Evolutionary Biology*, 9(5), 641–670. <https://doi.org/10.1046/j.1420-9101.1996.9050641.x>
- Gatesy, S. M., & Middleton, K. M. (1997). Bipedalism, flight, and the evolution of theropod locomotor diversity. *Journal of Vertebrate Paleontology*, 17, 308–329.
- Goswami, A., Smaers, J. B., Soligo, C., & Polly, P. D. (2014). The macroevolutionary consequences of phenotypic integration: From development to deep time. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1649), 20130254–20130254. <https://doi.org/10.1098/rstb.2013.0254>
- Heard, S. B., & Hauser, D. L. (1995). Key evolutionary innovations and their ecological mechanisms. *Historical Biology*, 10(2), 151–173. <https://doi.org/10.1080/10292389509380518>
- Hernandez, L. P., & Cohen, K. E. (2019). The role of developmental integration and historical contingency in the origin and evolution of Cypriniform trophic novelties. *Integrative and Comparative Biology*, 59(2), 473–488. <https://doi.org/10.1093/icb/icz056>
- Holzman, R., Collar, D. C., Price, S. A., Hulse, C. D., Thomson, R. C., & Wainwright, P. C. (2012). Biomechanical trade-offs bias rates of evolution in the feeding apparatus of fishes. *Proceedings of the Royal Society B*, 279, 1287–1292.
- Hulse, C. D., García de León, F. J., & Rodiles-Hernández, R. (2006). Micro- and macroevolutionary decoupling of cichlid jaws: A test of Liem's key innovation hypothesis. *Evolution*, 60(10), 2096–2109.
- Hulse, C. D., & García De León, F. J. (2005). Cichlid jaw mechanics: Linking morphology to feeding specialization. *Functional Ecology*, 19, 487–494.
- Hulse, C. D., Mims, M. C., Parnell, N. F., & Streebman, J. T. (2010). Comparative rates of lower jaw diversification in cichlid adaptive radiations. *Journal of Evolutionary Biology*, 23(7), 1456–1467. <https://doi.org/10.1111/j.1420-9101.2010.02004.x>
- Hulse, C. D., Roberts, R. J., Lin, A. S. P., Guldberg, R., & Streebman, J. T. (2008). Convergence in a mechanically complex phenotype: Detecting structural adaptations for crushing in cichlid fish. *Evolution*, 62(7), 1587–1599. <https://doi.org/10.1111/j.1558-5646.2008.00384.x>
- Hunter, J. P. (1998). Key innovations and the ecology of macroevolution. *Trends in Ecology and Evolution*, 13(1), 31–36. [https://doi.org/10.1016/s0169-5347\(97\)01273-1](https://doi.org/10.1016/s0169-5347(97)01273-1)
- Huttenberger, S. M., & Mitteroecker, P. (2011). Invariance and meaningfulness in phenotype spaces. *Evolutionary Biology*, 38(3), 335–351. <https://doi.org/10.1007/s11692-011-9123-x>
- Irisarri, I., Singh, P., Koblmüller, S., Torres-Dowdall, J., Henning, F., Franchini, P., Fischer, C., Lemmon, A. R., Lemmon, E. M., Thallinger, G. G., Sturmbauer, C., & Meyer, A. (2018). Phylogenomics uncovers early hybridization and adaptive loci shaping the radiation of Lake Tanganyika cichlid fishes. *Nature Communications*, 9(1), 3159. <https://doi.org/10.1038/s41467-018-05479-9>
- Joyce, D. A., Lunt, D. H., Gerner, M. J., Turner, G. F., Bills, R., & Seehausen, O. (2011). Repeated colonization and hybridization in Lake Malawi cichlids. *Current Biology*, 21(3), R108–R109. <https://doi.org/10.1016/j.cub.2010.11.029>
- Kaufman, L. S., & Liem, K. F. (1982). Fishes of the suborder Labroidei (Pisces: Perciformes): Phylogeny, ecology and evolutionary significance. *Breviora*, 472, 1–19.
- Keast, A. (1978). Trophic and spatial interrelationships in the fish species of an Ontario temperate lake. *Environmental Biology of Fishes*, 3(1), 7–31. <https://doi.org/10.1007/bf00006306>
- Kendall, M. G. (1938). A new measure of rank correlation. *Biometrika*, 30(1/2), 81–93. <https://doi.org/10.2307/2332226>
- Kendall, M. G. (1945). The treatment of ties in ranking problems. *Biometrika*, 33, 239–251. <https://doi.org/10.1093/biomet/33.3.239>
- Klingenberg, C. P. (2016). Size, shape, and form: Concepts of allometry in geometric morphometrics. *Development Genes and Evolution*, 226(3), 113–137. <https://doi.org/10.1007/s00427-016-0539-2>
- Labandeira, C. C. (1997). Insect mouthparts: Ascertaining the paleobiology of insect feeding strategies. *Annual Review of Ecology, Evolution, and Systematics*, 28, 153–193.
- Larouche, O., Hodge, J. R., Alencar, L. R. V., Camper, B., Adams, D. S., Zapfe, K., Friedman, S. T., Wainwright, P. C., & Price, S. A. (2020). Do key innovations unlock diversification? A case-study on the morphological and ecological impact of pharyngognathy in acanthomorph fishes. *Current Zoology*, 66(5), 575–588. <https://doi.org/10.1093/cz/zoaa048>
- Lauder, G. V. (1983). Functional design and evolution of the pharyngeal jaw apparatus in euteleostean fishes. *Zoological Journal of the Linnean Society*, 77(1), 1–38. <https://doi.org/10.1111/j.1096-3642.1983.tb01719.x>
- Lauder, G. V. (1990). Functional morphology and systematics: Studying functional patterns in an historical context. *Annual Review of Ecology and Systematics*, 21(1), 317–340. <https://doi.org/10.1146/annurev.es.21.110190.001533>
- Liem, K. F. (1973). Evolutionary strategies and morphological innovations: Cichlid pharyngeal jaws. *Systematic Zoology*, 22(4), 425–441. <https://doi.org/10.2307/2412950>
- Liem, K. F. (1990). *Evolutionary innovations*. University of Chicago Press.
- Liem, K. F., & Sanderson, S. L. (1986). The pharyngeal jaw apparatus of labrid fishes: A functional morphological perspective. *Journal of Morphology*, 187(2), 143–158. <https://doi.org/10.1002/jmor.1051870203>
- López-Fernández, H., Arbour, J. H., Winemiller, K. O., & Honeycutt, R. L. (2013). Testing for ancient adaptive radiations in neotropical cichlid fishes. *Evolution*, 67(5), 1321–1337. <https://doi.org/10.1111/evo.12038>
- Mabuchi, K., Miya, M., Azuma, Y., & Nishida, M. (2007). Independent evolution of the specialized pharyngeal jaw apparatus in cichlid and labrid fishes. *BMC Evolutionary Biology*, 7, 1–12.
- Mardia, K. V., Kent, J. T., & Bibby, J. M. (1979). *Multivariate analysis*. Academic Press.
- Marroig, G., & Cheverud, J. M. (2005). Size as a line of least evolutionary resistance: Diet and adaptive morphological radiation in New World monkeys. *Evolution*, 59(5), 1128–1142.
- Martin, C. H., Cutler, J. S., Friel, J. P., Dening Touokong, C., Coop, G., & Wainwright, P. C. (2015). Complex histories of repeated gene flow in Cameroon crater lake cichlids cast doubt on one of the clearest examples of sympatric speciation. *Evolution*, 69(6), 1406–1422. <https://doi.org/10.1111/evo.12674>
- Martinez, C. M., McGee, M. D., Borstein, S. R., & Wainwright, P. C. (2018). Feeding ecology underlies the evolution of cichlid jaw mobility. *Evolution*, 72(8), 1645–1655. <https://doi.org/10.1111/evo.13518>
- Matschiner, M., Böhne, A., Ronco, F., & Salzburger, W. (2020). The genomic timeline of cichlid fish diversification across continents. *Nature Communications*, 11(1), 1–8.
- Mayr, E. (1963). *Animal species and evolution*. Harvard University Press.
- McGee, M. D., Borstein, S. R., Neches, R. Y., Buescher, H. H., Seehausen, O., & Wainwright, P. C. (2015). A pharyngeal jaw evolutionary innovation facilitated extinction in Lake Victoria cichlids. *Science*, 350(6264), 1077–1079. <https://doi.org/10.1126/science.aab0800>
- Meier, J. I., Marques, D. A., Mwaiko, S., Wagner, C. E., Excoffier, L., & Seehausen, O. (2017). Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nature Communications*, 8(1), 14363. <https://doi.org/10.1038/ncomms14363>
- Meier, J. I., Stelkens, R. B., Joyce, D. A., Mwaiko, S., Phiri, N., Schlieven, U. K., Selz, O. M., Wagner, C. E., Katongo, C., & Seehausen, O.

- (2019). The coincidence of ecological opportunity with hybridization explains rapid adaptive radiation in Lake Mweru cichlid fishes. *Nature Communications*, 10(1), 5391. <https://doi.org/10.1038/s41467-019-13278-z>
- Miller, R. L., & Olson, E. C. (1958). *Morphological integration*. Chicago University Press.
- Montaña, C. G., & Winemiller, K. O. (2013). Evolutionary convergence in Neotropical cichlids and Nearctic centrarchids: Evidence from morphology, diet, and stable isotope analysis. *Biological Journal of the Linnean Society*, 109(1), 146–164. <https://doi.org/10.1111/bij.12021>
- Montaña, C. G., Winemiller, K. O., & Sutton, A. (2014). Intercontinental comparison of fish ecomorphology: Null model tests of community assembly at the patch scale in rivers. *Ecological Monographs*, 84(1), 91–107. <https://doi.org/10.1890/13-0708.1>
- Muñoz, M. M., Anderson, P. S. L., & Patek, S. N. (2017). Mechanical sensitivity and the dynamics of evolutionary rate shifts in biomechanical systems. *Proceedings of the Royal Society B: Biological Sciences*, 284, 20162325.
- Muñoz, M. M., Hu, Y., Anderson, P. S. L., & Patek, S. N. (2018). Strong biomechanical relationships bias the tempo and mode of morphological evolution. *Elife*, 7, 1–18.
- Murdoch, D. J., & Chow, E. D. (1996). A graphical display of large correlation matrices. *American Statistician*, 50(2), 178–180. <https://doi.org/10.1080/00031305.1996.10474371>
- Norton, S. F., & Brainerd, E. L. (1993). Convergence in the feeding mechanics of ecomorphologically similar species in the Centrarchidae and Cichlidae. *Journal of Experimental Biology*, 176(1), 11–29. <https://doi.org/10.1242/jeb.176.1.11>
- Ohno, S. (2013). *Evolution by gene duplication*. Springer Science & Business Media.
- Pavlicev, M., Cheverud, J. M., & Wagner, G. P. (2009). Measuring morphological integration using eigenvalue variance. *Evolutionary Biology*, 36(1), 157–170. <https://doi.org/10.1007/s11692-008-9042-7>
- Payne, J. L., & Wagner, A. (2019). The causes of evolvability and their evolution. *Nature Reviews Genetics*, 20(1), 24–38. <https://doi.org/10.1038/s41576-018-0069-z>
- Poelstra, J. W., Richards, E. J., & Martin, C. H. (2018). Speciation in sympatry with ongoing secondary gene flow and a potential olfactory trigger in a radiation of Cameroon cichlids. *Molecular Ecology*, 27(21), 4270–4288. <https://doi.org/10.1111/mec.14784>
- Pos, K. M., Farina, S. C., Kolmann, M. A., & Gidmark, N. J. (2019). Pharyngeal jaws converge by similar means, not to similar ends, when minnows (Cypriniformes: Leuciscidae) adapt to new dietary niches. *Integrative and Comparative Biology*, 59(2), 432–442. <https://doi.org/10.1093/icb/icz090>
- Price, S. A., Friedman, S. T., Corn, K. A., Martinez, C. M., Larouche, O., & Wainwright, P. C. (2019). Building a body shape morphospace of teleostean fishes. *Integrative and Comparative Biology*, 59(3), 716–730. <https://doi.org/10.1093/icb/icz115>
- R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://doi.org/https://www.R-project.org/>
- Rabosky, D. L., Chang, J., Title, P. O., Cowman, P. F., Sallan, L., Friedman, M., Kaschner, K., Garilao, C., Near, T. J., Coll, M., & Alfaro, M. E. (2018). An inverse latitudinal gradient in speciation rate for marine fishes. *Nature*, 559(7714), 392–395. <https://doi.org/10.1038/s41586-018-0273-1>
- Roberts, A. S., Farina, S. C., Goforth, R. R., & Gidmark, N. J. (2018). Evolution of skeletal and muscular morphology within the functionally integrated lower jaw adduction system of sculpins and relatives (Cottoidei). *Zoology*, 129, 59–65. <https://doi.org/10.1016/j.zool.2018.06.006>
- Roberts, A. S., Hodge, J. R., Chakrabarty, P., & Wainwright, P. C. (2021). Anatomical basis of diverse jaw protrusion directionality in ponyfishes (family Leiognathidae). *Journal of Morphology*, 282(3), 427–437. <https://doi.org/10.1002/jmor.21314>
- Ronco, F., Matschiner, M., Böhne, A., Boila, A., Büscher, H. H., El Tahaer, A., Indermaur, A., Malinsky, M., Ricci, V., Kahmen, A., Jentoft, S., & Salzburger, W. (2020). Drivers and dynamics of a massive adaptive radiation in cichlid fishes. *Nature*, 589(7840), 76–81. <https://doi.org/10.1038/s41586-020-2930-4>
- Ronco, F., & Salzburger, W. (2021). Tracing evolutionary decoupling of oral and pharyngeal jaws in cichlid fishes. *Evolution Letters*, 5(6), 625–635. <https://doi.org/10.1002/evl3.257>
- Salzburger, W. (2009). The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. *Molecular Ecology*, 18(2), 169–185. <https://doi.org/10.1111/j.1365-294X.2008.03981.x>
- Schaefer, S. A., & Lauder, G. V. (1986). Historical transformation of functional design: Evolutionary morphology of feeding mechanisms in loricioid catfishes. *Systematic Zoology*, 35(4), 489–508. <https://doi.org/10.2307/2413111>
- Schaefer, S. A., & Lauder, G. V. (1996). Testing historical hypotheses of morphological change: Biomechanical decoupling in loricioid catfishes. *Evolution*, 50(4), 1661–1675. <https://doi.org/10.1111/j.1558-5646.1996.tb03938.x>
- Schluter, D. (1996). Adaptive radiation along genetic lines of least resistance. *Evolution*, 50(5), 1766–1774. <https://doi.org/10.1111/j.1558-5646.1996.tb03563.x>
- Schluter, D. (2000). *The ecology of adaptive radiation*. Oxford University Press.
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 Years of image analysis. *Nature Methods*, 9(7), 671–675. <https://doi.org/10.1038/nmeth.2089>
- Schwenk, K., & Wagner, G. P. (2001). Function and the evolution of phenotypic stability: Connecting pattern to process. *American Zoologist*, 41(3), 552–563. <https://doi.org/10.1093/icb/41.3.552>
- Seehausen, O. (2004). Hybridization and adaptive radiation. *Trends in Ecology and Evolution*, 19(4), 198–207. <https://doi.org/10.1016/j.tree.2004.01.003>
- Seehausen, O. (2006). African cichlid fish: A model system in adaptive radiation research. *Proceedings of the Royal Society B: Biological Sciences*, 273, 1987–1998.
- Seehausen, O. (2015). Process and pattern in cichlid radiations—Inferences for understanding unusually high rates of evolutionary diversification. *New Phytologist*, 207(2), 304–312. <https://doi.org/10.1111/nph.13450>
- Seehausen, O., Terai, Y., Magalhaes, I. S., Carleton, K. L., Mrosso, H. D. J., Miyagi, R., Sluijs, I. V. D., Schneider, M. V., Maan, M. E., Tachida, H., Imai, H., & Okada, N. (2008). Speciation through sensory drive in cichlid fish. *Nature*, 455, 620–626.
- Simpson, G. G. (1944). *Tempo and mode in evolution*. Columbia University Press.
- Stiassny, M. L. J., & Jensen, J. S. (1987). Labroid intrarelationships revisited: Morphological complexity, key innovations, and the study of comparative diversity. *Bulletin of the Museum of Comparative Zoology*, 151, 269–319.
- Stroud, J. T., & Losos, J. B. (2016). Ecological opportunity and adaptive radiation. *Annual Review of Ecology, Evolution, and Systematics*, 47(1), 507–532. <https://doi.org/10.1146/annurev-ecolsys-121415-032254>
- Svardal, H., Quah, F. X., Malinsky, M., Ngatunga, B. P., Miska, E. A., Salzburger, W., Genner, M. J., Turner, G. F., & Durbin, R. (2020). Ancestral hybridization facilitated species diversification in the Lake Malawi cichlid fish adaptive radiation. *Molecular Biology and Evolution*, 37(4), 1100–1113. <https://doi.org/10.1093/molbev/msz294>
- Taylor, W. R., & Van Dyke, G. C. (1985). Revised procedure for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybiurn*, 9, 107–121.
- Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S*. Springer-Verlag.
- Verheyen, E., Slazburger, W., Snoeks, J., & Meyer, A. (2003). Origin of the superclade of cichlid fishes from Lake Victoria, East Africa. *Science*, 300, 325–329.
- Vermeij, G. J. (1973). Adaptation, versatility, and evolution. *Systematic Zoology*, 22(4), 466–477. <https://doi.org/10.2307/2412953>

- Vermeij, G. J. (2001). Innovation and evolution at the edge: Origins and fates of gastropods with a labral tooth. *Biological Journal of the Linnean Society*, 72(4), 461–508. <https://doi.org/10.1111/j.1095-8312.2001.tb01333.x>
- Wagner, C. E., Harmon, L. J., & Seehausen, O. (2012). Ecological opportunity and sexual selection together predict adaptive radiation. *Nature*, 487(7407), 366–369. <https://doi.org/10.1038/nature11144>
- Wagner, G. P., Pavlicev, M., & Cheverud, J. M. (2007). The road to modularity. *Nature Reviews Genetics*, 8(12), 921–931. <https://doi.org/10.1038/nrg2267>
- Wainwright, P. C. (2005). Functional morphology of the pharyngeal jaw apparatus. In R. Shadwick & G. V. Lauder (Eds.), *Biomechanics of Fishes*. San Diego, CA: Academic Press (pp. 77–101).
- Wainwright, P. C., Smith, W. L., Price, S. A., Tang, K. L., Sparks, J. S., Ferry, L. A., Kuhn, K. L., Eytan, R. I., & Near, T. J. (2012). The evolution of pharyngognath: A phylogenetic and functional appraisal of the pharyngeal jaw key innovation in labroid fishes and beyond. *Systematic Biology*, 61(6), 1001–1027. <https://doi.org/10.1093/sysbio/sys060>
- Wake, D. B., & Roth, G. (Eds.). (1989). *Dahlem workshop on complex organismal functions: Integration and evolution of vertebrates* (pp. 412–414). Wiley.
- Walker, J. A. (2007). A general model of functional constraints on phenotypic evolution. *American Naturalist*, 170(5), 681–689. <https://doi.org/10.1086/521957>
- Wei, T., & Simko, V. (2021). R package “corrplot”: Visualization of a correlation matrix.
- Zelditch, M. L., Swiderski, D. L., Sheets, H. D., & Fink, W. L. (2012). *Geometric morphometrics for biologists: A primer*. Academic Press.
- Zhang, J. (2003). Evolution by gene duplication: An update. *Trends in Ecology and Evolution*, 18(6), 292–298. [https://doi.org/10.1016/s0169-5347\(03\)00033-8](https://doi.org/10.1016/s0169-5347(03)00033-8)