ORIGINAL ARTICLE

Decoupled jaws promote trophic diversity in cichlid fishes

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Functional decoupling of oral and pharyngeal jaws is widely considered to have expanded the ecological repertoire of cichlid fishes. But, the degree to which the evolution of these jaw systems is decoupled and whether decoupling has impacted trophic diversification remains unknown. Focusing on the large Neotropical radiation of cichlids, we ask whether oral and pharyngeal jaw evolution is correlated and how their evolutionary rates respond to feeding ecology. In support of decoupling, we find relaxed evolutionary integration between the two jaw systems, resulting in novel trait combinations that potentially facilitate feeding mode diversification. These outcomes are made possible by escaping the mechanical trade-off between force transmission and mobility, which characterizes a single jaw system that functions in isolation. In spite of the structural independence of the two jaw systems, results using a Bayesian, state-dependent, relaxed-clock model of multivariate Brownian motion indicate strongly aligned evolutionary responses to feeding ecology. So, although decoupling of prey capture and processing functions released constraints on jaw evolution and promoted trophic diversity in cichlids, the natural diversity of consumed prey has also induced a moderate degree of evolutionary integration between the jaw systems, reminiscent of the original mechanical trade-off between force and mobility.

KEY WORDS: Adaptive radiation, integration, key innovation, macroevolution, MuSSCRat, RevBayes.

Ray-finned fishes comprise more than half of all vertebrate species and show extensive trophic diversity, feeding on nearly all aquatic metazoans, many plants, detritus, and microbes. Underlying this ecological diversity is a highly versatile feeding apparatus composed of two jaw systems: oral jaws that function in prey capture and pharyngeal jaws that are used in prey processing. The structural independence of these two systems and their capacity to physically decouple two primary feeding functions is recognized as having important implications for the diversification of ray-finned fishes (Liem 1973; Lauder 1982; 1983). Foremost among these is that the decoupling of functions between two structurally separate jaw systems could promote the independent evolution of morphological features associated with prey capture and processing, thereby enhancing the capacity for trophic diversification (Liem 1973). But, although the evolution of pharyngeal jaws is widely recognized as one of the classic examples of decoupling in evolutionary morphology (Lauder 1990;

Farina et al. 2019), fundamental predictions of its macroevolutionary consequences remain poorly explored (Hulsey 2006).

Functional decoupling, as a mechanism that promotes diversification, is regarded as a general principal in evolutionary morphology (Schaefer and Lauder 1986; Lauder 1990; Schwenk 2001). The core idea is that a single structural system (such as the oral jaw) that performs two functions must meet the performance demands of both, and this requirement may limit evolutionary changes to the system that trades off one function against the other. In fish feeding systems, the central design trade-off that has dominated discussion in the literature is between the ability to transmit force across jaw joints and both the speed and magnitude of jaw movements (Westneat 2004, 2005). This trade-off, a fundamental feature of levers and linkages, means that a single jaw system cannot simultaneously be modified for higher force transmission and greater transmission of motion (Fig. 1). For example, elasmobranchs capture and manipulate prey solely with

One jaw system
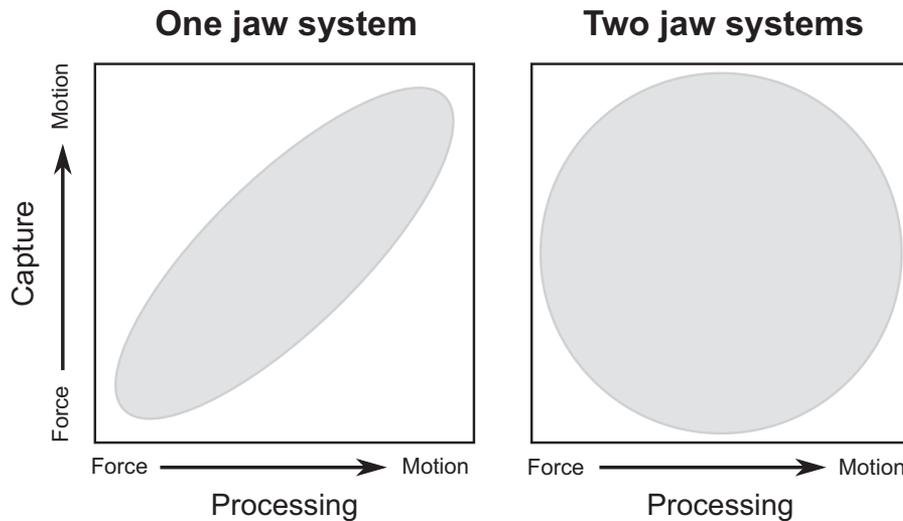
Two jaw systems

Figure 1. Hypothetical mechanical trade-off between force and motion modification in a one-jaw system such as the oral jaw (left), and escape from such a trade-off when prey capture and processing are performed by two jaw systems such as the oral and pharyngeal jaws (right).

oral jaws (Motta and Wilga 1995; Wilga and Motta 2000) that are subject to this trade-off, and it is unclear how, in the absence of a secondary jaw system, evolution might modify them to be simultaneously strong for forceful processing of structurally defended prey and also fast and highly kinetic for capturing elusive, mobile prey (Hulsey and García de León 2005; Cooper et al. 2010, 2017; Martinez et al. 2018). A consequence of physically decoupling prey capture and processing through the origin of a second jaw system is that the feeding mechanism of ray-finned fishes can theoretically create a novel functional combination where oral jaw mobility during prey capture is paired with forceful biting actions by the pharyngeal jaws, or high force-transmitting oral jaws paired with highly kinetic pharyngeal jaws (Fig. 1).

In the present study, we explore the consequences of decoupled prey capture and processing functions in an exemplar group of ray-finned fishes, the monophyletic cichlids of South and Middle America. Cichlids represent a particularly compelling example of decoupled feeding because they possess a derived condition of the pharyngeal jaw system, termed pharyngognathy, that increases functional versatility (Liem and Greenwood 1981; Stiassny and Jensen 1987). Indeed, the possibility that the spectacular diversity of cichlid fishes may be partly due to the release of constraints brought about by decoupling prey capture and processing functions and enhanced pharyngeal jaw functionality is the canonical example for evolutionary decoupling in the literature, in which it is discussed as a key innovation partly explaining the spectacular diversity of cichlid fishes through adaptive radiation (Liem 1973; Givnish 2015; Stroud and Losos 2016). In spite of considerable interest in this system, there have been few attempts to measure evolutionary integration between

oral and pharyngeal jaw traits in cichlids, and studies in this area have focused on a limited number of traits (Hulsey et al. 2006; Fraser et al. 2009).

We measure a suite of morphological and functional traits, characterizing both oral and pharyngeal jaws in 84 species of Neotropical cichlids and use phylogenetic comparative methods to explore predictions of decoupling as they have been applied to fish jaws. In the case of a single-jaw system, both prey capture and processing are performed by only one feeding apparatus (e.g., the oral jaws of elasmobranchs) and we therefore expect to see the mechanics of these two functions aligned. For example, high motion transmission and poor force transfer in prey capture will be associated with high motion transmission and poor force transfer in prey processing because the morphological traits that underlie these functions are largely shared within the same jaw system (Fig. 1). In the case of the decoupled two-jaw system found in cichlids, we ask whether evolutionary integration *between* the oral and pharyngeal jaws has been relaxed relative to the integration *within* each (Fig. 1). Second, we ask whether decoupling has allowed some cichlid lineages to circumvent the fundamental trade-off between force and mobility and evolve combinations of traits in the two jaw systems that are incompatible within a single-jaw system. Thus, we look for trait combinations that reflect adaptation for high oral jaw mobility in taxa with high pharyngeal jaw force transmission, or high oral jaw force transmission with high pharyngeal jaw kinesis (Fig. 1). Finally, to better understand the ecological constraints on the evolution of jaw mechanics, we evaluate the impact of feeding ecology on the evolutionary integration between the oral and pharyngeal jaw systems. Despite expected decoupling of oral

and pharyngeal jaws, we predict that selective pressures imposed by different prey types will result in some degree of coordinated evolution between jaw systems.

Material and Methods
SPECIMENS AND MEASUREMENTS

A total of 218 individuals representing 84 species (one to three individuals per species) were cleared and stained (Dingerkus and Uhler 1977) for examination of skeletal structures associated with oral and pharyngeal jaws. Specimens were either from existing museum collections or were specimens acquired through the pet trade (see Table S1 for museum accession numbers). The species sampled are widely distributed across the Neotropical cichlid phylogeny and representative of their morphological and trophic diversity (Table S1). Using digital calipers, we measured 16 traits, consisting of morphological variables that characterize the shape of the oral jaw system as well as several functional metrics derived from morphology (see Table S2 for detailed descriptions): protrusion, maximum gape, length of the dentigerous arm of the premaxilla, length of the ascending process of the premaxilla, length of the lower jaw, length of the oral and buccal cavities, anteroposterior and dorsoventral positions of the articular-quadrate joint (lower jaw joint) and the ligamentous connection forming the maxilla-nasal joint, the closing and opening mechanical advantage (MA) of the lower jaw (Westneat 1994), kinematic transmission (KT; Westneat 1990), kinesis, and kinematic asynchrony (KA). KT, kinesis, and KA are properties of the oral jaw four-bar linkage system that we calculated based on methods by Martinez and Wainwright (2019). Briefly, species-averaged oral jaw four-bar shapes were geometrically assembled in a standardized position from individual link measurements and were used to simulate linkage movements over 30 degrees of input rotation, representing mandibular abduction, with the R package LINKR (Olsen 2016). Ten four-bar shapes were extracted from these simulated movements, sampled at equal angular intervals of input rotation, and were aligned using generalized Procrustes analysis in the R package GEOMORPH (Adams et al. 2019). KT was calculated as the ratio of output rotation of the four-bar's maxillary link to input rotation of the mandible, measured sequentially between extracted four-bar shapes. Kinesis and KA are features of four-bar shape trajectories (i.e., the path traced through morphospace by an ordered series of motion shapes) that were generated by four-bar linkages during simulated movements (Martinez et al. 2018; Martinez and Wainwright 2019). Kinesis, a measure of four-bar mobility, was calculated as the length of the shape trajectory after 30 degrees of input rotation. KA reflects the relative level of temporal asynchrony of mobile components during the motion of the four-bar linkage and was calculated as the maximum deviation of the

shape trajectory from linear, scaled by the distance between the start and end motion shapes (Martinez and Wainwright 2019).

In addition to the oral jaw, we measured 11 traits that characterize shape and functional attributes of the pharyngeal jaw system (see Table S2 for detailed descriptions): length, depth, and width of the united fifth ceratobranchials (lower pharyngeal jaw; LPJ); length of the suture between left and right sides of the fifth ceratobranchials; area of the dentigerous surface on the fifth ceratobranchials; mean diameter of the three largest teeth on the lower pharyngeal jaw; diameter of the insertion site of the muscular sling on the lateral process of the fifth ceratobranchial; length, depth, and width of one of the paired fourth pharyngobranchials (right-side upper pharyngeal jaw; UPJ); and diameter of the dorsal facet of the upper pharyngeal jaw that articulates against a process on the underside of the neurocranium. Four additional traits were measured: standard body length, and the length, depth, and width of the head (Table S3). Morphological measurements from the oral and pharyngeal jaws were converted to log-shape-ratios with the geometric mean of head size:

$$\log\left(\text{trait}/\left(\text{head length} \times \text{head width} \times \text{head depth}\right)^{1/3}\right).$$

Because methods of size correction have the potential to influence the outcomes of our analyses, we compared results using log-shape-ratios to those based on residuals from phylogenetic regressions of log-transformed trait values against log-transformed standard body length, using the phyl.resid function implemented in the R package PHYTOOLS (Revell 2012). For this procedure and all subsequent comparative analyses, we used an existing phylogeny of cichlids (Burress and Tan 2017; Burress et al. 2019), pruned to the taxa in our study.

To estimate the impact of feeding ecology on the evolution of cichlid feeding mechanisms, we categorized species into four broad trophic guilds based on the major prey items found in their diets and how those prey are captured (adapted from the guilds defined in Burress et al. 2019): predators, sifters, grazers, and generalists. Predators almost exclusively consume evasive prey such as fishes. Sifters consume small prey items by plunging their snouts into the substrate. Their prey includes microcrustaceans and insect larvae from the substrate or interstitial organisms buried within the sediment. These species also consume, either intentionally or inadvertently, fractions of detritus and inorganic material as a result of their feeding mode. Grazers consume almost exclusively nonevasive prey, including attached organisms such as algae and molluscs. Attached prey are consumed by either scraping or picking. Finally, generalists consume mixtures of plant material and invertebrates, but generally not fishes, algae, or molluscs, and do not forage by sifting or scraping. Classifications were based on each species' primary feeding mode with consideration of the target prey (Říčan et al. 2016; Burress 2016;

Burress et al. 2019). All data are available on Dryad (Burress et al. 2020).

STATISTICAL ANALYSES

We assessed the evolutionary correlation, or integration, between oral and pharyngeal jaws in two ways. First, we calculated phylogenetic independent contrasts (Felsenstein 1985) for each trait using the pic function in APE (Paradis et al. 2004) and subsequently estimated the correlation coefficient for all pairwise combinations of traits from regressions through the origin. These pairwise correlations were partitioned into three groups: correlations among oral jaw traits, among pharyngeal jaw traits, and between oral and pharyngeal jaw traits. We also generated a null distribution of evolutionary correlations from a Brownian motion (BM) process by simulating a set of uncorrelated traits across the phylogeny using the fastBM function implemented in PHYTOOLS (Revell 2012). We then estimated pairwise correlations of simulated traits, using phylogenetic independent contrasts to build a null distribution of expected correlations under uncorrelated Brownian evolution. Pairwise evolutionary correlations were repeated across a distribution of 100 phylogenetic trees to account for uncertainty in topology and divergence times. We also calculated multivariate contrasts (McPeek et al. 2008) separately for oral and pharyngeal jaw traits and generated 100 corresponding null datasets consisting of 27 BM-simulated traits as described above. After separating these simulated traits into 16- and 11-trait sets to match the observed oral and pharyngeal jaw datasets, we calculated multivariate contrasts and generated a null distribution of multivariate evolutionary correlations. We chose to use BM as a null model, but there is an active discussion about alternatives (Butler and King 2004; Freckleton and Harvey 2006; Cooper et al. 2016; Uyeda et al. 2018).

Second, we calculated branch-specific rates of multivariate evolution separately for the oral and pharyngeal jaw systems using a relaxed morphological clock model that lets branches have different rate parameters implemented in RevBayes (Höhna et al. 2016). To achieve this, we used the relaxed local clock (Eastman et al. 2011), which assumes each branch either does or does not have a rate shift. When there is no shift along a branch, the rate of that branch is inherited directly from its ancestral branch. When there is a shift, the ancestral rate is multiplied by a rate shift parameter drawn from a prior distribution (i.e., the expected number of rate shifts/number of branches). The rate parameter at the root was drawn from a loguniform prior. The size of the rate shift was drawn from a lognormal distribution with a mean of 1 and a standard deviation such that rate shifts range about one order of magnitude. We estimated rates of oral and pharyngeal jaw evolution using a multivariate Brownian motion model for multiple continuous characters (Caetano and Harmon 2017).

Branch-specific Brownian rates were estimated using reversible-jump Markov chain Monte Carlo (MCMC) that sampled parameter values in proportion to their posterior probability. The rjMCMC was run for 500,000 generations with two independent runs and 10% burn-in. We repeated the analysis using different priors (i.e., 1, 5, and 10) on the number of rate shifts to determine its impact on posterior estimates of the number of rate shifts.

Finally, we tested for evolutionary integration driven by feeding ecology. We estimated the impact of trophic guild, a discrete trait, on the rates of oral and pharyngeal jaw evolution using a Bayesian, state-dependent, relaxed-clock model of multivariate Brownian Motion (MuSSCRat; May and Moore 2019) implemented in RevBayes (Höhna et al. 2016). MuSSCRat models the joint evolution of discrete and continuous characters evolving under a state-dependent multivariate Brownian motion process and, importantly, accommodates variation in the background rate of continuous character evolution (i.e., rate variation independent of the discrete character of interest). In this analysis, we assumed the discrete character evolves under a continuous-time Markov process and the continuous characters evolve under a multivariate Brownian motion process with rates that depend on the state of the discrete character. This method permits rates to vary along branches and among continuous characters. The priors for lineage-specific background rates of evolution were drawn from a shared lognormal distribution and standard deviation that permitted the rates prior to range an order of magnitude. The MCMC was run for 500,000 generations with 10% burn-in. We repeated the analysis using three different priors on the number of state changes (of the discrete character; 10, 20, and 30 state changes) and number of rate shifts (of the continuous characters; 1, 5, and 10 shifts) to determine their impact on posterior estimates of the mean evolutionary rate, number of state changes, number of rate shifts, and state-dependent evolutionary rates.

Species-specific rates of diversification, or tip rates, have been used to evaluate patterns of lineage diversification, but are increasingly used to describe patterns of morphological evolution (Harvey and Rabosky 2018; Title and Rabosky 2019). Tip rates are an estimate of the present-day evolutionary rate of a lineage, conditioned on past evolutionary history (Title and Rabosky 2019), whereas clade rates reflect deeper phylogenetic correlation. These two methods provide complementary insights into the correlated nature of oral and pharyngeal jaw evolution at different timescales. The concordance between the evolutionary rates and state-dependent rates of oral and pharyngeal jaw evolution was assessed by calculating mean clade rates for 18 nonoverlapping cichlid clades (Fig. S1) as well as tip rates for each species, and comparing their respective oral and pharyngeal jaw evolutionary rates using phylogenetic generalized least-squares regression, implemented with the gls function employed
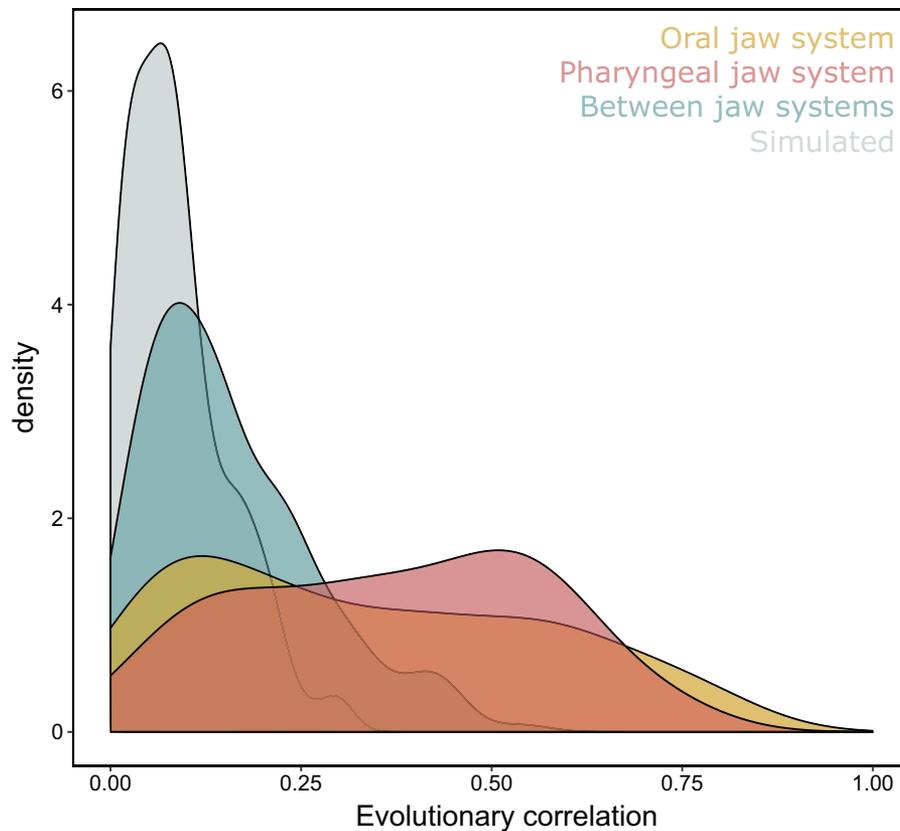
Figure 2. Evolutionary correlations within and between the oral and pharyngeal jaw systems. Distributions depict the effect sizes (correlation coefficients) between phylogenetic independent contrasts. The null distribution depicts evolutionary correlations that could result from a Brownian motion evolutionary process.

in the LMNE R package. In the case of clade rates, the tree was pruned to a representative tip for each clade.

Results

EVOLUTIONARY CORRELATIONS OF ORAL AND PHARYNGEAL JAWS

The evolutionary correlation of oral jaw traits varied, with approximately 63% of the pairwise relationships being more strongly correlated than would be expected under an uncorrelated BM evolutionary process (Fig. 2). By comparison, evolutionary correlations of pharyngeal jaw traits tended to be higher, with approximately 73% of the pairwise correlations being stronger than could be explained by a BM evolutionary process (Fig. 2). Finally, evolutionary correlations between oral and pharyngeal jaw traits mostly fell within the null distribution of values (Fig. 2), with only 29% of relationships stronger than expected from a BM evolutionary process (53 out of 180 pairwise correlations). These patterns were consistent when phylogenetic residuals were used to correct for size instead of log-shape-ratios (Fig. S2).

Significant correlations, when they occurred, tended to be negative relationships between oral jaw traits associated with size and protrusion (gape, protrusion, ascending process length, and premaxilla length) and pharyngeal jaw traits associated with biting strength (LPJ depth, muscular sling insertion, and UPJ facet; Table S3). The bite force potential of the oral jaws (MA closing) was negatively correlated with oral jaw kinesis as well as other secondary components of jaw mobility (i.e., gape and protrusion), consistent with constraints imposed by trade-offs in a one-jaw system (Fig. 3A). In comparison, several traits associated with the strength of the pharyngeal jaws (depth, dentigerous area, and tooth size of the LPJ, muscular sling insertion, and UPJ facet) were uncorrelated with most attributes of oral jaw mobility (KT, kinesis, and KA; Fig. 3B; Table S3).

There are several lineages that appear to capitalize on decoupling by combining high oral jaw mobility with strong pharyngeal jaws or force-modified oral jaws with motion-modified pharyngeal jaws (Fig. 3C). Multivariate contrasts of oral and pharyngeal jaw traits were significantly correlated ($r = 0.519$; $P < 0.0001$); however, this degree of evolutionary correlation was consistent with effect sizes simulated under uncorrelated BM ($P = 0.49$; Fig. 4). Branch-specific oral jaw evolutionary rates varied approximately sixfold across the phylogeny, whereas pharyngeal jaw evolutionary rates varied approximately 13-fold
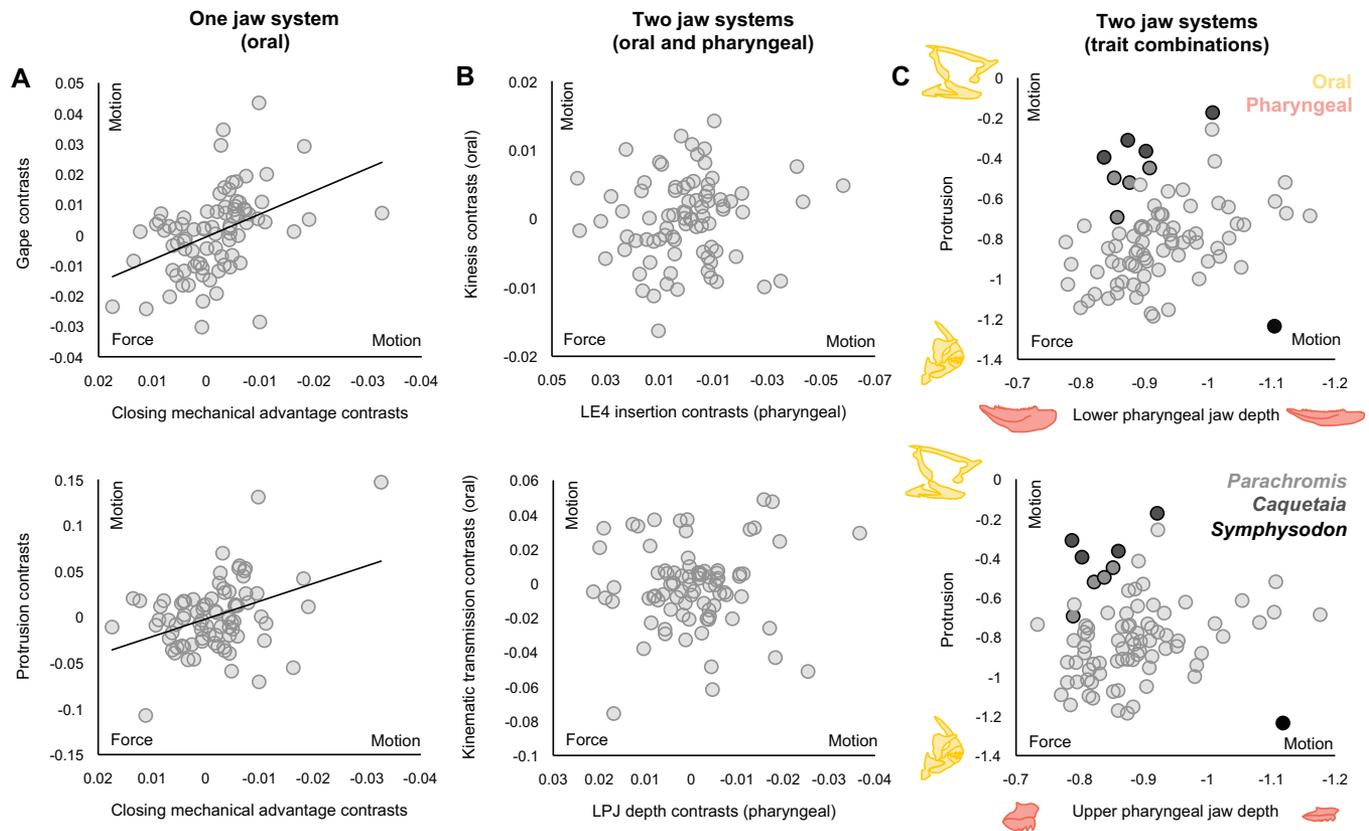
Figure 3. Trade-off between mobility and force transmission in a one-jaw system (A), the oral jaw, where prey capture is depicted by gape and protrusion and prey processing is depicted by the closing mechanical advantage of the lower jaw. A two-jaw system, including both the oral and pharyngeal jaws (B), provides release from the force-mobility constraint. Here, the pharyngeal jaws are depicted by the insertion of the muscular sling (fourth levator externus; LE4) on the lower pharyngeal jaw (LPJ) and depth of the LPJ. The oral jaws are depicted by kinesis and kinematic transmission. Some cichlids have taken advantage of the release of constraint that the two-jaw system offers to combine motion- and force-modified jaws (C). Here, the pharyngeal jaws are represented by LPJ depth and the oral jaws by protrusion. Values in A and B are phylogenetically independent contrasts. Values in C are size-corrected trait values. Note that the *x*-axes have been rotated such that force-modified is depicted by the origin in both axes.

(Fig. 5A). Clade rates of oral and pharyngeal jaws were correlated ($r = 0.588$; $P = 0.0287$; Fig. 5B), as were the tip rates ($r = 0.509$; $P = 0.002$; Fig. 5C). Posterior estimates of the number of rate shifts increased with the prior number of rate shifts (Fig. S3).

STATE-DEPENDENT ORAL AND PHARYNGEAL JAW EVOLUTION

Oral jaw evolutionary rates varied significantly according to feeding ecology (posterior probability = 1.0; Fig. 6A). Predators had faster evolutionary rates than grazers (1.6-fold), sifters (2.2-fold), and generalists (4.9-fold; Fig. 6A). Pharyngeal jaw evolutionary rates were also state dependent (posterior probability = 1.0; Fig. 6B). Predators and grazers had faster evolutionary rates than sifters (2.5-fold) and generalists (4.2-fold; Fig. 6B). In addition, state-dependent oral and pharyngeal jaw evolutionary rates were strongly correlated among clades ($r = 0.962$; $P < 0.0001$; Fig. 6C). State-dependent branch rates of oral and pharyngeal

jaw evolution varied 4.8-fold and 3.7-fold among branches, respectively. Posterior estimates of the mean evolutionary rate and the number of state changes as well as the state-dependent evolutionary rates were consistent across different prior numbers of state changes (Figs. S4 and S5) and rate shifts (Figs. S6 and S7). The generalist feeding guild was the ancestral state, with numerous transitions to the other guilds (Fig. S8).

Discussion

Although oral and pharyngeal jaws show some developmental integration through shared genetic networks (Fraser et al. 2009), the presence of structurally separate jaw systems is believed to largely decouple the functions of prey capture and prey processing in ray-finned fishes (Liem 1973). Functional decoupling enhances the potential for independent evolution of the two systems and provides a means to escape mechanical trade-offs found
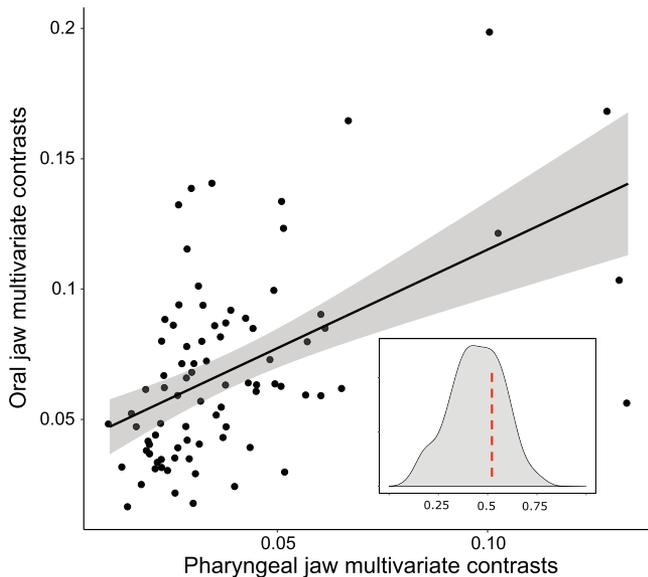
Figure 4. Evolutionary correlation between multivariate oral and pharyngeal jaw contrasts. The inset depicts the observed evolutionary correlation (dashed line) relative to those that could result from a Brownian motion process (density kernel).

within a single-jaw system performing both prey capture and processing. Our study reveals reduced evolutionary integration between the two jaw systems relative to that within each apparatus. In addition, trait combinations exist in some species reflecting this relaxed constraint in the form of functional adaptations that would be highly unlikely under the strong evolutionary integration of a one-jaw system. Although there is strong evidence that decoupling of functions has promoted the overall trophic diversification of Neotropical cichlids, the two different jaw systems respond similarly to feeding ecology, inducing a strong integration of oral and pharyngeal jaw evolution across the major trophic guilds found in this group.

DECOUPLING AS A MECHANISM THAT PROMOTES DIVERSIFICATION

A fundamental feature of levers and linkages is that they cannot simultaneously be modified for higher force transmission and greater transmission of motion (Muller 1996; Westneat 2004). In the case of ray-finned fishes, if prey capture and processing were carried out solely by the oral jaws, this trade-off would result in a strong association between the mechanical properties of prey capture and those for processing prey: strength in capture would inevitably be associated with strength in processing, whereas high kinesis and motion transfer in prey capture would be associated with the same features in prey processing (Fig. 1). The functional decoupling of prey capture and processing, mostly isolated between two different jaw systems, provides a release from this con-

straint by permitting combinations of capture and processing mechanics not allowed in the presence of the trade-off (Liem 1973).

Cichlids have two jaw systems, but each apparatus is subject to the same mechanical trade-offs as a one-jaw system. Indeed, we found evidence of the force-motion trade-off among oral jaw traits in Neotropical cichlids, as kinesis and other variables related to mobility (e.g., protrusion, premaxilla, and gape) were evolutionarily correlated with the closing mechanical advantage of the lower jaw, such that both prey capture and processing are either force modified or motion modified (Fig. 3; Table S3). However, we also see evidence of escape from this trade-off in some species when we considered both the oral and pharyngeal jaws, which appear to have compartmentalized prey capture and processing functions, respectively. Roughly half as many pairwise evolutionary correlations between jaw systems were significant as were within the oral jaw system alone (Fig. 2). Oral jaw mobility (kinesis) and the four-bar transmission coefficient (KT) were evolutionarily uncorrelated with almost all pharyngeal jaw traits (Fig. 3; Table S3). Isolated features of oral jaw mobility and function, such as protrusion, gape, and size (i.e., out-lever and premaxilla), were uncorrelated with about half of the pharyngeal jaw traits (Table S3). The frequency with which oral and pharyngeal jaw traits were uncorrelated highlights the expanded potential for trait combinations and their associated functions in decoupled systems, which may otherwise be caught in the force-motion trade-off found when prey capture and processing are performed by a single-jaw system and as reflected in stronger correlations between traits within each jaw system.

This study provides evidence that Neotropical cichlids have capitalized on the two-jaw system and evolved trait combinations that would appear to be incompatible if prey capture and processing were carried out by the same set of jaws. Some species exhibit a high degree of oral jaw mobility that is useful for suction feeding (Wainwright et al. 2001, 2015) and force-modified pharyngeal jaws that are useful for crushing and grinding tough prey (Hulsey 2006). The combination of these traits and functions results in an unusual feeding strategy in which the jaws are mechanically adapted differently, apparently providing the feeding versatility to capture evasive prey and also to process hard-shelled prey. Indeed, although some of these species, such as members of *Parachromis* and *Caquetaia*, consume principally evasive prey such as fishes, they also eat prey that require crushing such as clams, snails, and hard fruits (Winemiller 1989; Winemiller et al. 1995; Soria-Barreto et al. 2019). Another dramatic departure from mechanically aligned capture and processing systems occurs in discus (*Symphysodon*), which have force-modified oral jaws and gracile pharyngeal jaws. *Symphysodon* graze epiphytic algae (Crampton 2008), but their reduced pharyngeal jaws suggest they do not forcefully process it prior to ingestion, as do many other grazers (Xie 2001; Carr et al. 2006; Burress
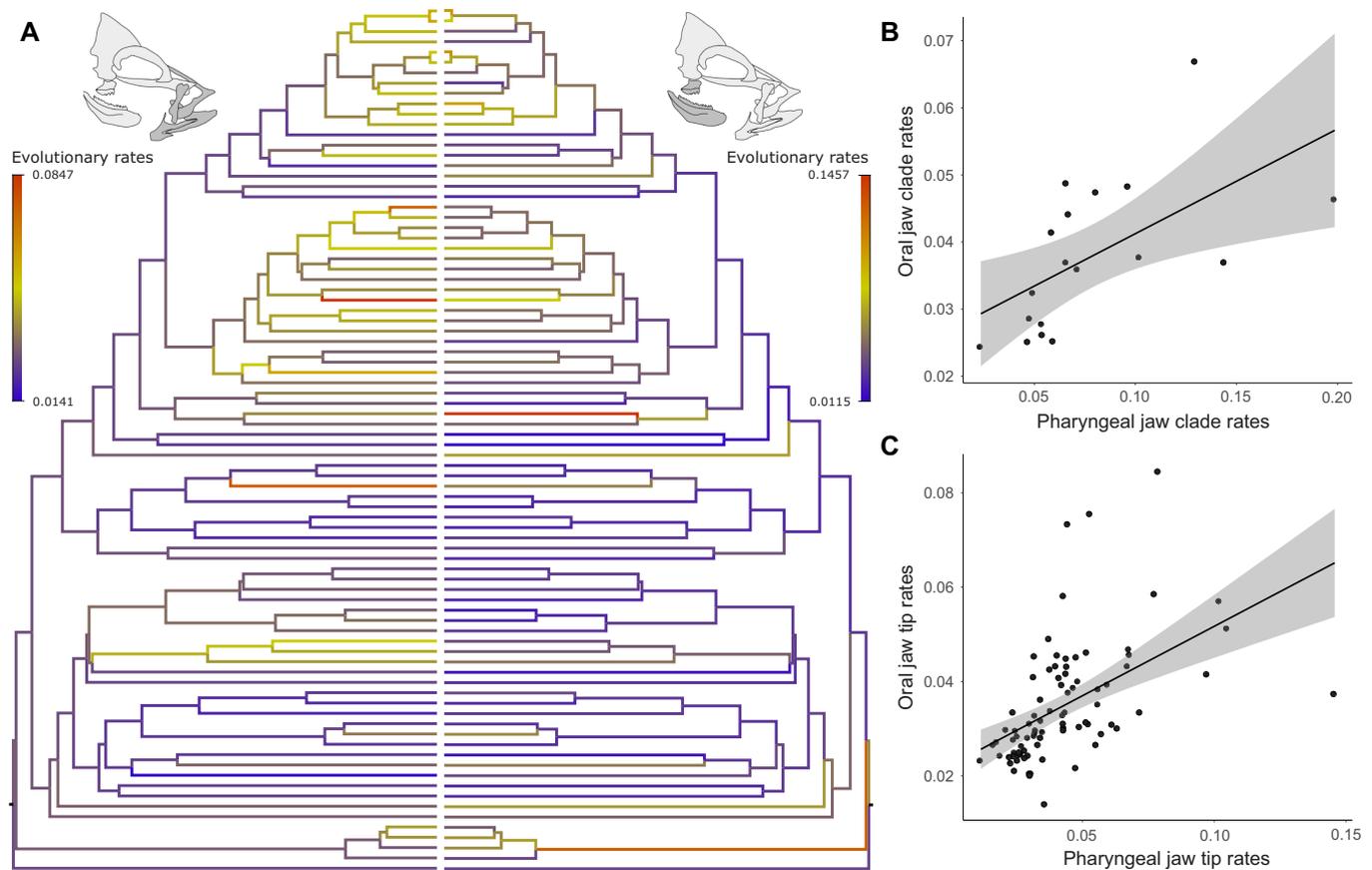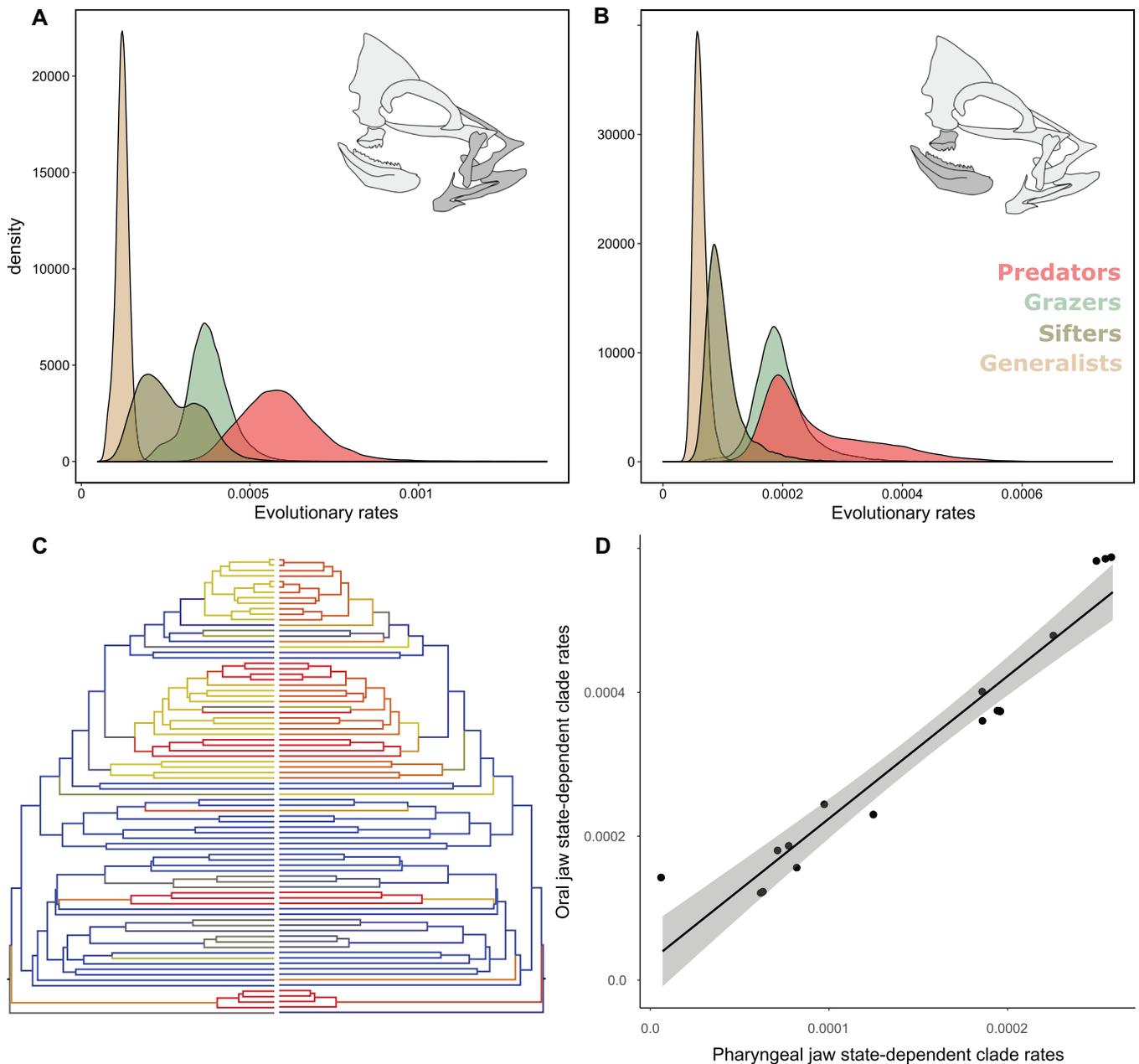
Figure 5. Rates of cichlid jaw evolution. Branch-specific rates of oral and pharyngeal jaw evolution across the Neotropical cichlid phylogeny (A). The relationship between clade rates (B) and tip rates (C) of oral and pharyngeal jaw evolution.

2016). These cases in which one-jaw system has been force modified and the other motion modified highlight the potential of decoupling in promoting trophic versatility and diversity.

INTEGRATION OF JAW SYSTEMS IS INDUCED BY FEEDING ECOLOGY

Despite the general pattern of weakly correlated evolution between the oral and pharyngeal jaw systems, using phylogenetic comparative methods that account for background rate variation, we identified an ecological dimension along which the evolution of the two jaw systems is highly integrated. Feeding ecology had a similar impact on the evolution of oral and pharyngeal jaws in that predators and grazers had elevated evolutionary rates, whereas generalists had slow rates (Fig. 6). Two of these guilds, predators and grazers, impose opposing functional demands on the two jaw systems, favoring mobility and force transmission, respectively. Many predatory cichlids make extensive use of suction to capture evasive prey (Norton and Brainerd 1993; Wainwright et al. 2001; Higham et al. 2007) and several lineages have highly specialized jaw systems for feeding upon fish prey. *Petenia*, some *Caquetaia*, and *Acaronia* use extreme oral jaw protrusion (Waltzek and Wainwright 2003) that likely enhances

prey capture performance when suction feeding on elusive prey (Bellwood et al. 2015), gracile pharyngeal jaws that may ease pharyngeal gape constraints (Burress et al. 2015), and feed almost exclusively upon fishes (Winemiller 1989; Cochran-Biederman and Winemiller 2010; Pease et al. 2018). In contrast, grazers principally feed on attached prey, scraping (e.g., algae) or picking prey from rock surfaces that are subsequently crushed by the pharyngeal jaws (e.g., snails). Several lineages have become highly specialized at biting with the oral and pharyngeal jaws. *Talamancaheros*, *Hypsophrys*, and *Uaru* have stout, compact oral jaws with specialized dentition for scraping algae from rocks (Casciotta and Arratia 1993; Winemiller et al. 1995). These taxa exhibit the lowest oral jaw kinesis, smallest gape, and least protrusion in our dataset (Table S1). They also possess stout pharyngeal jaws for crushing snails (Hulsey 2006) or processing cellulose-rich prey, such as algae, by rupturing cells (Xie 2001; Carr et al. 2006; Burress 2016). The ecological dimension that contrasts suction feeding upon evasive prey versus biting attached prey has been previously identified as a major axis of diversity in Neotropical cichlids (Winemiller et al. 1995; Arbour and López-Fernández 2014, 2016; Burress et al. 2019; Arbour et al. 2020). Thus, despite the evolutionary independence provided

Figure 6. Oral and pharyngeal jaw evolution in response to feeding ecology. State-dependent rates of oral (A) and pharyngeal (B) jaw systems estimated with a Bayesian, state-dependent, relaxed-clock model of multivariate Brownian motion. Branch-specific state-dependent rates of oral (left) and pharyngeal jaw (right) evolution across the phylogeny (C). The relationship between clade rates (D) of state-dependent oral and pharyngeal jaw evolution.

by decoupled prey capture and processing systems in cichlids, many species co-modify the jaw systems for similar functions (i.e., specialize in mobility or force generation). This tendency results in the two jaw systems being highly integrated along this ecological dimension (Fig. 6), resulting in a pattern reminiscent of the expectation under a one-jaw system (Fig. 1). The degree to which the oral and pharyngeal jaw systems are evolutionarily correlated in Neotropical cichlids is due to the correlated demands of capturing and processing different types of prey.

We posit that the high degree of integration between the state-dependent evolutionary rates is due to similar functional demands on prey capture and processing imposed by each of these trophic guilds. The capture of elusive prey requires suction or ram feeding and is associated with mobile oral jaws, often with high jaw protrusion (Waltzek and Wainwright 2003; Higham et al. 2007), and gracile pharyngeal jaws that appear to function primarily in transporting prey from the buccal cavity to the esophagus (Casciotta and Arratia 1993; Burress 2016).

Feeding on attached prey requires biting capture modes and is associated with compact jaws, higher mechanical advantage of jaw muscles, and less jaw protrusion, but also strong pharyngeal jaws that crush and forcefully bite prey such as molluscs and algae (Casciotta and Arratia 1993; Burress 2016). Although the presence of separate jaw systems for prey capture and processing allows other combinations of traits to evolve, the natural diversity of prey drives strong evolutionary integration between oral and pharyngeal jaws in terms of motion and force transmission. Another factor is that there is a high degree of jaw diversity within these feeding guilds (Fig. S8), in part facilitated by decoupling of the jaw systems allowing many trait combinations. Predators, for example, range from laterally compressed fish that suction feed using extensive jaw protrusion (e.g., *Petenia splendida* and *Caquetaia myersi*; Waltzek and Wainwright 2003), to large-bodied, elongate predators that feed using a high attack velocity (e.g., *Cichla* and *Crenicichla*; Norton and Brainerd 1993; Wainwright et al. 2001), and species that have intermediate morphology, yet consume large fractions of fishes (e.g., *Parachromis*; Winemiller et al. 1995). These groups exhibit dramatically different adaptations to feeding on fish and their diverse designs led to high rates of jaw evolution associated with a predatory feeding ecology. This diversity among predatory cichlids is in contrast to patterns seen in another major radiation of Neotropical freshwater fishes, characiforms, which show repeated convergence by piscivores on an elongate body form (Burns and Sidlauskas, 2019). A high degree of morphological disparity among piscivores has also been shown in wrasses (Labridae; Bellwood et al. 2005). Grazers similarly range from elongate forms that feed from rocky surfaces in lotic conditions (e.g., *Talamancaheros* and *Tomocichla*; Říčan et al. 2016) to discoid forms that graze from organic surfaces such as plants and wood in lentic conditions (e.g., *Symphysodon* and *Uaru*; Crampton 2008).

Conclusion

Integration within a jaw system is induced by mechanical trade-offs that prevent simultaneous modification of traits to enhance the transmission of both force and motion. In other words, within a jaw system functional integration is maintained by the shared anatomical features of that system. The evolution of a second jaw system enhances evolutionary versatility of fishes by structurally decoupling prey capture and processing. This decoupling provides a release from the trade-off because two structurally independent jaw systems can be modified to have different functional properties. We found that some cichlids capitalize on this possibility by combining jaws modified for force transmission and mobility. In spite of this potential for independent evolution of the jaw systems, a moderate degree of integration between the jaw systems is induced by feeding ecology. The contrast between an integrated single-jaw system with an intrinsic constraint on its functional anatomy (i.e., the force-mobility trade-off) and a constraint brought about by an extrinsic factor, feeding ecology, which shaped the pattern of integration between oral and pharyngeal jaw systems, highlights the complex evolutionary interaction between jaw functional morphology and the natural diversity of prey.

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Supporting Information

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

Table S1. Continuous and discrete character data used in the study.

Table S2. Descriptions of the oral and pharyngeal jaw measurements.

Table S3. Correlation matrix between the oral and pharyngeal jaw systems.

Figure S1. Delimitation of the 18 clades (in blue) used to calculate clade rates.

Figure S2. Comparison of evolutionary correlations within and between the oral and pharyngeal jaw systems depending if log shape ratios (as in the main text) or phylogenetic residuals were used during size correction of oral and pharyngeal jaw traits.

Figure S3. Posterior estimates of the mean rate and number of rate shifts across different priors on the number of rate shifts during the estimation of branch-specific rates of morphological evolution.

Figure S4. Posterior estimates of the mean rate and number of state changes variation across different priors on the number of state changes/shifts during the MuSSCRat analyses.

Figure S5. Posterior estimates of the state rates (branch-specific rates associated with each trophic guild) across different priors on the number of state changes/shifts during the MuSSCRat analyses.

Figure S6. Posterior estimates of the mean rate and number of rate shifts across different priors on the number of rate shifts during the MuSSCRat analyses.

Figure S7. Posterior estimates of the state rates (branch-specific rates associated with each trophic guild) across different priors on the number of rate shifts during the MuSSCRat analyses.

Figure S8. The evolutionary history of feeding ecology used to estimate state-dependent rates of evolution.