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RESEARCH ARTICLE

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Organismal form constrains the evolution of complex lever systems in Neotropical cichlid four-bar linkages

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Abstract

The diversification of functional traits may be limited by the intrinsic constraints of organismal form (i.e., constructional constraints), owing to the differential investment in different anatomical structures. In this study, we test whether overall organismal form impacts the evolution of shape and function in complex lever systems. We examined the relationship between four-bar shape and overall head shape in two four-bar linkage systems: the oral-jaw and hyoid-neurocranium systems in Neotropical cichlids. We also investigated the strength of form-function mapping in these four-bar linkages and the impact of constraining head shape on these correlations. We quantified the shape of the head and two four-bar linkages using geometric morphometrics and compared these with the kinematic transmission coefficient of each linkage system. The shapes of both linkages were strongly correlated with their mechanical properties, and head shape appears to constrain the shape of both four-bar linkages. Head shape induced greater integration between the two linkages, was associated with stronger form-function correlations and higher rates of evolution in biomechanically important features. Head shape constraints may also contribute to a weak but significant trade-off in linkage kinematics. Elongation of the head and body, in particular, appears to minimize the impact of this trade-off, possibly through maximizing anterior–posterior space availability. However, the strength of relationships between shape and function, and the impact of head shape differed between the two linkages, with the hyoid four-bar in general showing stronger form-function relationships despite being more independent from head shape constraints.

KEYWORDS

biomechanics, fish, geometric morphometrics, macroevolution, morphology

1 | INTRODUCTION

The evolution of organismal form and function may be constrained by traits that are inherent to the biology of the organism, such as developmental patterns (e.g., heterochrony; Quillévéré et al., 2002), allometry (Cardini et al., 2015) and biomechanical or physiological trade-offs (e.g., metabolic-size trade-offs and lever mechanical outputs; Fonseca-Azevedo & Herculano-Houzel, 2012; Holzman et al., 2012). These intrinsic constraints differ from extrinsic constraints like ecological opportunity (Mahler et al., 2010), which may only be experienced in certain environments or following certain events (biogeographic, extinction, etc.). Intrinsic constraints relating to

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the differential somatic investment in specific anatomical features may be termed "constructional" (Arbour & López-Fernández, 2018; Barel, 1984; Gorb, 1995; Hulsey et al., 2018; Hulsey & Hollingsworth Jr, 2011; Ubukata et al., 2008). For example, with limited space available for jaw muscles, selection may prioritize the size of different muscle subdivisions in different species (Arbour & López-Fernández, 2018; Herrel et al., 2008; Hulsey & Hollingsworth Jr, 2011). Neotropical cichlids showed greater investment (through larger size) into the AM1 and AM2 divisions with increasing proportional adductor mandibular size, while comparatively AM3 size proportionately decreased (Arbour & López-Fernández, 2018). Understanding the functional consequences of size constraints may be fairly straightforward in such a case; for example, muscle mass is a major predictor of the maximum force produced by a muscle (Herrel et al., 2008; Landry & Turnbull, 1971; Santana et al., 2010; Weijs, 1980; Westneat, 2003). However, the mapping between form and function (i.e., the translation or relationship of shape to functional output) may not always be so simple. The principle of many-to-one-mapping defines those instances where many morphological traits may represent very similar (or the same) functional outputs (e.g., the kinematic transmission coefficient [KT] describing output to input rotation in four-bar linkages; Alfaro et al., 2005; Wainwright et al., 2005).

Four-bar linkages are complex lever systems made of four connected elements (links), which can be fixed in place at one link (Hu et al., 2017; Muller, 1996; Westneat, 1994). Rotation of any given unfixed link results in a known rotation of all other links. A number of biomechanical systems in organismal design function as a four-bar linkage, such as fish jaws (Westneat, 1994), mantis shrimp claws (Hu et al., 2017), bird wings, and the human knee (Burgess, 2021). Four-bar linkages are defined as having an input link (force is applied, such as through a muscle or tendon), an output link (rotates as a result of the input link), a fixed link (usually representing connection to the relatively immobile body), and a coupler link (which helps to transmit motion between the input and output). The behavior of four-bar linkages can be quantified using the KT coefficient, which measures the ratio between rotation of the output link over a set rotation of the input link. High values of KT (>1) are motion advantaged—the resulting motion of the output link is higher than the rotation of the input link. KT is impacted by both the length of the links and their initial positions relative to each other. Like mechanical advantage of single lever systems, KT values represent an inherent trade-off between the transmission of force and velocity, thus linkage configurations can be optimized for the transmission of force or motion but not both simultaneously (Alfaro et al., 2005; Camp & Brainerd, 2015; Wainwright & Richard, 1995). Four-bar linkages exhibit "many-two-one-mapping," where similar KT values may be produced by a range of different four-bar linkage shapes. Evolutionarily, this may produce relatively "flat" regions of a functional adaptive landscape (where performance/fitness shows low to no variability with changes in shape), in which biological form may diversify with little impact on performance, partially disconnecting the diversity of morphology and function (Wainwright et al., 2005).

Many spiny-rayed fishes possess multiple four-bar linkages within the skull alone, responsible for opening and closing/contracting of the oral-jaws, buccal cavity, or opercular system (Camp & Brainerd, 2015; Muller, 1996; Westneat, 1994). Two in particular, the oral-jaw and the hyoid-neurocranium (henceforth just hyoid) four-bar linkages have been found to perform consistently with expectations from 2D models (Figure 1), while others require more sophisticated modeling techniques (Camp & Brainerd, 2015; Muller, 1996; Westneat, 1994). The oral-jaw four-bar linkage connects rotation of the lower jaw to rotation of the maxilla. The oral jaw four bar linkage protrudes the premaxilla through the action of the maxilla (Hulsey & Garcia De Leon, 2005). It is driven by contraction of the adductor mandibulae during mouth closing and the sternohyoideus (via the interopercular ligament) during mouth opening (Alfaro et al., 2005; Hulsey et al., 2008; Martinez & Sparks, 2017; Wainwright et al., 2004, 2007). The hyoid four-bar linkage connects depression of the hyoid and dorsal rotation of the neurocranium, which work together simultaneously to expand the buccal cavity during feeding. In a previous study of Neotropical cichlids, high values of hyoid KT were associated with ram-feeding taxa, with rapid buccal expansion during prey capture (Arbour & López-Fernández, 2014). The hyoid four-bar linkage is driven by both the sternohyoideus (which comprises the coupler link) and the body musculature (largely the epaxials; Camp et al., 2015; Muller, 1996; Wainwright et al., 2004, 2007). Thus, the two four-bar systems are indirectly linked through the action of the sternohyoideus.

Neotropical cichlid fishes (Cichlidae Cichlinae) are a diverse subfamily of freshwater fishes found in South and Central America, with current estimates of more than 700 species (Albert & Reis, 2011; López-Fernández et al., 2013; McMahan et al., 2013). Neotropical cichlids feed on a variety of foods (aquatic and terrestrial insects, other fish, plankton, aquatic plants, detritus, and fruit) and show numerous dietary specializations associated with morphological and behavioral adaptations (Arbour et al., 2020; Burress, 2016; Cochran-Biederman & Winemiller, 2010; Crampton, 2008; López-Fernández et al., 2014; Pease

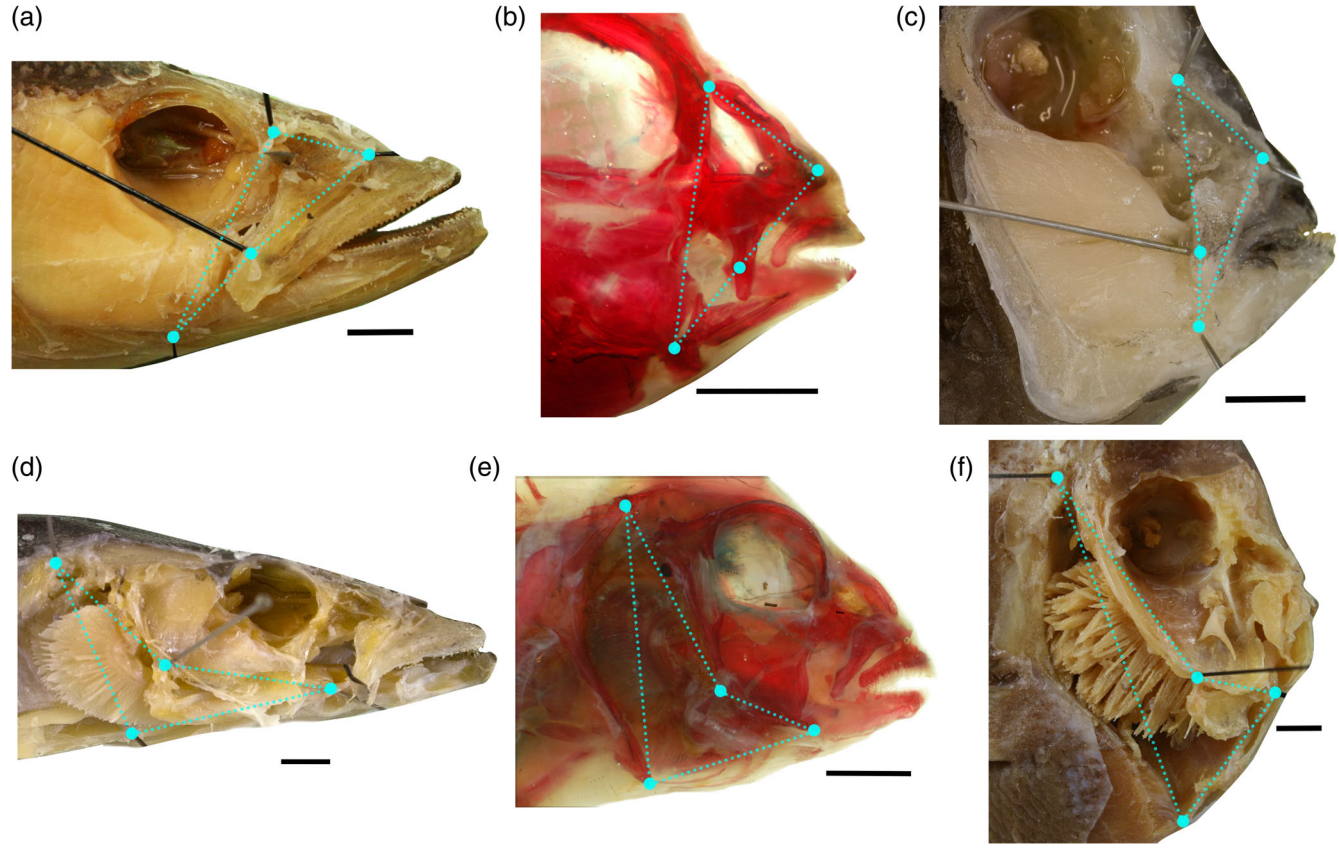


FIGURE 1 The (a–c) oral-jaw four-bar linkage and the (d–f) hyoid-neurocranium four-bar linkages in different species of Neotropical cichlids. Dashed lines show the four links and points correspond to the landmarks described in Tables 1 and 2 and also shown in Figure 2. (a) *Crenicichla saxatilis*, (b) *Biotodoma wavrini*, (c) *Uaru amphiacanthoides*, (d) *Crenicichla multispinosa*, (e) *Andinoacara rivulatus*, and (f) *Symphysodon aequifasciatus*.

et al., 2018; Weller et al., 2016; Winemiller et al., 1995). Previous comparative analyses showed that variation in four-bar kinematic values was high, and comparable to other fish model systems such as labrids and centrarchids (Arbour & López-Fernández, 2014; Hulsey et al., 2006; Wainwright et al., 2004).

The two four-bar linkages represent major structures located across the head, and Cichlinae shows a tremendous diversity in head shapes (Figure 2). Among this diverse, subclades are piscivorous species showing convergence toward elongate bodies and head with large, terminal mouths (*Crenicichla, Cichla, Parachromis*), substrate-sifting species tending toward triangular heads with sub-terminal mouth positions (*Geophagus, Gymnogeophagus, Thorichthys, Retroculus*), deep-bodied detritus feeders with short faces and small, sturdy jaws (*Uaru*, *Symphysodon, Herichthys*), and dwarf taxa with fairly elongate bodies but with comparably short faces (*Biotoecus, Dicrossus, Apistogramma*), among many more morphologies (Arbour et al., 2020; López-Fernández et al., 2012, 2013, 2014; Pease et al., 2018; Winemiller et al., 1995). The relative "investment" in the size and positioning of each of the links of each four-bar system in the space of the head may reflect a form of constructional constraint driven by overall head shape. The extent to which such associations may impact functional variation in complex systems like four-bar linkages is unknown and may be different from simpler systems (e.g., single levers, muscle size) previously analyzed (Arbour & López-Fernández, 2018; Hulsey et al., 2007; Hulsey & Hollingsworth, 2011).

In this study, we examine the impact of the intrinsic constraint of organismal form on the shape and evolutionary patterns of complex lever systems. Specifically, we quantify the form-function associations between head shape and the shape of two four-bar linkage systems, the oral-jaw and hyoid, in Cichlinae. Our overall objectives were to (1) determine how much shape and functional diversity of the four-bar linkages are constrained by head shape across Cichlinae, (2) quantifying the relationship between form and function in the two four-bar linkages, and (3) determine if the oral-jaw and hyoid four-bar linkages are integrated, and whether that integration is partly influenced by the overall constructional constraint of head shape. We hypothesize that shape variation is

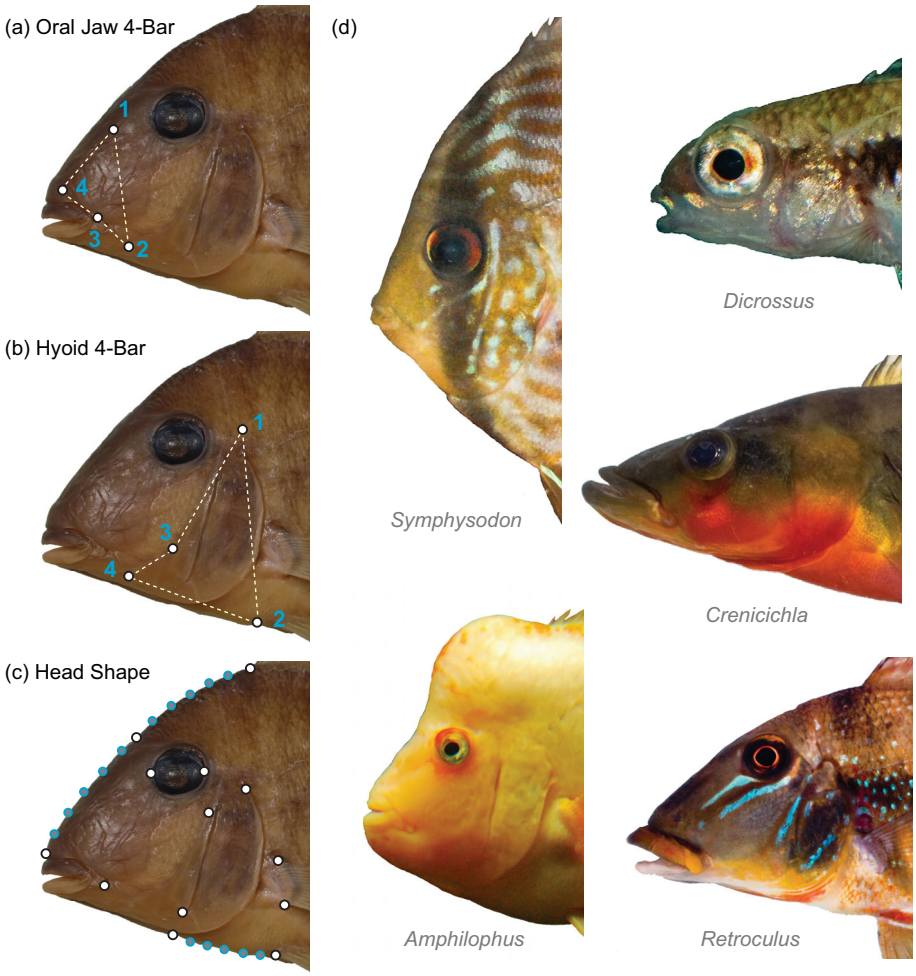


FIGURE 2 Approximate locations of the landmarks used for the (a) oral-jaw four-bar linkage, (b) hyoid four-bar linkage, and (c) head shape (see Tables 1 and 2; Table S1 for descriptions of landmarks). Dashed lines show the links. Locations of linkage landmarks were determined from dissected specimens, not externally. (d) Head shape diversity in Neotropical cichlids. *Source*: Photo credits to J. Arbour and H. Lopez-Fernandez.

linked between the two four-bar linkages as a result of partitioning of the cranial skeleton (e.g., widening the oral-jaw linkage would necessitate a caudal repositioning of the interhyal portion of the hyoid linkage, and while the length of the hyoid bar is limited by space availability between the lower jaw and associated elements). Furthermore, we predict that head shape constraints facilitate inter-linkage shape variation, which would be supported by a reduction in integration after accounting for covariation with head shape.

2 | METHODS

2.1 | Data collection

Previous analyses have generally compared only the relative lengths of the four-bar elements, (Hulsey & Garcia De Leon, 2005; Wainwright et al., 2005), and in cases where positioning has been incorporated the angle between the input link (lower jaw) and the fixed link (neurocranium–suspensorium) was standardized across species prior to analysis (Martinez & Sparks, 2017). However, while this maintains the relative ratios of the lengths of the links and some of the orientations, these do not necessarily represent biologically meaningful starting orientations, and motion transfer (KT) is not necessarily equal across the range of rotation of four-bar linkage elements (Corn et al., 2021; Martinez et al., 2018; Muller, 1996). Thus, for both shape and biomechanical analyses, we use the starting orientation as they occurred in the specimen, rather than standardizing to a single value.

We used geometric morphometrics to quantify four-bar linkage shape and head shape across 64 species of cichlid (1–4 specimens per species). We followed the same landmarks used in Arbour and López-Fernández (2014) to quantify the KT of each four-bar linkage, and the same dissection photos were used from that previous study (Figure 1). The four landmarks used for each four-bar linkage are listed in Table 1. Two-dimensional landmarks coordinates were obtained from dissection images using TPSdig2 (Rohlf, 2015). The links characterized by each pair of landmarks are described in Table 2.

Estimates of four-bar KT were taken from the data from Arbour and López-Fernández (2014), which were

TABLE 1 Landmark locations for the digitization of four-bar linkage shape in Neotropical cichlids.

	Hyoid	Oral
1	Post-temporal—supra-cleithrum joint	Intersection of nasal and neurocranium
2	Ventral most tip of the cleithrum	Articular-quadrate joint of the lower jaw
3	Intersection of the interhyal and suspensorium at symplectic/hyomandibula	Soft tissue connection between the angular process of the lower jaw and the maxilla
4	Anterior tip of the hypohyal	Articulation of the maxilla and nasal bones

Note: See Table 2 for the assignment of landmarks to specific links.

TABLE 2 Description of the two four-bar linkages analyzed from Neotropical cichlid skulls.

	Anatomical structures involved	Connecting landmarks
Oral jar four-bar		
Input	Lower jaw	2:3
Output	Maxilla	3:4
Coupler	Nasal	4:1
Fixed	Neurocranium–suspensorium	1:2
Hyoid-neurocranium four-bar		
Input	Neurocranium	3:1
Output	Sternohyoideus	2:4
Coupler	Hyoid bar	3:4
Fixed	Pectoral girdle	1:2

Note: Connecting landmarks refers to the numbered landmarks in Table 1.

also used in subsequent comparative analyses of Cichlinae functional evolution (Arbour et al., 2020; Arbour & López-Fernández, 2016). In brief, for the oral-jaw four-bar linkage, KT was calculated as the resulting rotation of the maxilla (output) compared to the nasal link (coupler) given a 30° rotation of the lower jaw from the starting position, relative to the fixed link (neurocranium–suspensorium). For the hyoid, KT was calculated as the depression of the hyoid (rotation of the output sternohyoideus link, compared to the fixed link, the pectoral girdle) as a result of a 5° upward rotation of the input link relative to the fixed link. Following Wainwright et al. (2004), the hyoid KT calculation included a 10% reduction in the length of the sternohyoideus link, to account for muscle shortening during contraction of the muscle during hyoid depression (as was previously applied in the previously cited study; Arbour & López-Fernández, 2014).

Head shape (including elements of the anterior body like the pectoral girdle) was quantified using 13 landmarks and 3 curves comprising sliding semi-landmarks from the left side of the specimen (Table S1) from photographs taken prior to dissection. Each curve was bounded by 2 landmarks and contained 5 semi-landmarks; curve 1 extended from landmark 9 to 10 along the dorsal profile of the head, curve 2 extended from landmark 10 to 11 again along the dorsal profile of the head, and curve 3 extended from landmark 12 to 13 along the ventral profile.

2.2 | Geometric morphometric analysis

We used generalized Procrustes analysis (GPA) to remove the impact of position, rotation, and scale from the landmark configurations (Adams & Otarola-Castillo, 2013; Bookstein, 1997; Zelditch et al., 2004). The function "gpagen" from the R package "geomorph" was used to individually align each of the oral four-bar, hyoid four-bar, and lateral head shape datasets (Adams & Otarola-Castillo, 2013). After GPA alignment, landmark configurations for each dataset were averaged across each of the 64 species in the dataset. Given that the landmark datasets all showed significant phylogenetic signal, as quantified using the "geomorph" function "physignal" (K from 0.53 to 0.64, all p values = 0.001) we used phylogenetically corrected analyses to verify that none of the results of the above analyses were driven by evolutionary relatedness alone. We summarized shape variation in each landmark dataset using a phylogenetic principal component analysis (using a covariance matrix) as implemented in the "phytools" function "phyl.pca." We used a time-calibrated version of the Ilves et al. (2018) phylogeny, previously implemented in phylogenetic comparative analyses of trait evolution (Arbour et al., 2020). We examined scree plots to select critical axes for each (Horn, 1965; Jackson, 1993; Peres-Neto et al., 2005).

2.3 | The impact of head shape on four-bar linkage shape

We assessed possible constraining effects of head shape on four-bar linkage shape using a phylogenetically corrected, two block partial least squares (TB-PLS), which can be used to find an axis that maximizes covariation between two datasets. The function "phylo.integration" in the R package "geomorph" is capable of incorporating geometric morphometric landmark data (either comparing two shape datasets,

or a shape dataset to a different variable[s]) and accounting for evolutionary relatedness. We tested for a relationship (quantified by the PLS correlation coefficient *r*, denoted r-PLS below) between each of the aligned four-bar linkages landmark datasets and the lateral head shape dataset.

To test whether overall head shape impacts four-bar linkage shape (i.e., the constructional constraints on four-bar shape), we produced a set of head-shape-corrected landmark coordinates. We regressed each of the oral four-bar and hyoid four-bar landmarks against the critical principal component (PC) axes of head shape variation (PC1–3), which represented the majority of head shape variation (77%). The regressions were carried out using the function "procD.pgls" from the R package "geomorph." We obtained the residuals of each of these regressions and added them to the consensus confirmation of each dataset (calculated using the function "mshape"; Adams & Otarola-Castillo, 2013). These new landmark configurations were considered to represent four-bar shape free of head shape variation.

Differences between the original and shape corrected landmarks should represent the impact of the intrinsic constraint of head shape form–function relationships. We compared the original and head-shape-corrected configurations using TB-PLS as described above. We assessed the amount of shape variation in the four-bar linkages using the decrease (from 1) in the correlation coefficient (comparing before and after shape correction).

2.4 | Form-function mapping and constructional constraints on four-bar shape

We assessed the strength of the relationship (mapping) between shape and function in each of the four-bar linkages using phylogenetically corrected TB-PLS (see above), between their landmarks and their corresponding KT value. We also tested whether KT values were associated with head shape. To determine how covariation with head shape has impacted form-function mapping in the two four-bar linkage systems, we also used TB-PLS to assess the relationship between the KT values and the head-shape-corrected landmark coordinates for the two four-bar linkages. Lastly, we determined the evolutionarily corrected relationship between the biomechanics (KT) of both linkages using robust regression on phylogenetic independent contrasts (PIC), using the R functions "lmrob" (robustbase) and "pic" (ape; Felsenstein, 1985; Paradis et al., 2004; Rousseeuw et al., 2014; Slater & Pennell, 2014). PICs were regressed through the origin.

2.5 | Integration and modularity of the four-bar linkages

We tested for the degree of integration between the landmark datasets using TB-PLS. We also investigated whether the four-bar linkages were modular in their shape variation, that is, whether shape covariation was stronger within linkages than across linkages. We used the covariance ratio (CR) to assess the degree of modularity between the oral-jaw and hyoid four-bar linkages. The CR statistic is the ratio of shape covariation between modules compared to the covariation within modules, and the *p*-value associated with this test is generated by calculating the CR value across landmarks that have been randomly assigned to each module. We used the R function "modularity.test" to calculate the CR statistic and associated *p* values. For both tests of integration and modularity, we examined whether significant patterns were driven by head shape variation, by repeating all analyses using the head-shape-corrected arrays (see above for description).

2.6 | Lability of four-bar linkage shape

We tested whether different elements of each of the four-bar linkages (i.e., the landmarks) evolved at different rates. We used "compare.multi.rates.evol" function in the R package "geomorph," to calculate the multivariate Brownian Motion (BM) evolutionary rate parameter (σ^2_{multi}). Significance of the differences in BM evolutionary rates is quantified in this function as the highest divided by the lowest BM rate and compared to a range of ratios generated under a simulated, single rate BM process. The frequency of BM simulations with rate ratios higher than the observed value is considered the *p*-value for the test. It is important to note that since the landmarks for each linkage were aligned prior to analysis, the relative rates between the two linkages do not reflect their true ratio in their original data space.

3 | RESULTS

3.1 | Head and four-bar shape variation

The first two PC axes (PC1 = 51.3% and PC2 = 27.5%) of shape variation in the oral-jaw linkage, were predominantly associated with the length and orientation of the nasal (coupler) link and anterior–posterior expansion or compression of the four-bar linkage (Figure 3b). The relative positions of the maxilla and lower jaw links (input and output links) were also important to both axes, with

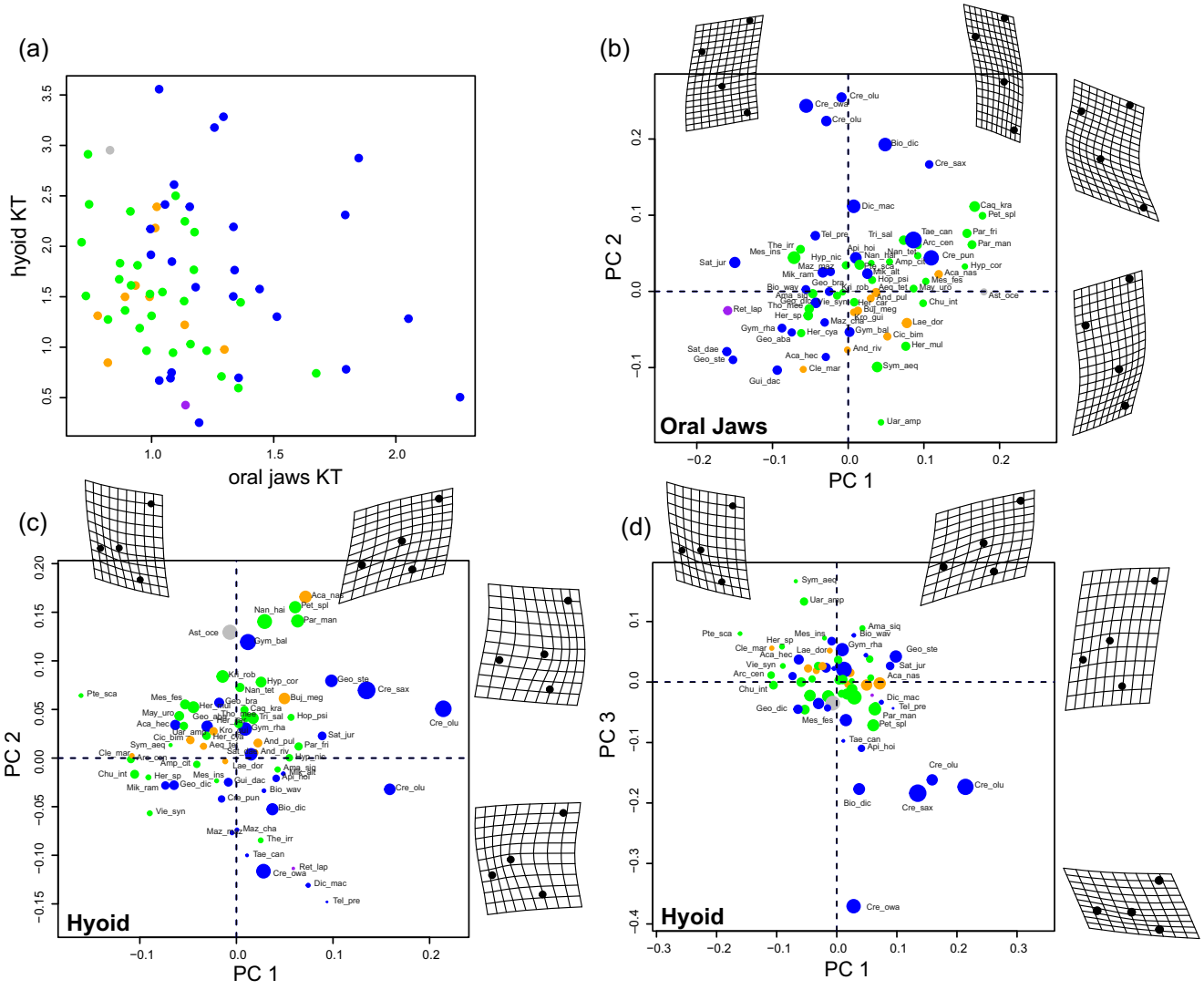


FIGURE 3 Diversity of four-bar linkage shapes and biomechanical coefficients. (a) Kinematic transmission coefficient values from the two four-bar linkage systems, the oral-jaw and hyoid linkages. (b) Results of a principal component analysis (PCA) of oral jar four-bar shape using geometric morphometrics. (c, d) Results of a PCA of hyoid four-bar shape. Colors indicate tribe membership: blue = Geophagini, green = Heroini, orange = Cichlasomatini, gray = Astronotini, and purple = Retroculini.

either more parallel or more angled links. Higher KT values were clustered high on PC2 but were predominantly found in geophagine species. However, geophagine cichlids qualitatively showed a greater diversity of shapes than Heroini or Cichlasomatini (other tribes represent few species), albeit with Heroini showing the most extreme "compressed" shapes on PC2.

The first two PC axes (PC1 = 34.8%, PC2 = 32.88%) of hyoid four-bar linkage shape comprised predominantly variation in the lengths of the hyoid bar (input) and interhyal links (coupler; Figure 3c). Comparatively, PC3 (24.1%) varied predominantly in overall anterior–posterior elongation of the linkage and the depth of the cleithrum (fixed link), respectively. Hyoid KT was generally highest in PC space associated with shorter pectoral girdles and long hyoid bars (high PC1 and PC2, and low PC3; Figure 3d). Extreme hyoid four-bar shapes and high KT values were more prevalent among the Geophagini (particularly among the *Crenicichla* species included) than the other major tribes of Cichlinae (Figure 3, blue).

Head shape variation largely followed findings from previous studies (Arbour & López-Fernández, 2014, 2018; López-Fernández et al., 2013). The first PC axis (44.9% of shape variation) was associated with a gradient from tall, blunt-headed species (*Symphysodon* and *Uaru*) to vertically narrowed but elongated heads (e.g., *Crenicihla* and *Cichla*; Figure S1). PC2 (20.9%) was associated with the position of the eye and orientation of the mouth (species

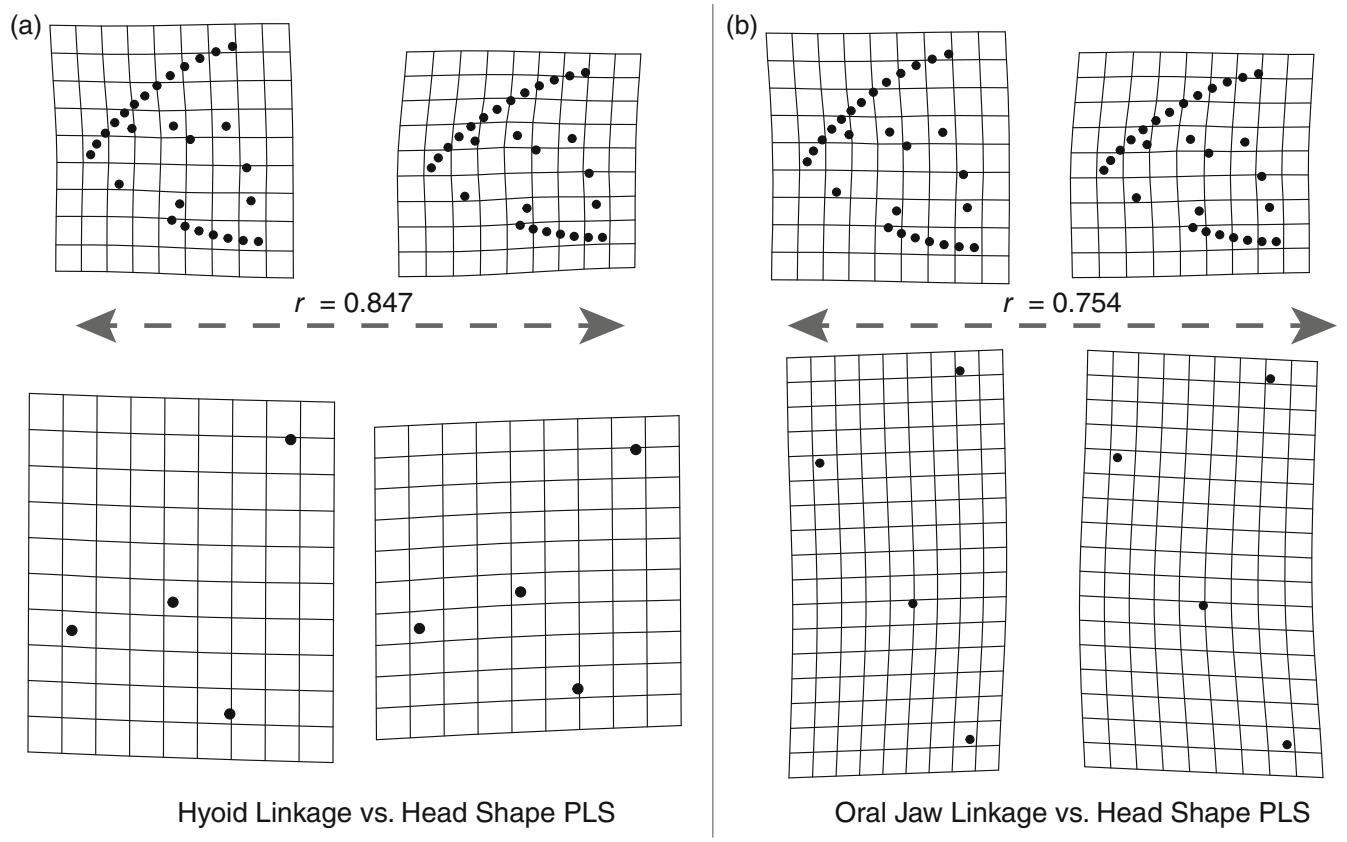


FIGURE 4 Relationship between head shape and four-bar linkage shape across the (a) oral-jaw and (b) hyoid-neurocranium four-bar linkages in Neotropical cichlids. Results of a two block partial least squares (TB-PLS) analysis of each four-bar landmark dataset against head shape. *r*, PLS-correlation coefficient.

with high set eyes and subterminal/inferior mouths vs. species with lower set eyes and more terminal mouths). PC3 comprised little shape variation (~11%), but largely described more minor changes in mouth position and orientation.

3.2 | The impact of head shape on four-bar linkage covariation

The shape of both four-bar linkages was found to be strongly associated with head shape, with PLS correlation coefficients of 0.754 and 0.847 (both p values <0.001), for the oral-jaws and hyoid, respectively. In both cases, the major aspect of head shape associated with linkage shape was elongation (shorter heights paired with greater lengths, similar to head shape PC1 in Figure S1). Shape changes associated with head shape were subtle in the four-bar linkages. But in general, more elongated heads were associated with oral-jaw linkages with more horizontally oriented nasal links, and more posteriorly located lower jaw joints with more parallel lower jaw (input) and maxilla (output) links (Figure 4b). Head elongation was also associated with longer hyoid bar, and a slightly shorted cleithrum (Figure 4a). When compared with the original landmark configurations, the correlation coefficients for the head-shape-corrected oral and hyoid four-bar linkage shapes were 0.775 and 0.963, respectively (both $p = 0.001$). Thus, oral four-bar shape was more strongly driven by head shape (as evidenced by the lower correlation to the original dataset after correction).

3.3 | Correspondence between form and function, and the constraining impact of head shape

We found a significant negative association between the KT values of the two linkages (slope = −0.901, $p = 0.002$, $R^2 = 13.37\%$) using robust regression of PICs, with an unusual (downweighted) contrast at the base of *Crenicichla-Teleocichla* (Figure S2). Combinations of both high KT values were rare (as opposed to either high hyoid with low oral KT, vice versa, or both low values), and predominantly occurred in the aforementioned clade

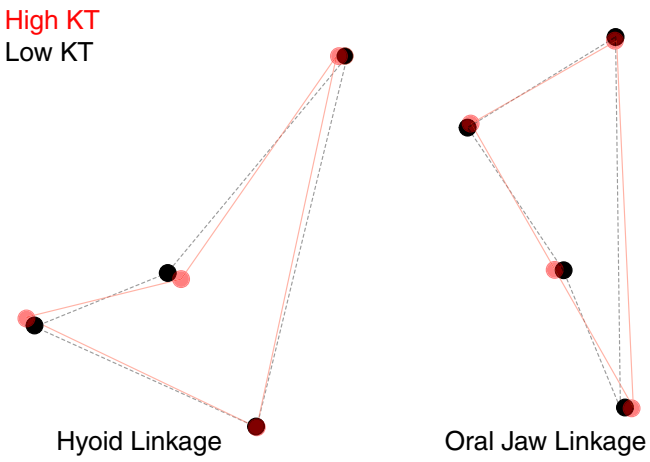


FIGURE 5 Four-bar linkage shape changes associated with changes in kinematic transmission coefficient (KT) values. Shape changes taken from two block partial least squares analyses of KT and landmark configurations. Red shows shapes associated with high KT values, and black shows those associated with low KT values for both shapes.

of highly elongated taxa (Figure 3). Additionally, Geophagini showed higher diversity of KT values and combinations than either Cichlasomatini or Heroini, though low oral-jaw KT values were more common in Heroini (Figure 3). In all cases, however, fewer species represented force-advantaged systems for either KT (values <1), and only two species were force advantaged in both linkages (*Cleithracara maronii* and *Vieja melanurus*). High mobility was important in both systems across all major tribes despite the apparent weak trade-off in KT values.

The strength of the relationship between form and function differed between the two four-bar linkages. Both oral-jaw and hyoid four-bar shapes were found to be significantly associated KT values (Hyoid $r = 0.911$, Oral-jaw $r = 0.757$, $p = 0.001$). Notably, the strength of association between KT and shape was higher for the hyoid linkage than the oral-jaw linkage. High hyoid KT values were associated primarily with longer hyoid bars, while oral KT values were associated with more subtle changes in the orientations of the input (lower jaw) and output (maxilla) links. High oral KT was associated with more parallel input and output links (Figure 5). In both cases, the elements of shape variation associated with KT values were also features associated with head shape (see Figure 4 and results above).

Overall head shape showed a moderate but significant relationship with shape in both linkages ($r = 0.575$ vs. 0.566 for hyoid and oral-jaws, respectively, both $p = 0.001$), which was weaker than the relationship between linkage shape and KT (see above). Both of the four-bar linkages remained significantly correlated with their respective KT values even after correcting for variation driven by head shape ($r = 0.878$ and 0.598, for hyoid and oral-jaws, respectively, both $p = 0.001$). However, both linkages showed a weakening of the form-function relationship. Correcting for head shape variation also had a stronger impact on form-function mapping on the oral-jaw four-bar linkage (larger decrease in r, after correction for head shape).

3.4 | Integration and modularity of four-bar linkages

We found significant shape covariation (integration) between the two four-bar landmark datasets ($r = 0.785$, $p = 0.001$). Elements of hyoid length and the orientation of the maxilla and lower jaw links were important to intra-linkage covariation (Figure S3), similar to covariation with KT and head shape. Hyoid four-bar linkages with short hyoid and hyomandibula were associated with an anterior–posteriorly compressed oral-jaw four-bar with a more acute angle between the maxilla and lower jaw links (Figure S3). Comparatively, hyoid four-bar linkages with longer hyoid and hyomandibula links were associated with a slightly more elongated oral-jaw four-bar linkage, with more parallel maxilla and lower jaw links and a longer nasal. Correcting for head shape also led to a weakening in shape integration between the two four-bar linkages, although the correlation between linkages remained significant ($r = 0.632$, $p = 0.001$).

The oral-jaw and hyoid four-bar linkages exhibited significant within-linkage modularity (CR = 0.872, $p = 0.02$). Modularity was strengthened by accounting for the shape variation driven by overall head shape (after correction CR = 0.729, $p = 0.02$). Both integration and modularity analyses support that a significant part of the covariation between the two four-bar linkage shapes is associated with the overall shape of the head.

3.5 | Evolutionary lability of four-bar shape

Both the oral-jaw and hyoid four-bar linkages showed heterogeneous evolutionary rates (Table 3). In the oral-jaw four-bar linkage, the highest rates were observed at the connection between the lower jaw and maxilla (Figure 6a), and in the hyoid linkage, shape change was highest in the hyoid bar, particularly at the landmark representing the articulation of the interhyal with the rest of the suspensorium. These corresponded to landmarks also important to form-

TABLE 3 Results from "compare.multi.evol.rates" function from the R package "geomorph."

	Original		Shape correction	
	Rate ratio	***p*-value**	**Rate ratio**	***p*-value**
Oral-jaw	1.65	0.004	2.16	0.001
Hyoid	2.95	0.001	5.33	0.001

Note: Rate ratio is the highest evolutionary rate (σ^2) calculated across all landmarks compared to the lowest. The *p*-value is calculated from Brownian motion simulated shape data and is equal to the number of simulations producing a higher rate ratio than observed.

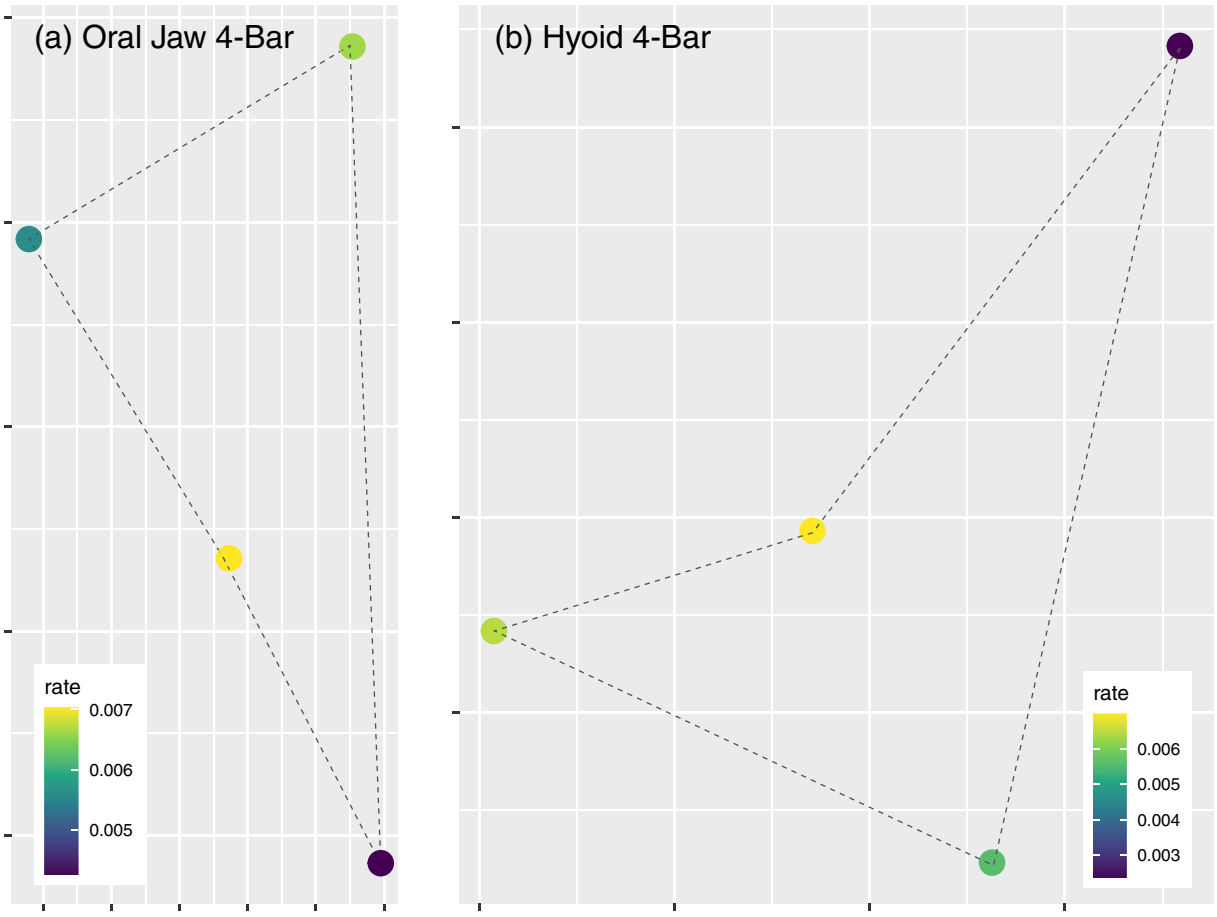


FIGURE 6 Multivariate evolutionary rate (Brownian Motion—σ) per landmark in the (a) oral-jaw and (b) hyoid four-bar linkages. Rates are not comparable between the two four-bar linkages, as landmarks were corrected for scale prior to analysis. Colors show the relative rates of multivariate shape evolution on the consensus shape configuration per linkage.

function covariation, and covariation with head shape (see PLS results). The supra-cleithrum—post-temporal joint in the hyoid linkage and the articular-quadrate joint (the joint of the lower jaw) showed the lowest rates of evolution (Figure 6b). However, the hyoid four-bar linkage showed much stronger heterogeneity (Table 3). Additionally, evolutionary rate variation was higher after correction for head shape (Table 3), particularly for the hyoid four-bar linkage. Overall, trends of regions of high and low rates of evolution remained the same as prior to correction.

4 | DISCUSSION

4.1 | Constructional versus biomechanical constraints on four-bar shape

Organisms represent integrated systems of anatomical structures which must be shaped and act in concert to accomplish the necessary functions of the individual (e.g., feeding, locomotion, reproduction, evasion, etc.). Such functional and anatomical integration may impose

constraints on morphological evolution. A previous study found that constructional constraints impact the diversification of elements of the feeding apparatus across the subfamily Cichlinae (Arbour & López-Fernández, 2018). This prior work focused on the adductor mandibulae, the major jaw closing muscle, and the relative impacts of biomechanical and constructional constraints, finding that constructional constraints were more important. However, the form-function mapping of biting mechanics in the lower jaw system is simple compared to four-bar linkage dynamics found in other elements of the fish skull.

We show that such constructional constraints also impact the evolution of form and function in complex lever systems like four-bar linkages, in addition to simple systems like simple levers and muscle size. Across all analyses in this study, head shape was shown to covary with the shape of the four-bar linkages across Cichlinae, in ways that were also important to the form-function relationships of both linkages. More moderate correlations (r-PLS = 0.62) were found between oral-jaw four-bar shape and body shape in the African Etropline and Ptychochromine cichlid clades (Martinez & Wainwright, 2019). Notably, this analysis standardized the angle between the fixed and input link, which did not reflect the actual starting positions of the four-bar link systems, possibly partially obfuscating shape relationships (Martinez & Sparks, 2017). Head shape contributed to shape integration between the linkages in our study, and also limited the overall evolutionary lability of both four-bar linkages, generating a tendency toward producing more evenly distributed shape change (Figure 6; Table 3). As shape was significantly correlated with function in both the four-bar linkages, constructional constraints on four-bar shape have the potential to limit the potential kinematic outputs possible across Cichlinae.

The patterns of shape covariation associated with the head and four-bar linkages support the role of constructional constraints in shape covariation and relationships with biomechanical outputs in Cichlinae four-bar linkages. High hyoid KT values were associated with elongate, ram-feeding piscivores in Cichlinae (Arbour & López-Fernández, 2014), and extreme jaw protrusion (associated largely with piscivory in Cichlinae) was associated with high oral-jaw KT values (Hulsey & Garcia De Leon, 2005), suggesting a similar pressure for kinematic mobility in ram-feeding mechanics in Cichlinae. Therefore, if the shape of the linkages was predominantly driven by biomechanical constraints, and selection on these linkages were themselves driving covariation with head shape, we might expect to see a positive relationship in KT values, with velocity or force-advantaged systems being mutually selected (e.g., high mobility in both systems in piscivores). However, after correcting for evolutionary relatedness, we saw a weak but significant negative association between the two KT values, such that greater force transmission (relatively) in the oral-jaws was associated with greater velocity transmission in the hyoid system, and vice versa. In both the hyoid and oral-jaw linkages, anterior–posterior widening was found to contribute to high KT values, both in PLS results and in regions of morphospace associated with high KT values (Figures 3 and 4). If space in the Cichlinae skull is limiting, then these opposing space requirements (i.e., both requiring anterior–posterior expansion to generate high mobility) could generate a weak negative relationship between KT values. Consistent with this, only those species with very elongated heads (*Crenicichla* and *Teleocichla*) were found to possess a combination of relatively higher oral KT and hyoid KT values (Figure 2, upper right region of KT vs. KT plot). Indeed, this is supported by the atypical combination of hyoid KT PIC and oral-jaw KT PIC, suggesting these taxa depart from the general trade-off experienced by other Cichlinae. Prior analyses of adductor mandibulae size also found an opposite pattern to that predicted by biomechanical constraints and consistent with constructional ones (Arbour & López-Fernández, 2018).

4.2 | Morphological, ecological, and taxonomic correlates of four-bar function

Body and head elongations appear to play a significant role in constraining variation in the shape of the four-bar linkages. Body elongation is a major axis of shape variation across Cichlinae as well as many other groups of fish (Claverie & Wainwright, 2014; López-Fernández et al., 2013; Sallan & Friedman, 2011). There are numerous critical consequences of body elongation in fishes, including changing swimming mechanics (Blake, 2004; Webb, 1984), predator avoidance (Bronmark & Miner, 1992), feeding mechanics (Camp et al., 2015; Carroll & Wainwright, 2009), habitat use (Claverie & Wainwright, 2014), and many others. Thus, there may be strong selection on overall body form, which also limits diversification in the four-bar linkage shapes. Body depth in fishes is also tightly linked to the height of the cleithrum (pectoral fin girdle). Hulsey et al. (2018) suggested that the depth of the body may coevolve as a result of selection pressure on pectoral fin traits linked to cleithrum depth, a so-called "evolutionary spandrel." The cleithrum also represents the fixed link within the hyoid four-bar linkages, and the relative sizes of the fixed and hyoid-bar links appeared most important to both body shape versus four-bar shape

correlations and form-function mapping within the four-bar system. How the interplay of the relative pressures of feeding mechanics (hyoid) and swimming mechanics (body elongation, pectoral fin shape) impacts the distribution of head, four-bar, and body shape diversity through the shared use of the cleithrum is an unexplored but potentially fruitful area of exploration in the examination of constructional constraints.

Prior theoretical distributions of KT values in labrids showed a log-normal shape (Alfaro et al., 2005), however, only the oral-jaw KT values showed a strong right skew in our dataset (data not shown, hyoid KT distribution was normal). Admittedly, such log-normal distribution was generated from simulated but biologically feasible oral-jaw linkages (specifically maxillary KT; Alfaro et al., 2005), thus it could be that the typical configurations of this linkage in Percomorph fishes tend to make achieving high KT values in the oral-jaw linkages more "difficult" (i.e., rarer morphologies) compared with the hyoid system.

Combinations of relatively high KT values in both the oral and hyoid linkages were only found in very elongate-bodied species, including the large bodied, piscivorous pike-cichlids (*Crenicichla*) but also some among elongate dwarf cichlids (*Taeniacara, Biotoecus*, *Crenicara*) which tend to be leaf-litter inhabitants "picking" small aquatic invertebrates (Arbour & López-Fernández, 2014; López-Fernández et al., 2012; Lowe-McConnell, 1969, 1991; Winemiller et al., 1995). In a previous study, Hyoid KT was much more strongly associated with diet than oral KT, particularly in trade-offs involving fish and other evasive prey items (vs. vegetation and detritus; Arbour et al., 2020). Oral KT was by contrast associated with the consumption of small invertebrates (meiofauna and microcrustracea); dietary elements important to the dwarf genera. Thus, it is possible that there are also body size implications to the optimization of different four-bar systems for achieving extreme KT values in some linkage systems.

Additionally, such extreme KT values and four-bar shapes were not equally distributed across the Cichlinae tribes examined. In particular, Geophagini showed much greater shape and functional diversity (Figure 2). Geophagini represents the most species rich tribe of Cichlinae, and unlike the next two largest tribes is only found in South America. Geophagini shows tremendous body shape diversity, including highly elongate forms, and a high diversity of body size (López-Fernández et al., 2012, 2013; Steele & López-Fernández, 2014; Winemiller et al., 1995). Indeed, release from competition with Geophagini likely spurred greater phenotypic diversification among Heroini in Central America (Arbour & López-Fernández, 2016). Thus, the exploration of extremely elongate forms in this clade appears to have significant consequences for the generation of novel biomechanical properties in cranial kinesis (Hulsey & Garcia De Leon, 2005; López-Fernández et al., 2013). Notably, the two largest genera of Cichlinae (>90 sp. each), both of which are found within Geophagini, are from these two morphological types: the variably sized *Crenicichla* and the dwarf *Apistogramma*.

4.3 | Modularity and integration between four-bar linkages

Among African cichlids, previous studies have suggested the "preorbital" region represents a separate morphological module from the rest of the head (Parsons et al., 2011). The relative independence of this region is believed to have contributed to rapid and convergent shape adaptations associated with feeding morphology (Cooper et al., 2010). Although this has not been examined within the Neotropical clade Cichlinae, both clades show a number of overlapping trophic adaptations and morphological variation, and both clades likely share some of the same intrinsic mechanics underlying trophic diversification (Arbour & López-Fernández, 2014; Cooper et al., 2010; Higham et al., 2007; Hulsey et al., 2006; López-Fernández et al., 2013; Winemiller et al., 1995). The oral-jaw four-bar linkage in both clades would generally be defined by structures located within the "pre-orbital" module, while the hyoid four-bar linkage elements would generally not be. Levels of integration and morphological variability may vary between different modules (Goswami & Finarelli, 2016). It is possible that variation in intra-module shape integration has impacted the evolutionary lability of the two linkage systems. High integration within the pre-orbital region may contribute to the high trophic-associated morphological diversity in the clade and have contributed to the more "even" pattern of evolutionary rates in the oral-jaw linkage compared to the hyoid. Indeed, a number of studies have suggested integration may be associated with fast shape diversification, through the coordination of shape change across trait complexes (Arbour et al., 2021; Felice et al., 2018).

4.4 | Form-function mapping of four-bar linkages in cichlids

Four-bar linkages have been a model system for understanding the link between morphological and mechanical diversification, especially in the light of "many-to-one-mapping" (multiple morphologies translating to the same

functional output; Alfaro et al., 2005; Martinez & Wainwright, 2019; Wainwright, 2007; Wainwright et al., 2005). Interestingly the shape of the four-bar linkages was highly correlated with their respective mechanical output. While neither shows an exact correlation between shape and KT value, and therefore both systems still exemplify "many-to-one-mapping," PLS correlation coefficients were very high especially for the hyoid four-bar linkage (>0.85), suggesting that specific four-bar-linkage configurations have correspondingly specific performance outputs.

The highest variation in evolutionary rates was associated with the joint between the input and output links in the oral-jaw linkage, and in the joint between the coupler and output links in the hyoid linkage. These were also associated with variation in KT values (Figure 5) and in covariation with head shape. This differs from previous studies on Mantis shrimp, which found that the strongest form-function mapping was associated with variation in the input link (Muñoz et al., 2017). However, they similarly found that features (e.g., the input link) most strongly associated with mechanical outputs show the highest evolutionary rates. The two four-bar linkage systems showed divergent patterns in the constraints imposed by head shape and in form-function mapping. The hyoid four-bar linkage showed stronger form-function mapping than the oral-jaw four-bar linkage, and head shape constructional constraints appear to have a weaker impact on hyoid four-bar linkage kinematics than oral-jaw shape. Interestingly, an analysis of evolutionary rates in simple lever systems in several families (including cichlids) also showed higher rates of evolutionary in traits most strongly associated with biomechanical trade-offs (Holzman et al., 2012). Perhaps, the weaker constructional constraints on hyoid four-bar shape enable tighter form-function mapping compared to the oral-jaw four-bar system and permit greater evolutionary lability associated with biomechanical constraints.

Analyses of form-function mapping in four-bar linkages in fish have almost completely focused on the oral-jaw linkage and tend to be from a limited number of families, including this study (Alfaro et al., 2005; Hu et al., 2017; Hulsey & Garcia De Leon, 2005; Hulsey & Wainwright, 2002; Martinez & Sparks, 2017; Martinez & Wainwright, 2019; Muller, 1996; Wainwright et al., 2005; Westneat, 1991, 1994). Comparatively, the hyoid linkage is must less explored. This unintended bias may have significant impacts of our understanding of how selection on mechanical outputs impacts morphological diversification. For example, integration between body shape and four-bar shape may allow rapid change across trait complexes (Arbour et al., 2021; Felice et al., 2018). For instance, Martinez and Sparks (2017) note that many-to-one-mapping may help to improve trait lability without compromising KT optimization. Prior studies in labrids showed that oral-jaw four-bar shape tended to be grouped around redundant KT values, rather than diversifying across all biologically feasible four-bar configurations (Alfaro et al., 2005). Comparatively, the stronger form-function mapping of the hyoid-four-bar system may change these expectations. Hyoid KT values showed much clearer regionalization in four-bar shape space, allowing for reduced redundancy compared with oral-jaw KT values.

Furthermore, even within our own study we consider only one mechanical property of each linkage, even though realistically, all moving elements may have functional consequences—both hyoid depression and cranial elevation are important properties for buccal expansion and suction feeding for example. A recent analysis by Martinez and Wainwright (2019) also introduced the concept of shape change across the movement of the oral-jaw four-bar linkage as a previously unexplored property of morphological evolution. Overall, we feel it important to stress that the morphological and macroevolutionary patterns of oral-jaw four-bar linkage diversification (especially those studied in few taxonomic groups and just one mechanical property) may not be generalizable to all similarly complex functional systems.

AUTHOR CONTRIBUTIONS

Jessica Arbour: Conceptualization; methodology; formal analysis; supervision; visualization; writing – original draft; writing – review and editing; investigation. **Faith Rumpp:** Conceptualization; methodology; writing – original draft; data curation; formal analysis; investigation. **Hernán López-Fernández:** Writing – original draft; writing – review and editing; conceptualization.

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