Author Manuscript

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

AUTHORS: Jessica Arbour[1*], Faith Rumpp[1] and Hernán López-Fernández[2]

[1] Department of Biology, Middle Tennessee State University, 1301 E Main Street, Murfreesboro TN

[2] Department of Ecology and Evolutionary Biology, University of Michigan, 1105 N University Avenue, Ann Arbor, MI

* corresponding author - jessica.arbour@mtsu.edu

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ar.25231

This article is protected by copyright. All rights reserved.

Author Manuscript

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 (KT) 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/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.

Author Manuscript

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, Debat, & Auffray, 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 the differential somatic investment in specific anatomical features may be termed "constructional" (Barel, 1984; Gorb, 1995; Ubukata *et al.*, 2008; Hulsey & Hollingsworth Jr, 2011; Arbour & López-Fernández, 2018; Hulsey, Holzman, & Meyer, 2018). For example, with limited space available for jaw muscles, selection may prioritize the size of different muscle subdivisions in different species (Herrel *et al.*, 2008; Hulsey & Hollingsworth Jr, 2011; Arbour & López-Fernández, 2018). 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; e.g., muscle mass is a major predictor of the maximum force produced by a muscle (Landry & Turnbull, 1971; Weijs, 1980; Westneat, 2003; Herrel *et al.*, 2008; Santana, Dumont, & Davis, 2010). 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 describing output to input rotation in four bar linkages; Alfaro, Bolnick, & Wainwright, 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 (Westneat, 1994; Muller, 1996; Hu, Nelson-Maney, & Anderson, 2017). 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 behaviour of four-bar linkages can be quantified using the kinematic transmission coefficient, or KT, 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 both by 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 (Wainwright & Richard, 1995; Alfaro *et al.*, 2005; Camp & Brainerd, 2015). 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

Author Manuscript

Author Manuscript

which biological form may diversify with little impact to 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 (Westneat, 1994; Muller, 1996; Camp & Brainerd, 2015). 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 (Fig. 1), while others require more sophisticated modelling techniques (Westneat, 1994; Muller, 1996; Camp & Brainerd, 2015). The oral jaw four bar linkage connects rotation of the lower jaw to rotation of the maxilla and indirectly the protrusion of the pre-maxilla, and is associated with the ability to protrude the upper jaw (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 (Wainwright *et al.*, 2004, 2007; Alfaro *et al.*, 2005; Hulsey *et al.*, 2008; Martinez & Sparks, 2017). 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 both by the sternohyoideus (which comprises the coupler link) and the body musculature (largely the epaxials) (Muller, 1996; Wainwright *et al.*, 2004, 2007; Camp, Roberts, & Brainerd, 2015). 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

Author Manuscript

(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, fruit) and show numerous dietary specializations associated with morphological and behavioral adaptations (Winemiller, Kelso-Winemiller, & Brenkert, 1995; Crampton, 2008; Cochran-Biederman & Winemiller, 2010; López-Fernández *et al.*, 2014; Burress, 2016; Weller, McMahan, & Westneat, 2016; Pease, A. Mendoza-Carranza, M. Winemiller *et al.*, 2018; Arbour *et al.*, 2020). 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 (Wainwright *et al.*, 2004; Hulsey, García de León, & Rodiles-Hernández, 2006; Arbour & López-Fernández, 2014).

The two four bar linkages represent major structures located across the head, and Cichlinae show a tremendous diversity in head shapes (Fig. 2). Among this diverse subclades are piscivorous species showing convergence towards elongate bodies and head with large, terminal mouths (*Crenicichla, Cichla, Parachromis*), substrate-sifting species tending towards 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 (Winemiller *et al.*, 1995; López-Fernández *et al.*, 2012, 2013, 2014; Pease, A. Mendoza-Carranza, M. Winemiller *et al.*, 2018; Arbour *et al.*, 2020). 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 (Hulsey, Mims, & Streelman, 2007; Hulsey & Hollingsworth Jr, 2011; Arbour & López-Fernández, 2018).

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 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.

METHODS

DATA COLLECTION

Previous analyses have generally only compared the relative lengths of the four bar elements, (Wainwright *et al.*, 2005; Hulsey & Garcia De Leon, 2005), and in cases where positioning has been incorporated the angle between the input link (lower jaw) and the fixed link

Author Manuscript

(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 (Muller, 1996; Martinez *et al.*, 2018; Corn *et al.*, 2021). 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 et al (Arbour & López-Fernández, 2014) to quantify the kinematic transmission coefficient (KT) of each four-bar linkage, and the same dissection photos were used from that previous study (and see Fig. 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 is described by Table 2.

Estimates of four-bar kinematic transmission coefficients (KT) were taken from the data from Arbour et al. (2014), which were also used in subsequent comparative analyses of Cichlinae functional evolution (Arbour & López-Fernández, 2016; Arbour *et al.*, 2020). 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-degree 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-degree upward rotation of the input link

Author Manuscript

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 (and previously applied in the initial morphological 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.

GEOMETRIC MORPHOMETRIC ANALYSIS

We used Generalized Procrustes Analysis to remove the impact of position, rotation and scale from the landmark configurations (Bookstein, 1997; Zelditch *et al.*, 2004; Adams & Otarola-Castillo, 2013). 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"

Author Manuscript

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, Jackson, & Somers, 2005).

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 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.

Author Manuscript

Author Manuscript

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).

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 two block 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 two block 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, Claude, & Strimmer, 2004; Rousseeuw *et al.*, 2014; Slater & Pennell, 2014). PICs were regressed through the origin.

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, i.e.,

Author Manuscript

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).

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 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.

RESULTS

HEAD AND FOUR-BAR SHAPE VARIATION

Author Manuscript

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 anterio-posterior expansion or compression of the four bar linkage (Fig. 3B). The relative positions of the maxilla and lower jaw links (input and output links) were also important to both axes, with 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) (Fig. 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; Fig. 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 (Fig. 3, blue).

Head shape variation largely followed findings from previous studies (López-Fernández *et al.*, 2013; Arbour & López-Fernández, 2014, 2018). 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 elongate heads (e.g., *Crenicihla* and *Cichla*; Fig. S1). PC2 (20.9%) was associated with the position of the eye and orientation of the mouth (species 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.

THE IMPACT OF HEAD SHAPE ON FOUR BAR LINKAGE COVARIATION

The shape of both four bar linkages were 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 Fig. S1). Shape changes associated with head shape were subtle in the four bar linkages. But in general, more elongate 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 (Fig. 4B). Head elongation was also associated with longer hyoid bar, and a slightly shorted cleithrum (Fig. 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).

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 *Crencichla-Teleocichla* (Fig. S2). Combinations of both

Author Manuscript

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 of highly elongate taxa (Fig. 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 (Fig. 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 shape were found to be significantly associated KT values (Hyoid r = 0.911, Oral jaw r = 0.757, p values = 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 (Fig. 5). In both cases the elements of shape variation associated with KT values were also features associated with head shape (see Fig. 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.

Author Manuscript

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).

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 (Fig. 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 angel between the maxilla and lower jaw links (Fig. S3). Comparatively, hyoid four-bar linkages with longer hyoid and hyomandibula links were associated with a slightly more elongate 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.

EVOLUTIONARY LABILITY OF FOUR BAR SHAPE

Both the oral jaw and hyoid four bar linkages showed heterogenous evolutionary rates, (Table 3). In the oral jaw four bar linkage, the highest rates were observed at the connection

Author Manuscript

between the lower jaw and maxilla (Fig. 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-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 (Fig. 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.

DISCUSSION

CONSTRUCTIONAL VS. 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.

Author Manuscript

Author Manuscript

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 towards producing more evenly distributed shape change (Fig. 6 and Table 3). As shape was significantly correlated with function in both 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 supports 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

Author Manuscript

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 (Fig. 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 (Fig 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 tradeoff 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).

MORPHOLOGICAL, ECOLOGICAL AND TAXONOMIC CORRELATES OF FOUR BAR FUNCTION

Body and head elongation 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 fishes (Sallan & Friedman, 2011; López-Fernández *et*

Author Manuscript

al., 2013; Claverie & Wainwright, 2014). There are numerous critical consequences of body elongation in fishes, including changing swimming mechanics (Webb, 1984; Blake, 2004), predator avoidance (Bronmark & Miner, 1992), feeding mechanics (Carroll & Wainwright, 2009; Camp *et al.*, 2015), 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) suggest that the depth of the body may co-evolve 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-neurocranium four-bar linkages, and the relative sizes of the fixed and hyoid-bar links appeared most important to both body-shape vs. 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 used 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 (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.

Author Manuscript

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 (Lowe-McConnell, 1969, 1991; Winemiller *et al.*, 1995; López-Fernández *et al.*, 2012; Arbour & López-Fernández, 2014). 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 (Fig 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 (Winemiller *et al.*, 1995; López-Fernández *et al.*, 2012, 2013; Steele & López-Fernández, 2014). 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*.

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, Cooper, & Albertson, 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 (Winemiller *et al.*, 1995; Hulsey *et al.*, 2006; Higham *et al.*, 2007; Cooper *et al.*, 2010; López-Fernández *et al.*, 2013; Arbour & López-Fernández, 2014). 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 preorbital 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 (Felice, Randau, & Goswami, 2018; Arbour, Curtis, & Santana, 2021).

Author Manuscript

Author Manuscript

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; Wainwright *et al.*, 2005; Wainwright, 2007; Martinez & Wainwright, 2019). Interestingly the shape of the four bar linkages was highly correlated with their respective mechanical output. While neither show 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 were 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 (Fig. 5) and in covariation with head shape. This differs from previous studies in Mantis shrimp, which found that the strongest form-function mapping was associated with variation in the input link (Muñoz, Anderson, & Patek, 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

Author Manuscript

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 (Westneat, 1991, 1994; Muller, 1996; Hulsey & Wainwright, 2002; Alfaro *et al.*, 2005; Wainwright *et al.*, 2005; Hulsey & Garcia De Leon, 2005; Hu *et al.*, 2017; Martinez & Sparks, 2017; Martinez & Wainwright, 2019). Comparatively the hyoid-neurocranium 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 (Felice *et al.*, 2018; Arbour *et al.*, 2021). 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.

ACKNOWLEDGEMENTS

We are grateful to E. Holm, M. Burridge, M. Zur and D. Stacey (Royal Ontario Museum), J. Armbruster and D. Werneke (Auburn University Museum), M. Sabaj-Perez and J. Lundberg (Academy of Natural Sciences of Drexel University), and R. Reis and C. Lucena (Museu de Zoologia da Pontificia Universidade Catolica do Rio Grande do Sul) for access to specimens under their care.This work was funded, in part, by an Natural Science and Engineering Research Council of Canada (NSERC) Graduate Scholarship to J.H.A., an NSERC Discovery Grant and funds from the University of Michigan to H.L.F., and funds from ORSP from Middle Tennessee State University to J.H.A.

Author Manuscript

Author Manuscript

REFERENCES

Adams DC & Otarola-Castillo E. 2013. geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution* 4: 393–399.

Albert J & Reis RE. 2011. *Historical Biogeography of Neotropical Freshwater Fishes*. University of California University Press.

Alfaro ME, Bolnick DI & Wainwright PC. 2005. Evolutionary consequences of many-to-one mapping of jaw morphology to mechanics in labrid fishes. *The American Naturalist* 165: E140–E154.

Arbour JH, Montaña CG, Winemiller KO, Pease AA, Soria-barreto M, Cochran-biederman JL & López-fernández H. 2020. Macroevolutionary analyses indicate that repeated adaptive shifts towards predatory diets affect functional diversity in Neotropical cichlids. : 1–18.

Arbour JH, Curtis AA & Santana SE. 2021. Sensory adaptations reshaped intrinsic factors underlying morphological diversification in bats. *BMC Biology* 19: 1–13.

Arbour JH & López-Fernández H. 2014. Adaptive landscape and functional diversity of Neotropical cichlids: implications for the ecology and evolution of Cichlinae (Cichlidae; Cichliformes). *Journal of Evolutionary Biology* 27: 2431–42.

Arbour JH & López-Fernández H. 2016. Continental cichlid radiations: functional diversity reveals the role of changing ecological opportunity in the Neotropics. *Proceedings of the Royal Society B* 283: 20160556.

Arbour JH & López-Fernández H. 2018. Intrinsic Constraints on the Diversification of Neotropical Cichlid Adductor Mandibulae Size. *Anatomical Record* 301: 216–226.

Barel CDN. 1984. Form-relations in the context of constructional morphology: the eye and suspensorium of lacustrine Cichlidae (Pisces: Teleostei). *Netherlands Journal of Zoology* 34:

Author Manuscript

439–502.

Blake RW. 2004. Fish functional design and swimming performance. *Journal of Fish Biology* 65: 1193–1222.

Bookstein FL. 1997. Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. *Medical image analysis* 1: 225–243.

Bronmark C & Miner JG. 1992. Predator-Induced Phenotypical Change in Body Morphology in Crucian Carp Author. *Science* 258: 1348–1350.

Burgess S. 2021. A review of linkage mechanisms in animal joints and related bioinspired designs. *Bioinspiration and Biomimetics* 16.

Burress ED. 2016. Ecological diversification associated with the pharyngeal jaw diversity of Neotropical cichlid fishes. *Journal of Animal Ecology* 85: 302–313.

Camp AL & Brainerd EL. 2015. Reevaluating Musculoskeletal Linkages in Suction-Feeding Fishes with X-Ray Reconstruction of Moving Morphology (XROMM). *Integrative and Comparative Biology* 55: 36–47.

Camp AL, Roberts TJ & Brainerd EL. 2015. Swimming muscles power suction feeding in largemouth bass. *Proceedings of the National Academy of Sciences of the United States of America* 112: 8690–8695.

Cardini A, Polly D, Dawson R & Milne N. 2015. Why the Long Face? Kangaroos and Wallabies Follow the Same 'Rule' of Cranial Evolutionary Allometry (CREA) as Placentals. *Evolutionary Biology* 42: 169–176.

Carroll AM & Wainwright PC. 2009. Energetic limitations on suction feeding performance in centrarchid fishes. *The Journal of experimental biology* 212: 3241–51.

Claverie T & Wainwright PC. 2014. A morphospace for reef fishes: elongation is the dominant

Author Manuscript

axis of body shape evolution. *PloS one* 9: e112732.

Cochran-Biederman JL & Winemiller KO. 2010. Relationships among habitat, ecomorphology and diets of cichlids in the Bladen River, Belize. *Environmental Biology of Fishes* 88: 143–152.

Cooper WJ, Parsons K, McIntyre A, Kern B, McGee-Moore A & Albertson RC. 2010. Bentho-pelagic divergence of cichlid feeding architecture was prodigious and consistent during multiple adaptive radiations within African rift-lakes. *PloS one* 5: e9551.

Corn KA, Martinez CM, Burress ED & Wainwright PC. 2021. A Multifunction Trade-Off has Contrasting Effects on the Evolution of Form and Function. *Systematic biology* 70: 681–693.

Crampton WGR. 2008. Ecology and life history of an Amazon floodplain cichlid: the discus fish *Symphysodon* (Perciformes: Cichlidae). *Neotropical Ichthyology* 6: 599–612.

Felice RN, Randau M & Goswami A. 2018. A fly in a tube: Macroevolutionary expectations for integrated phenotypes. *Evolution*: 2580–2594.

Felsenstein J. 1985. Phylogenies and the comparative method. *The American Naturalist* 125: 1–15.

Fonseca-Azevedo K & Herculano-Houzel S. 2012. Metabolic constraint imposes tradeoff between body size and number of brain neurons in human evolution. *Proceedings of the National Academy of Sciences of the United States of America* 109: 18571–18576.

Gorb SN. 1995. Design of the predatory legs of water bugs (Hemiptera: Nepidae, Naucoridae, Notonectidae, Gerridae). *Journal of Morphology* 223: 289–302.

Goswami A & Finarelli JA. 2016. EMMLi: A maximum likelihood approach to the analysis of modularity. *Evolution; international journal of organic evolution* 70: 1622–1637.

Herrel A, Smet A De, Aguirre LF & Aerts P. 2008. Morphological and mechanical determinants of bite force in bats: do muscles matter? *The Journal of Experimental Biology* 211: 86–91.

Author Manuscript

Higham TE, Hulsey CD, Rícan O & Carroll a M. 2007. Feeding with speed: prey capture evolution in cichilds. *Journal of evolutionary biology* 20: 70–8.

Holzman R, Collar DC, Price SA, Hulsey CD, Thomson RC & Wainwright PC. 2012. Biomechanical trade-offs bias rates of evolution in the feeding apparatus of fishes. *Proceedings of the Royal Society B* 279: 1287–1292.

Horn JL. 1965. A rationale and test for the number of factors in factor analysis. *Psychometrika* 32: 179–185.

Hu Y, Nelson-Maney N & Anderson PSL. 2017. Common evolutionary trends underlie the four-bar linkage systems of sunfish and mantis shrimp. *Evolution* 71: 1397–1405.

Hulsey CD, Roberts RJ, Lin ASP, Guldberg R & Streelman JT. 2008. Convergence in a mechanically complex phenotype: detecting structural adaptations for crushing in cichlid fish. *Evolution* 62: 1587–99.

Hulsey CD & Garcia De Leon FJ. 2005. Cichlid jaw mechanics: linking morphology to feeding specialization. *Functional Ecology* 19: 487–494.

Hulsey CD, García de León FJ & Rodiles-Hernández R. 2006. Micro- and macroevolutionary decoupling of cichlid jaws: a test of Liem's key innovation hypothesis. *Evolution* 60: 2096–2109.

Hulsey CD & Hollingsworth Jr PR. 2011. Do constructional constraints influence cyprinid (Cyprinidae: Leuciscinae) craniofacial coevolution? *Biological Journal of the Linnean Society* 103: 136–146.

Hulsey CD, Holzman R & Meyer A. 2018. Dissecting a potential spandrel of adaptive radiation: Body depth and pectoral fin ecomorphology coevolve in Lake Malawi cichlid fishes. *Ecology and Evolution* 8: 11945–11953.

Author Manuscript

Hulsey CD, Mims MC & Streelman JT. 2007. Do constructional constraints influence cichlid craniofacial diversification? *Proceedings. Biological sciences / The Royal Society* 274: 1867–1875.

Hulsey CD & Wainwright PC. 2002. Projecting mechanics into morphospace: disparity in the feeding system of labrid fishes. *Proceedings of the Royal Society B* 269: 317–26.

Ilves KL, Torti D & López-Fernández H. 2018. Exon-based phylogenomics strengthens the phylogeny of Neotropical cichlids and identifies remaining conflicting clades (Cichliformes: Cichlidae: Cichlinae). *Molecular Phylogenetics and Evolution* 118: 232–243.

Jackson D. 1993. Stopping rules in principal component analysis: a comparison of heuristical and statistical approaches. *Ecology* 74: 2204–2214.

Landry SO & Turnbull WD. 1971. Mammalian Masticatory Apparatus. *Journal of Mammalogy* 52.

López-Fernández H, Winemiller KO, Montaña C & Honeycutt RL. 2012. Diet-morphology correlations in the radiation of South American geophagine cichlids (Perciformes: Cichlidae: Cichlinae). *PloS one* 7: e33997.

López-Fernández H, Arbour JH, Winemiller KOKO & Honeycutt RLRL. 2013. Testing for Ancient Adaptive Radiations in Neotropical Cichlid Fishes. *Evolution* 67: no-no.

López-Fernández H, Arbour JH, Willis S, Watkins C, Honeycutt RLRL & Winemiller KOO. 2014. Morphology and efficiency of a specialized foraging behavior, sediment sifting, in neotropical cichlid fishes. *PloS One* 9: e89832.

Lowe-McConnell RH. 1969. The cichlid fishes of Guyana, South America, with notes on their ecology and breeding behaviour. *Zoological Journal of the Linnean Society* 48: 255–302.

Lowe-McConnell RH. 1991. Ecology of cichlids in South American and African waters,

Author Manuscript

excluding the African Great Lakes. In: Keenleyside MHA, ed. *Cichlid Fishes: Behaviour, ecology and evolution*. London: Chapman Hall, 61–85.

Mahler DL, Revell LJ, Glor RE & Losos JB. 2010. Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles. *Evolution; international journal of organic evolution* 64: 2731–45.

Martinez CM, McGee MD, Borstein SR & Wainwright PC. 2018. Feeding ecology underlies the evolution of cichlid jaw mobility. *Evolution* 72: 1645–1655.

Martinez CM & Sparks JS. 2017. Malagasy cichlids differentially limit impacts of body shape evolution on oral jaw functional morphology. : 2219–2229.

Martinez CM & Wainwright PC. 2019. Extending the Geometric Approach for Studying Biomechanical Motions. *Integrative and Comparative Biology* 59: 684–695.

McMahan CD, Chakrabarty P, Sparks JS, Smith WL & Davis MP. 2013. Temporal patterns of diversification across global cichlid biodiversity (Acanthomorpha: Cichlidae). *PloS one* 8: e71162.

Muller M. 1996. A novel classification of planar four-bar linkages and its application to the mechanical analysis of animal systems. *Philosophical transactions of the Royal Society B* 351: 689–720.

Muñoz MM, Anderson PSL & Patek SN. 2017. Mechanical sensitivity and the dynamics of evolutionary rate shifts in biomechanical systems. *Proceedings of the Royal Society B: Biological Sciences* 284.

Paradis E, Claude J & Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20: 289-290.

Parsons KJ, Cooper WJ & Albertson RC. 2011. Modularity of the Oral Jaws Is Linked to

Author Manuscript

Repeated Changes in the Craniofacial Shape of African Cichlids. *International Journal of Evolutionary Biology* 2011: 1–10.

Pease, A. Mendoza-Carranza, M. Winemiller K, Pease AA, Mendoza-Carranza M & Winemiller KO. 2018. Feeding ecology and ecomorphology of cichlid assemblages in a large Mesoamerican river delta. *Environmental Biology of Fishes* 101: 867–879.

Peres-Neto PR, Jackson D a. & Somers KM. 2005. How many principal components? stopping rules for determining the number of non-trivial axes revisited. *Computational Statistics & Data Analysis* 49: 974–997.

Quillévéré F, Debat V & Auffray JC. 2002. Ontogenetic and evolutionary patterns of shape differentiation during the initial diversification of Paleocene acarininids (planktonic foraminifera). *Paleobiology* 28: 435–448.

Rohlf FJ. 2015. The tps series of software. *Hystrix* 26: 1–4.

Rousseeuw P, Croux C, Todorov V, Ruckstuhl A, Salibian-Barrera M, Verbeke T, Koller M & Maechler M. 2014. robustbase: Basic Robust Statistics.

Sallan LC & Friedman M. 2011. Heads or tails: staged diversification in vertebrate evolutionary radiations. *Proceedings. Biological sciences / The Royal Society*: 2025–2032.

Santana SE, Dumont ER & Davis JL. 2010. Mechanics of bite force production and its relationship to diet in bats. *Functional Ecology* 24: 776–784.

Slater GJ & Pennell MW. 2014. Robust regression and posterior predictive simulation increase power to detect early bursts of trait evolution. *Systematic biology* 63: 293–308.

Steele SE & López-Fernández H. 2014. Body size diversity and frequency distributions of neotropical cichlid fishes (Cichliformes: Cichlidae: Cichlinae). *PLoS ONE* 9: e106336.

Ubukata T, Tanabe K, Shigeta Y, Maeda H & Mapes RH. 2008. Piggyback whorls: A new

Author Manuscript

theoretical morphologic model reveals constructional linkages among morphological characters in ammonoids. *Acta Palaeontologica Polonica* 53: 113–128.

Wainwright PC, Bellwood DR, Westneat MW, Grubich JR & Hoey AS. 2004. A functional morphospace for the skull of labrid fishes: patterns of diversity in a complex biomechanical system. *Biological Journal of the Linnean Society* 82: 1–25.

Wainwright PC, Alfaro ME, Bolnick DI & Hulsey CD. 2005. Many-to-One Mapping of Form to Function: A General Principle in Organismal Design? *Integrative and Comparative Biology* 45: 256–262.

Wainwright PC, Carroll AM, Collar DC, Day SW, Higham TE & Holzman R. 2007. Suction feeding mechanics, performance, and diversity in fishes. *Evolution; international journal of organic evolution* 47: 96–106.

Wainwright PC. 2007. Functional versus morphological diversity in macroevolution. *Annual Review of Ecology, Evolution, and Systematics* 38: 381–401.

Wainwright PC & Richard BA. 1995. Predicting patterns of prey use from morphology of fishes. *Environmental Biology of Fishes* 44: 97–113.

Webb PW. 1984. Form and Function in Fish Swimming. *Scientific American* 251: 72–82.

Weijs WA. 1980. Biomechanical models and the analysis of form: A study of the mammalian masticatory apparatus. *Integrative and Comparative Biology* 20: 707–719.

Weller HI, McMahan CD & Westneat MW. 2016. Dirt-sifting devilfish: winnowing in the geophagine cichlid Satanoperca daemon and evolutionary implications. *Zoomorphology*: 1–15.

Westneat MW. 1991. Linkage Biomechanics and evolution of the unique feeding mechanism of Epibulus Insidiator (Labridae: Teleostei). *Journal of Experimental Biology* 159: 165–184.

Westneat MW. 1994. Transmission of force and velocity in the feeding mechanisms of labrid

fishes (Teleostei, Perciformes). *Zoomorphology* 114: 103–118.

Westneat MW. 2003. A biomechanical model for analysis of muscle force, power output and lower jaw motion in fishes. *Journal of Theoretical Biology* 223: 269–281.

Winemiller KO, Kelso-Winemiller LC & Brenkert AL. 1995. Ecomorphological diversification and convergence in fluvial cichlid fishes. *Environmental Biology of Fishes* 44: 235–261.

Zelditch ML, Swiderski DL, Sheets HD & Fink WL. 2004. *Geometric morphometrics for biologists: a primer* (London, Ed.). Elsevier Academic Press.

Author Manuscript

Author Manuscript

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 Table 1-2 and also shown in Fig. 2. A) *Crenicichla saxatilis*, B) *Biotodoma wavrini*, C) *Uaru amphiacanthoides*, D) *Crenicichla multispinosa*, E) *Andinoacara rivulatus*, F) *Symphysodon aequifasciatus*.

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 Table 1-2 and S1for descriptions of landmarks). Dashed lines show the links. Note: locations of linkage landmarks were determined from dissected specimens, not externally. D) Head shape diversity in Neotropical cichlids. Photo credits to J. Arbour and H. Lopez-Fernandez.

Figure 3: Diversity of four bar linkage shapes and biomechanical coefficients. A) KT values from the two four-bar linkage systems, the oral-jaw and hyoid linkages. B) Results of a 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, Grey = Astronotini, Purple = Retroculini.

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 PLS analysis of each four-bar landmark dataset against head shape. r = PLS-correlation coefficient.

Author Manuscript

Figure 5: Four-bar linkage shape changes associated with changes in KT values. Shape changes taken from two block PLS 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.

Figure 6: Multivariate evolutionary rate (BM – 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.

Table 1: Landmark locations for the digitization of four-bar linkage shape in Neotropical cichlids. See Table 2 for the assignment of landmarks to specific links.

	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

Table 2: Description of the two four bar linkages analyzed from Neotropical cichlid skulls.

Connecting landmarks refers to the numbered landmarks in table 1.

	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

Author Manuscript

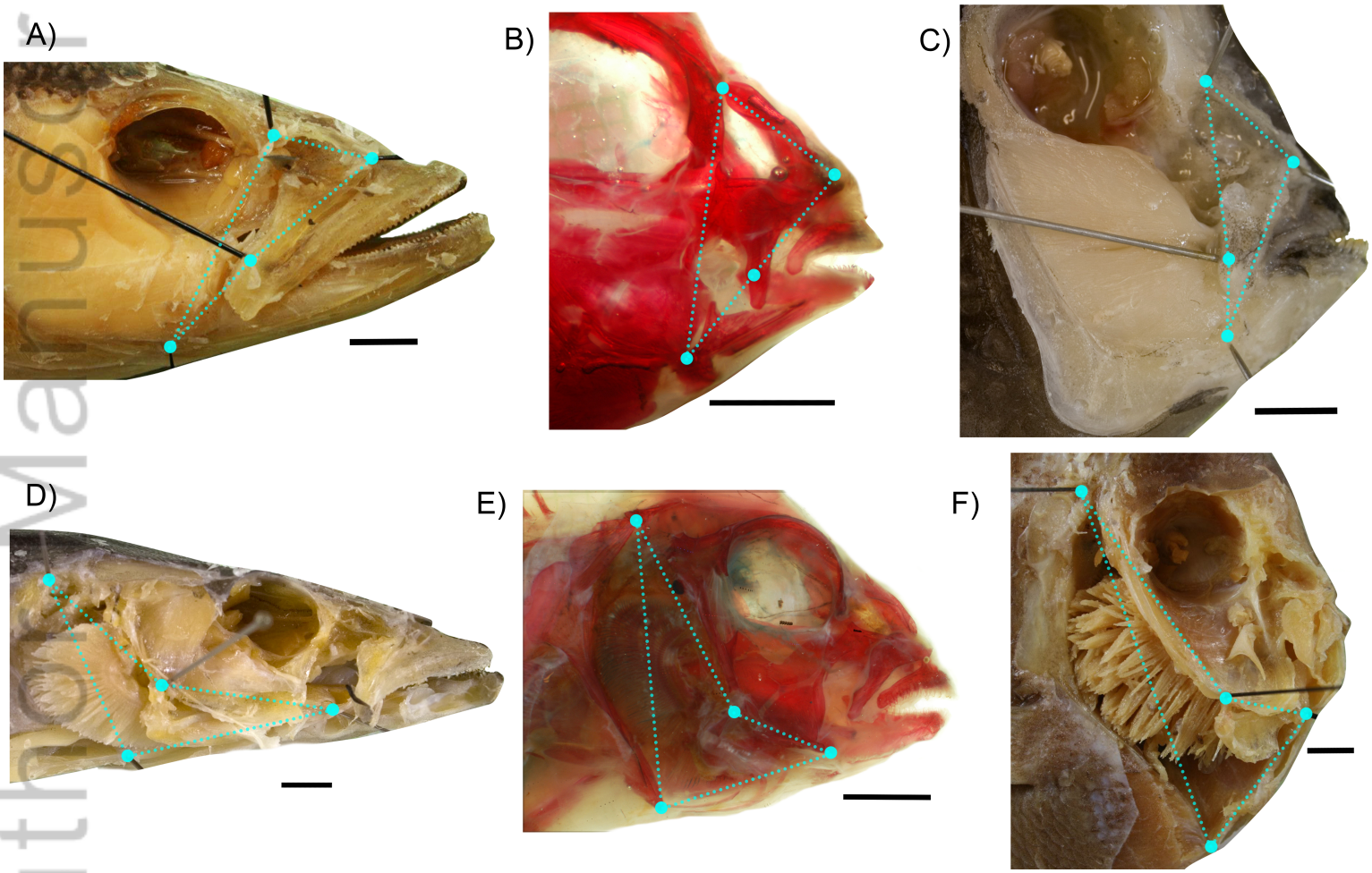
Author Manuscript

Table 1: Results from "compare.multi.evol.rates" function from the R package "geomorph". 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.

	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

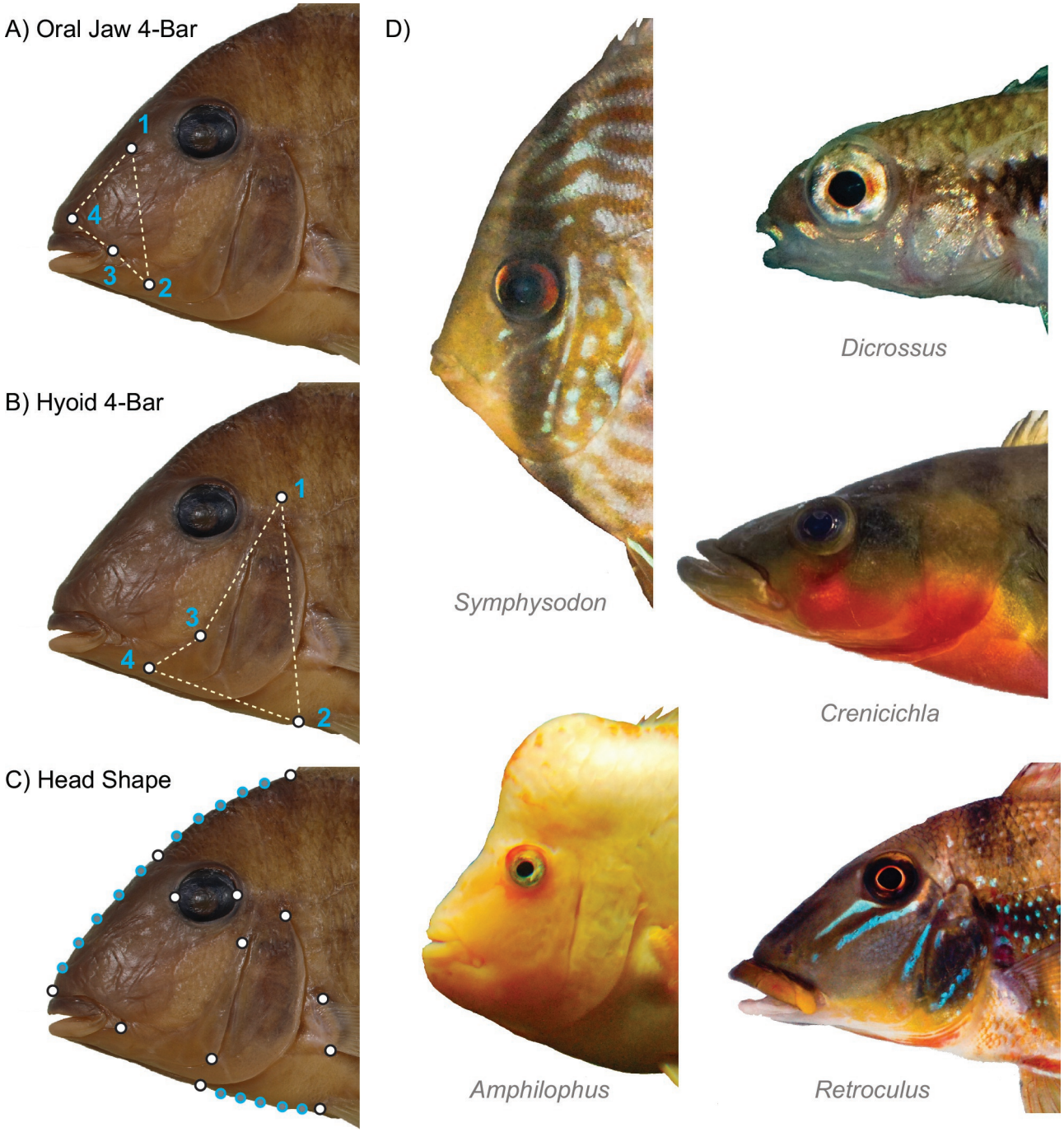
Author Manuscript

Author Manuscript



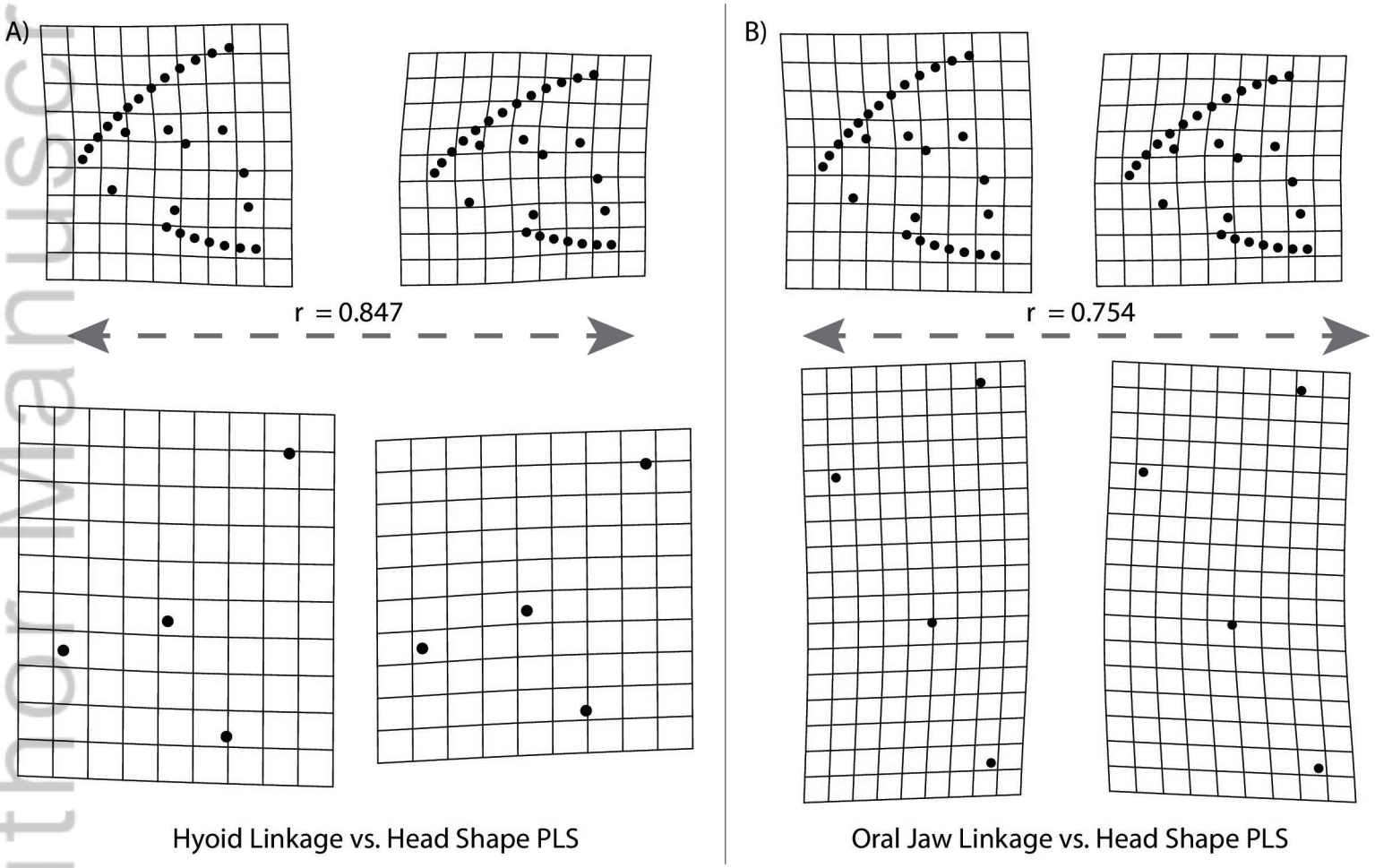
AR_25231_fig 1 - 4 bars.tif

Author Manuscript



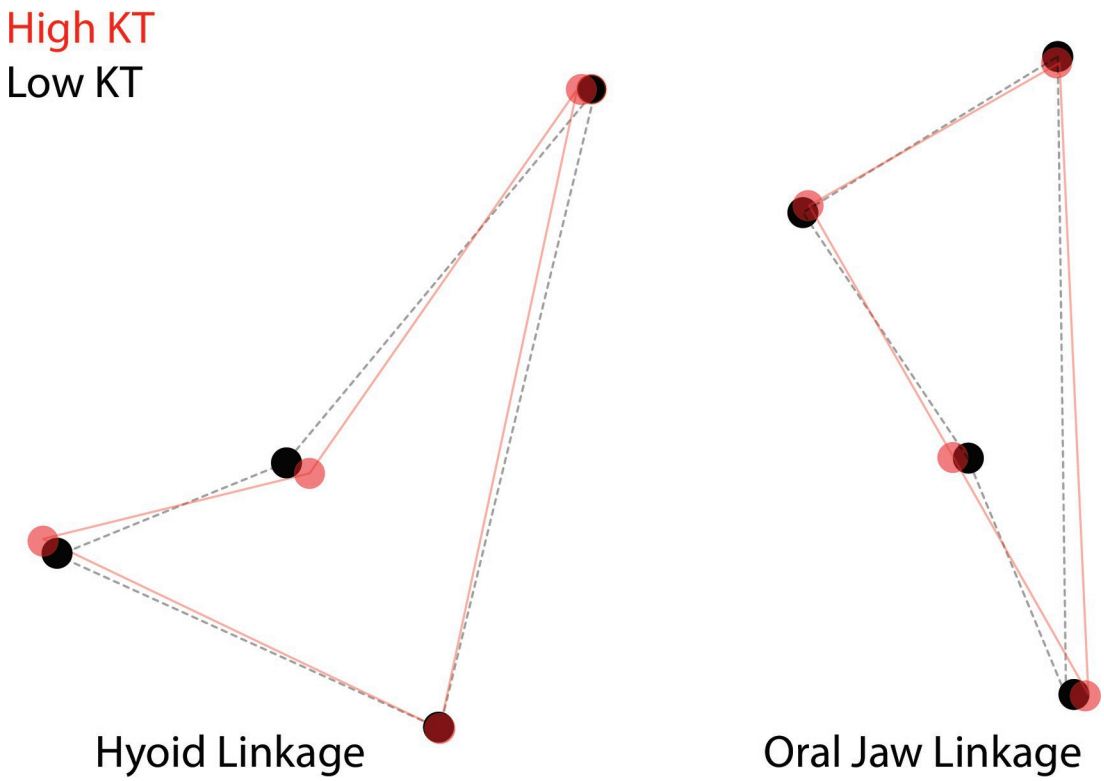
ar_25231_fig 2 - intro plot.eps

Author Manuscript



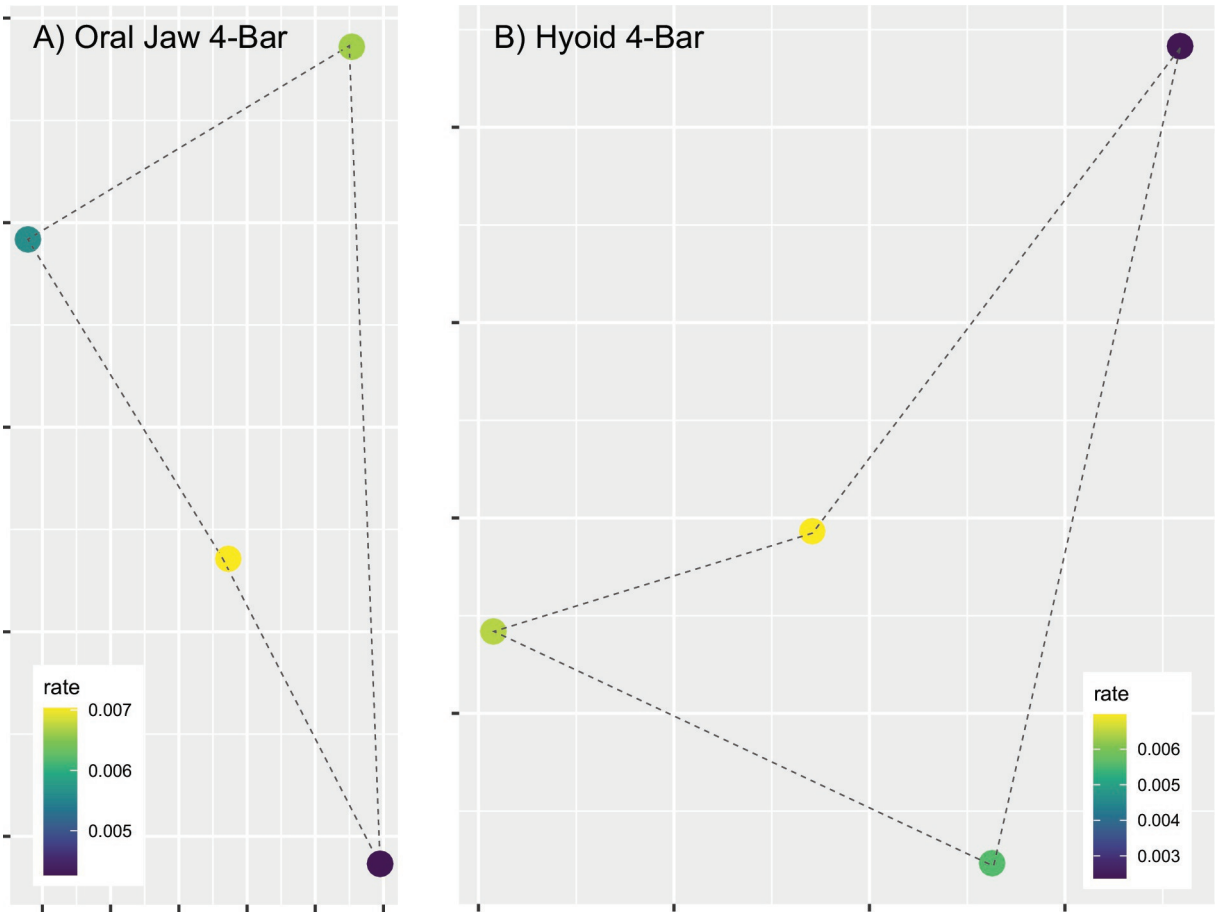
ar_25231_fig 4 - linkage vs head shape pls.eps

Author Manuscript



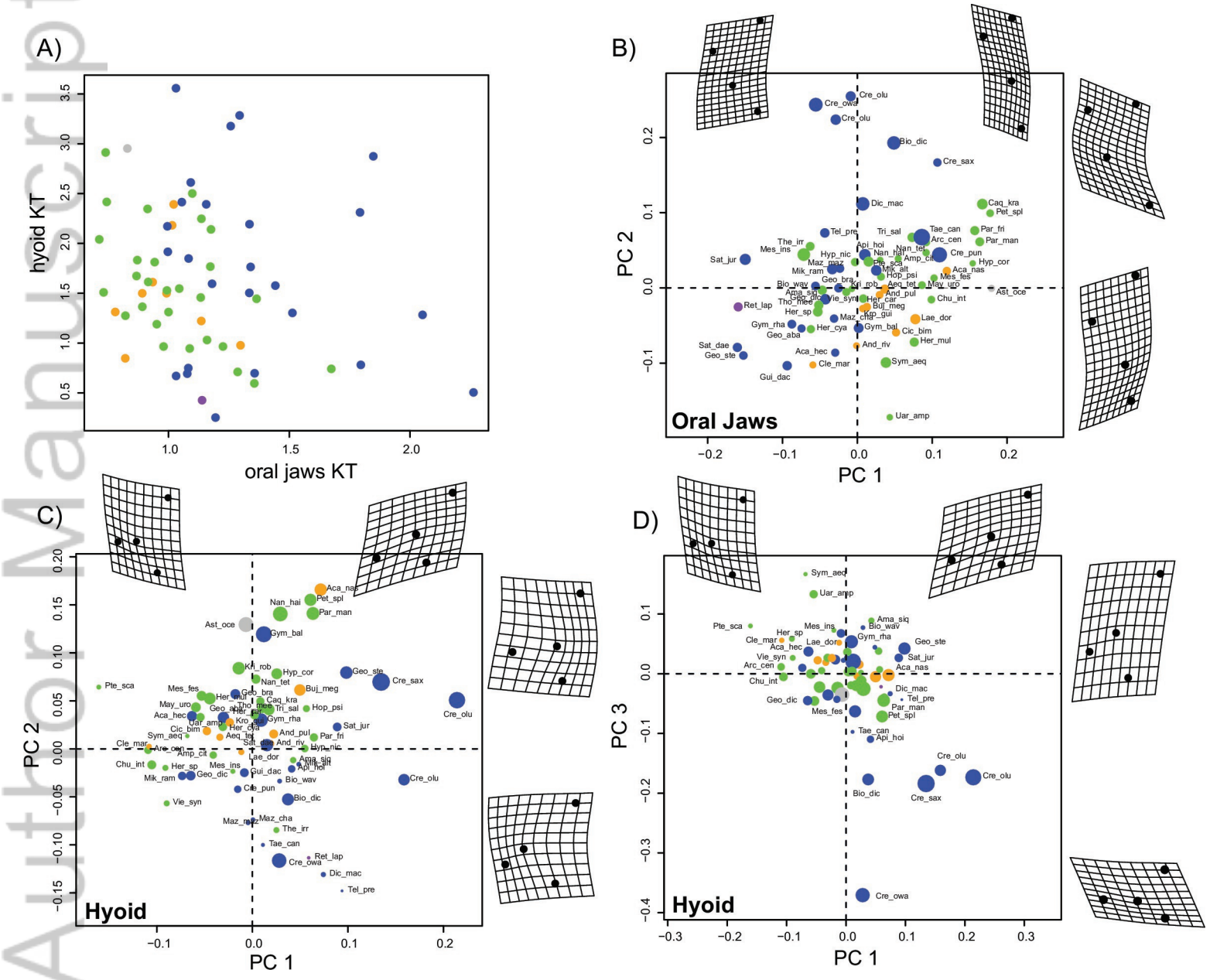
ar_25231_fig 5 - shape vs kt.eps

Author Manuscript



ar_25231_fig 6 - evolutionary rate plot.eps

Author Manuscript



ar_25231_fig 3 - pca and kt plots.eps

Table 1: Landmark locations for the digitization of four-bar linkage shape in Neotropical cichlids. See Table 2 for the assignment of landmarks to specific links.

	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

Author Manuscript

Table 2: Description of the two four bar linkages analyzed from Neotropical cichlid skulls.

Connecting landmarks refers to the numbered landmarks in table 1.

	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

Author Manuscript

Author Manuscript

Table 1: Results from "compare.multi.evol.rates" function from the R package "geomorph". 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.

	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

Author Manuscript