Malagasy cichlids differentially limit impacts of body shape evolution on oral jaw functional morphology

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Patterns of trait covariation, such as integration and modularity, are vital factors that influence the evolution of vertebrate body plans. In functional systems, decoupling of morphological modules buffers functional change in one trait by reducing correlated variation with another. However, for complex morphologies with many-to-one mapping of form to function (MTOM), resistance to functional change may also be achieved by constraining morphological variation within a functionally stable region of morphospace. For this research, we used geometric morphometrics to evaluate the evolution of body shape and its relationship with jaw functional morphology in two independent radiations of endemic Malagasy cichlid (Teleostei: Cichlidae). Our results suggested that the two subfamilies used different strategies to mitigate impacts of body shape variation on a metric of jaw function, maxillary kinematic transmission (MKT): (1) modularity between cranial and postcranial morphologies, and (2) integration of body and jaw evolution, with jaw morphologies varying in a manner that limits change in MKT. This research shows that, unlike modularity, MTOM allows traits to retain strong evolutionary covariation while still reducing impacts on functionality. These results suggest that MTOM, and its influence on the evolution of correlated traits, is likely much more widespread than is currently understood.

KEY WORDS: Cichlidae, Madagascar, integration, modularity, many-to-one mapping.

Morphological traits comprising vertebrate body plans often interact, such that variation of one trait may constrain or facilitate the evolution of others (Goswami and Polly 2010; Sanger et al. 2011). Integration refers to the degree of covariation between traits and is a pervasive attribute of organismal design that occurs at multiple scales (Zelditch 1988; Zelditch and Carmichael 1989; Klingenberg 2014). A related concept, modularity, is characterized by localized integration and relative decoupling from other traits (Klingenberg 2008; Klingenberg 2014). The compartmentalization of traits into modules allows for different functional units to evolve at least semiautonomously and has been shown to influence diversification. In mantis shrimp, for example, higher modularity of raptorial appendages was associated with elevated rates of morphological evolution (Claverie and Patek 2013). Disparate patterns of modularity may also evolve in related species

to satisfy selection for different functional roles. Parsons et al. (2012) found that lower jaw modularity of Lake Malawi cichlids differed for species with suction and biting feeding types. It is clear that the ability for trait correlations to evolve is a powerful tool for generating specialization and novelty, but it is also important to keep in mind that any change in the degree of integration or modularity occurs within the context of a form-function relationship and is therefore regulated by it.

For complex biomechanical systems, consisting of three or more components, it is expected that a single functional output may be achieved by multiple morphological configurations, termed MTOM of form to function. MTOM is a nonbiological feature of the form-function landscape, but may influence the distribution of morphological and mechanical diversity within clades (Wainwright et al. 2005). While examples of MTOM have been observed in systems such as soricid shrew jaws (Young et al. 2007) and anolis lizard legs (Vanhooydonck et al. 2006), many documented biological accounts are in reference to the oral jaw fourbar linkage of teleost fishes (Westneat 1990; Wainwright et al.
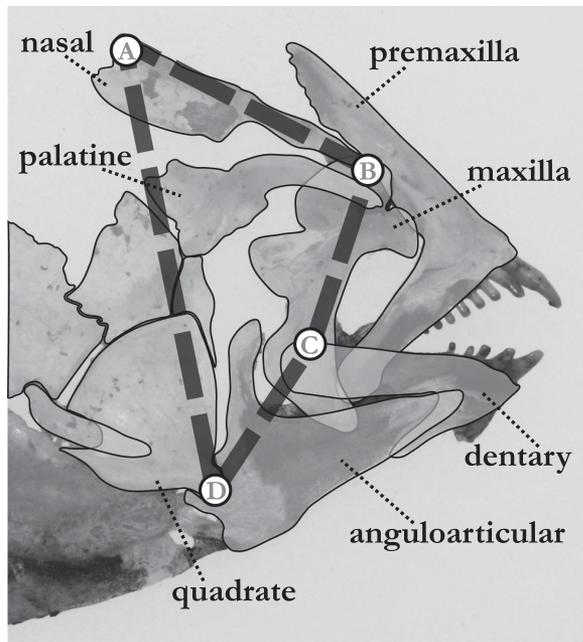
Figure 1. Configuration of the oral jaw fourbar linkage used in this study and its associated skeletal elements. The fourbar is composed of the nasal link ($\overline{AB}$), maxillary link ($\overline{BC}$), lower jaw link ($\overline{CD}$), and fixed/hyomandibular link ($\overline{AD}$). Vertices of the four-bar represent landmarks analyzed with geometric morphometrics. Specimen pictured: *Paretroplus polyactis* (AMNH 88192SD).

2004; Alfaro et al. 2005; Hulsey and García De León 2005; Collar and Wainwright 2006; Parnell et al. 2008). The fourbar linkage is a biomechanical model, representing a skeletal complex that is responsible for much of the anterior jaw kinesis that is produced during feeding (Fig. 1). While this feature is one component of a larger integrated feeding system, it is both functionally and ecologically important for prey capture (e.g., Westneat 1994; Westneat 1995). A mechanical output of the oral jaw fourbar, MKT, measures motion transfer efficiency within linkages, with larger values associated with mechanically faster jaws and more mobile prey and lower values with slower jaws and greater bite force (Westneat et al. 2005). In new world cichlids, for example, MKT was related to the capacity of jaws to generate anterior protrusion for suction feeding (Hulsey and García De León 2005).

Previous studies have shown that fourbar linkages display MTOM with respect to the mechanical trait, MKT, and that there are a number of expectations from the ensuing relationship between form and function in these systems (Alfaro et al. 2004; Alfaro et al. 2005; Wainwright et al. 2005). First, the ability of multiple forms to achieve a single value of MKT will result in regions of the oral jaw fourbar morphospace over which function remains stable. Second, when treated as continuous variables, relationships (e.g., regression or correlation) between fourbar shape and MKT will be weak. The reason for this is that, while we know MKT to be estimated in perfect and deterministic fashion from

fourbar morphology, MTOM precludes convergence on (and prediction of) a single fourbar shape for a given value of MKT. Third, because it is possible to diversify with respect to fourbar shape without a comparable increase in MKT, a mismatch can occur in the accumulation of morphological versus mechanical diversity within clades. Finally, *if* shape variation is constrained within a mechanically stable region of morphospace, then there is potential for a secondary function to be optimized with minimal impact on primary functionality.

The above expectations have intriguing implications for jaw diversification in fishes, including the role of MTOM in the evolution of the overall body plan, where competing selective demands on cranial (trophic) and postcranial (habitat) morphologies may differ (Streelman and Danley 2003; Sallan and Friedman 2011; Aguilar-Medrano et al. 2016). In cichlids, one of the largest and most diverse vertebrate families (Turner 2007), associations between body shape, trophic morphology, and feeding ecology have been identified (Rüber and Adams 2001; Kassam et al. 2003; Trapani 2003; Clabaut et al. 2007; Muschick et al. 2012). However, it is not clear if the complex form-function landscape of the oral jaw fourbar linkage has influenced patterns of covariation between cranial and postcranial morphologies at the clade level.

For this study, we focused on the evolution of oral jaw morphology in the endemic cichlids of Madagascar and its relationship with body shape, which ranges from deep to shallow-bodied forms (Fig. 2). The 30 species of Malagasy cichlid (including one undescribed, new species) represent about 1.8% of species diversity within Cichlidae (based on total valid species from Eschmeyer et al. 2016). However, together with three additional South Asian species, they comprise two of four cichlid subfamilies, Ptychochrominae (five genera; *Katria*, *Oxylapia*, *Paratilapia*, *Ptychochromis*, and *Ptychochromoides*) and Etroplinae (three genera; *Etroplus*, *Paretroplus*, and *Pseudetroplus*) (Fig. 2). While the age of these subfamilies has been debated, studies agree that Ptychochrominae is sister to the mainland African and Neotropical cichlids (Pseucochrenilabrinae and Cichlinae, respectively) and Etroplinae is sister to the clade containing Ptychochrominae plus the African and Neotropical cichlids (Sparks and Smith 2004; Friedman et al. 2013; McMahan et al. 2013).

In contrast to the more often studied cichlid radiations from mainland Africa, many of which diversified within large lake systems, most Malagasy cichlids have highly restricted and isolated distributions, occurring within a single river drainage or lake (Sparks 2008). There are only three species that are known to have more expansive ranges within Madagascar, *Paratilapia polleni*, *Paretroplus polyactis*, and *Ptychochromis grandidieri* (Stiassny and Gerstner 1992; Sparks 2008). In addition, dietary information is largely restricted to data collected from type series and species' descriptions. Most species appear to be generalist feeders, with common prey including small gastropods and bivalves, fibrous
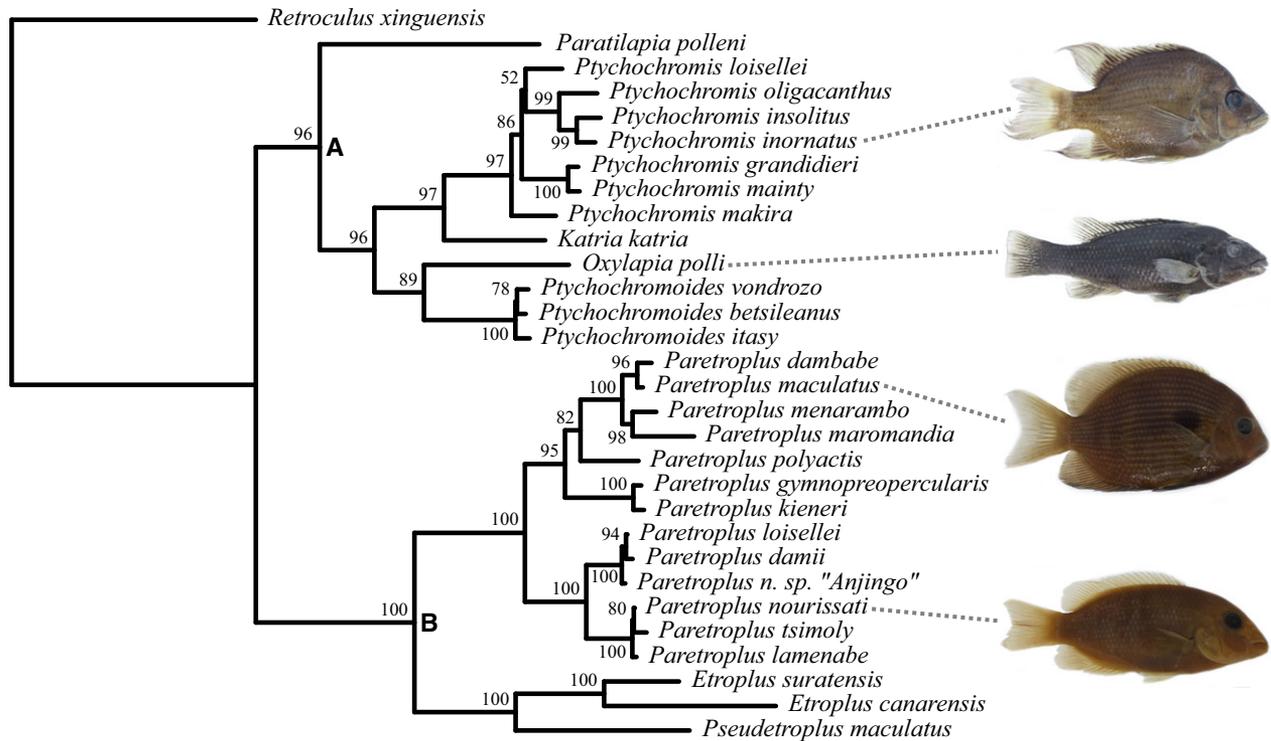
Figure 2. Maximum likelihood (ML) tree of ptychochromine (clade A) and etropline (clade B) cichlids, with ML bootstrap support values at nodes. Images of select species are included to illustrate similar morphological variation from shallow to deep-bodied forms in each subfamily.

plant material, algae, and insects (Sparks 2008; personal observations of authors). *Paratilapia polleni* is the sole species that regularly eats larger, mobile prey, such as amphibians and other fishes (Stiassny and Gerstner 1992).

The overall objective of this research was to study patterns of evolutionary covariation between body and jaw morphologies in two independent radiations of Malagasy cichlid. To do this, we first evaluated body shape diversity, rates of evolution, and associations with habitat. Next, we compared oral jaw fourbar morphologies and their associated mechanical outputs between subfamilies. Finally, we assessed the degree of body and jaw shape covariation in the two subfamilies (integration), as well as the relative independence of cranial and postcranial regions of the body plan (modularity). We predicted that the nature of oral jaw variation, its form-function landscape, and its relationship with body shape would be consistent with the expectations of systems with MTOM, outlined above.

Materials and Methods
SPECIMEN ACQUISITION AND IMAGING
For this research, we examined specimen collections from the American Museum of Natural History (AMNH), the University of Michigan Museum of Zoology (UMMZ), and other natural history collections. Institutional abbreviations used in this manuscript fol-

low Sabaj Pérez (2014). To analyze data in a comparative framework, we only included species of Malagasy cichlid for which we had molecular data and that could be placed in a phylogenetic context. In all, we evaluated the morphologies of 26 species, including 25 of 29 valid species and one additional new species belonging to the genus *Paretroplus*. For body shape analyses, we photographed 213 specimens in lateral view on the side that yielded the best morphological representation of overall body shape (Table S1). For comparisons of oral jaw skeletal morphologies, we used 163 specimens that were either radiographed or cleared and stained (CS) and photographed (Table S2). Often, the same specimen was used for external and internal morphometrics, but this was not always possible (e.g., not all external body features could be identified in CS specimens).

PHYLOGENETIC ANALYSES
Phylogenetic relationships were inferred for the cichlid subfamilies Ptychochrominae and Etroplinae, with *Retroculus xinguensis* designated as the outgroup. We used four nuclear and mitochondrial genes (16S, CO1, H3, and Tmo-4C4) that were sequenced and published in previous work (Sparks 2004; Sparks and Smith 2004; Stiassny and Sparks 2006; and Martinez et al. 2015). For full details on procedures for alignment and phylogenetic inference used in this study, refer to Martinez et al. (2015). Briefly, we used to RAxML version 8 (Stamatakis 2014) to reconstruct
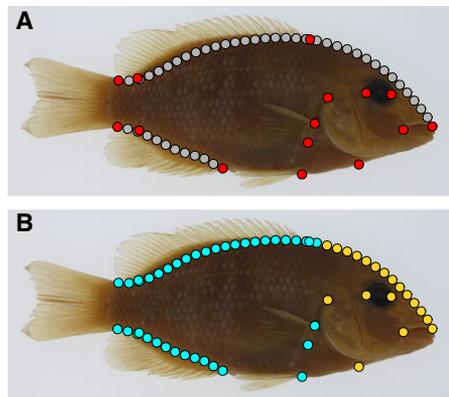
Figure 3. (A) Landmarks used to evaluate cichlid body shape with geometric morphometrics. Fixed landmarks are shown in red and sliding semilandmarks in gray. (B) Partitioning of landmarks used for analysis of phylogenetic modularity, with cranial landmarks in yellow and postcranial in light blue. Specimen pictured: *Paretroplus nourissati* (AMNH 229555).

maximum likelihood (ML) trees based on a concatenated alignment of the above sequences, with the GTRGAMMA model under full ML optimization. Node support was based on 1000 bootstrap searches.

BODY SHAPE

Variation of body forms was evaluated with geometric morphometrics, a landmark-based method for shape comparisons. Body shape was defined by 15 fixed landmarks and 43 sliding semilandmarks (Fig. 3A), which were digitized in TpsDig2 (Rohlf 2015). Fixed landmarks were placed on morphological features that could be identified to a single homologous point across specimens (Bookstein 1991), while sliding semilandmarks were used to define curves along body margins (Gunz and Mitteroecker 2013). Unless otherwise specified, all analyses following digitization were done in the *geomorph* package (Adams and Otarola-Castillo 2013) in R (R Core Team 2013), using average shapes of species. *P*-values, whenever reported, were based on 10,000-iteration permutation tests. Comparative analyses assumed a Brownian motion model of trait evolution.

To visualize body shape variation, we plotted specimens in phylomorphospace, simultaneously displaying morphological diversity and phylogenetic relationships. We used phylogenetic Procrustes ANOVA to test whether body shape differed between subfamilies. Morphological disparity (= shape diversity) was then compared between subfamilies as the Procrustes variance in body shapes. We tested for phylogenetic signal in body shape across all Malagasy cichlids and within each subfamily, based on a multivariate *K* statistic (Adams 2014). Significance for phylogenetic signal was assessed by comparison of the observed value of *K* to a null distribution of *K*s, generated from permutation tests. In

addition, we compared rates of morphological evolution among cichlids grouped by subfamily. Lastly, we used phylogenetic Procrustes ANOVA to determine if body shape differed between broad habitat categories, which were assigned as lake, river, or both (i.e., lake and river). Habitat types came from primary literature, IUCN documents (Available at http://www.iucnredlist.org), and personal observations of author JSS (Table S3). For this analysis, we only performed a combined test containing all species, as sample sizes were too small within individual subfamilies to subdivide among three habitat types. Additionally, we removed the recently described species, *Ptychochromis mainty*, because habitat information was not known (Martinez et al. 2015).

ORAL JAW SHAPE

We used a fourbar linkage model to compare the overall form of oral jaws (Fig. 1). Morphological features used to construct the fourbar linkage vary slightly from study to study. We used a common arrangement with vertices at the center of the quadrate-articular joint, the ligamentous connection of the maxilla to the dorsal process of the dentary, the dorsal head of the maxilla at its ligamentous connection to the nasal, and the posteriordorsal extent of the nasal at its ligamentous connection to the neurocranium (Westneat 2006). Sometimes the latter two points are defined at the extremes of the palatine, along its anteroposterior axis. However, we did not use this method as the palatine of Malagasy cichlids is either fused to (*Katria*, *Paratilapia*, *Ptychochromis*, *Ptychochromoides*) or is broadly connected to (*Paretroplus* and *Oxylapia*) the ectopterygoid and endopterygoid, reducing or eliminating independent mobility relative to the suspensorium. Manual opening and closing of jaws in CS specimens verified that the nasal did, in fact, rotate and that there was minimal mobility of the palatine. The same linkage was used in previous studies on new world cichlids, where species exhibit similar morphological conditions (Hulsey and García De León 2005; Parnell et al. 2008). Consequently, we did not include the South Asian etroplines (Genera, *Etroplus* and *Pseudetroplus*) in this study as their jaws clearly possessed highly mobile palatines, suggesting the alternative fourbar model was more appropriate for them.

We measured the lengths of links from digital photographs (for CS) and x-rays of specimens carefully positioned in lateral view. To standardize their shapes, we divided fourbars into two triangles and used trigonometry to recreate fourbar shapes at 15 degrees between the fixed suspensorial link and lower jaw link (Fig. 1). Vertices were used as type I, fixed landmarks in geometric morphometrics. Fourbar shapes were averaged by species and visualized in a phylomorphospace that was overlaid on a contour plot of the mechanical trait, MKT (additional details below). To obtain MKT values for the contour plot, fourbar shapes were estimated at regular intervals across morphospace (Fig. S1) in TpsRelw (Rohlf 2015). As we did for body shape

analyses, we tested for differences in jaw shapes between subfamilies, compared morphological disparities among subfamilies, assessed phylogenetic signal, and compared rates of morphological evolution.

ORAL JAW MECHANICS

Jaw mechanics were directly measured from fourbar configurations as MKT, which is the ratio of output rotation of the maxilla to input rotation from the lower jaw. We used the linkR package (Olsen 2016) in R to calculate MKT values from species' fourbar shapes. Specifically, we applied 30 degrees of input rotation and measured the ratio of maxillary to lower jaw rotation over 100 equal intervals, then averaged these values for MKT. We used phylogenetic generalized least squares (PGLS) regression to test for relationships between jaw (fourbar) shape and function (MKT). Again, we tested for phylogenetic signal and compared rates of evolution between subfamilies.

RELATIONSHIPS BETWEEN BODY AND JAW SHAPES

We evaluated relationships between body and jaw shapes for all species combined and also within each subfamily with phylogenetic two-block partial least squares (2B-PLS) analysis. This tests for covariation between two sets or blocks of data (Rohlf and Corti 2000). To assess whether relationships differed between subfamilies, we also compared effect sizes from the separate 2B-PLS tests. In addition, to determine whether relationships between body and jaw shapes could be explained by allometry, rather than actual covariation of jaw and body shapes, we performed an additional set of tests; here, we calculated residuals from PGLS regressions of jaw and body shapes on the natural logarithm of body centroid size, and treated them as allometry-adjusted shape data (e.g., McCord and Westneat 2016). We used body centroid size as the independent variable for both regressions to generate shape data that were adjusted by a common factor that represented overall organismal size. We then tested for covariation between adjusted jaw and body shapes with 2B-PLS.

As a complement to the comparisons of body and jaw shapes, we also tested for modularity between cranial and postcranial body regions, within a comparative framework (Fig. 3B). The degree of modularity, or independence of modules, was based on the covariance ratio (CR) of Adams (2016), which compares within and among-module covariance. Significance was based on comparison of the observed CR to a distribution of CRs generated by randomizing landmark placement into each module.

Results

PHYLOGENETIC ANALYSES

Our ML reconstruction strongly supported monophyly of etropline and ptychochromine subfamilies (Fig. 2), with a tree topol-
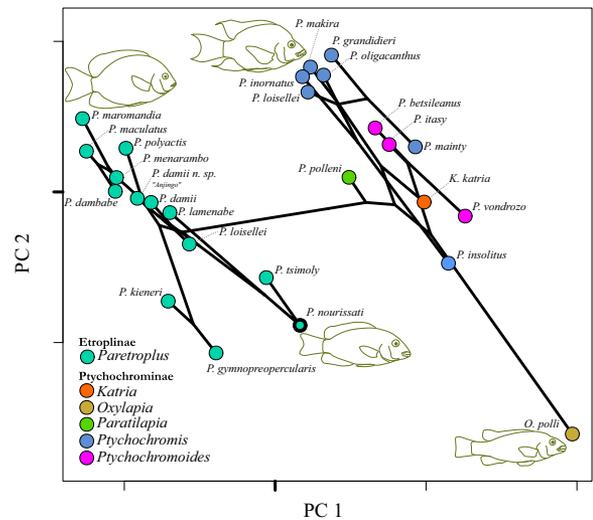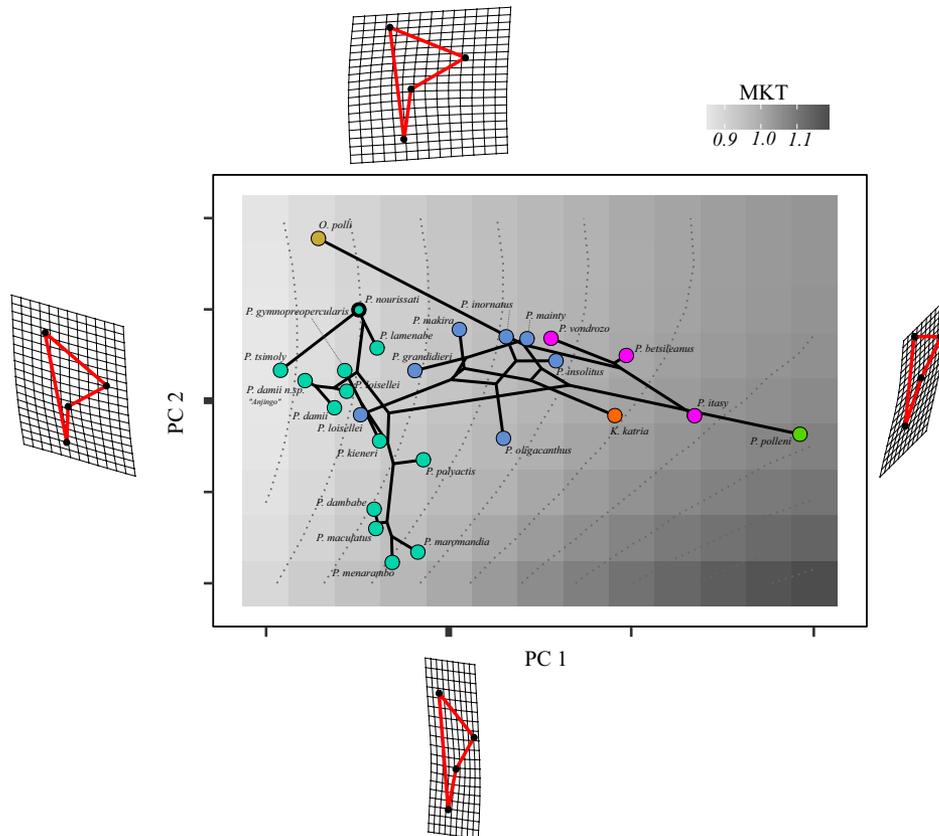

Figure 4. Principle components (PCs) 1 and 2 of body phylomorphospace in Malagasy cichlids. Shapes of representative species are shown for reference. PCs shown account for 83.3% of total body shape variation.

ogy that was largely consistent with that of previous work (Sparks and Smith 2004; Stiassny and Sparks 2006; Sparks and Schelly 2011; Martinez et al. 2015).

BODY SHAPE

Body shapes of cichlid subfamilies were well differentiated with respect to the first two principle components (PCs) of phylomorphospace, which accounted for 83.3% of overall shape variation (Fig. 4). Although morphologies were significantly different ($F_{1,24} = 0.20, P = 0.0001$), the directions of variation through shape space were largely parallel in Etroplinae and Ptychochrominae. In each subfamily, deep-bodied species occupied one end of a morphological trajectory, including the etroplines, *Paretroplus maromandia*, *P. polyactis*, and *Paretroplus maculatus* and several of the ptychochromine species from the genus *Ptychochromis*, such as *P. grandidieri* and *Ptychochromis makira*. On the opposite ends of body shape trajectories were the more shallow-bodied etropline species *Paretroplus nourissati* and *Paretroplus gymnopreopercularis* and the ptychochromines, *Ptychochromoides vondrozo*, *Ptychochromis insolitus* (although this species is based on a single subadult specimen), and *Oxylapia polli*. While morphologically extreme, *O. polli* followed the general trajectory for its subfamily, with a highly streamlined/shallow body shape that is consistent with its characterization as a rheophilic habitat specialist (Loiselle 2004). Within the etroplines, the most deep-bodied species came from a single clade and shallow-bodied species were from two clades that, besides being similarly shallow, occupied separate locations in morphospace (Fig. 4).

Despite having an apparent morphological outlier in *O. polli*, body shape disparity in the ptychochromines was not statistically

Figure 5. Principle components (PCs) 1 and 2 of oral jaw fourbar phylomorphospace in Malagasy cichlids plotted simultaneously with contours of the mechanical trait, maxillary kinematic transmission (MKT). Jaw shape variation on corresponding PCs shown as warp grids. PCs shown account for 94.1% of total shape variation.

greater than in the etroplines (variance = 0.0029 and 0.0015, respectively; $P = 0.25$). There was significant phylogenetic signal in body shape for all cichlids combined ($K = 0.23$, $P = 0.0001$) and for Etroplinae ($K = 0.20$, $P = 0.049$), but not for Ptychochrominae ($K = 0.30$, $P = 0.11$). Additionally, the rate of body shape evolution (σ^2) was 0.0020 in Etroplinae and 0.0013 in Ptychochrominae, which was not statistically different ($P = 0.17$). A phylogenetic Procrustes ANOVA revealed that there were no statistically significant differences in body shapes between habitat types ($F_{2,22} = 1.55$, $P = 0.068$).

ORAL JAW SHAPE

Where Malagasy subfamilies displayed body variation that was parallel in shape space, their oral jaw fourbar shapes were roughly orthogonal to each other (Fig. 5). Despite a small amount of overlap in jaw shapes, subfamilies had significantly different morphologies ($F_{1,24} = 0.48$, $P = 0.0001$). The first two principle components of phylomorphospace (PCs 1 and 2) accounted for 94.1% of total shape variance. PC1 was characterized by nearly uniform shearing of jaw shapes (warp grids in Fig. 5) that impacted the relative length of the nasal link, the angle of the nasal link, and the angle of the maxillary link. All etroplines were concen-

trated at lower PC1 values, with more sharply angled nasal links relative to the suspensorium and also a shallowing of the maxilla toward the horizontal. The ptychochromines displayed considerable variation on PC1 that ranged from *Ptychochromis loisellei* and *O. polli*, which were more similar in overall jaw shape to the etropline form, to *P. polleni*, which had a short nasal link that was deflected upward and a maxilla that was rotated toward the vertical. Variation on PC2, rather than a shear, was defined primarily by compression and expansion of the jaw (warp grids in Fig. 5). Most of the variation in etroplines occurred with respect to PC2, ranging from short jaws with a dorsoventrally expanded suspensorium to longer jaws with a dorsoventrally compressed suspensorium.

Shape disparity of jaws was roughly two and a half times larger in Ptychochrominae (0.0063) than in Etroplinae (0.0024), but a permutation test indicated that the difference was not statistically significant ($P = 0.074$). Given that the P-value was not much greater than 0.05, it is conceivable that small sample sizes led to a lack statistical power to distinguish shape disparity between subfamilies. Phylogenetic signal of jaw shape was significant for all species ($K = 0.45$, $P = 0.0001$), for Etroplinae ($K = 0.45$, $P = 0.0001$) and for Ptychochrominae ($K = 0.50$,
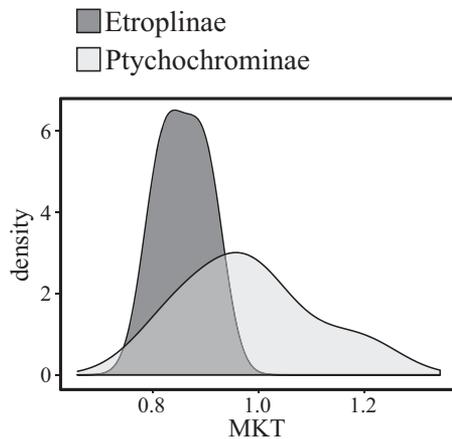
Figure 6. Comparisons of maxillary kinematic transmission (MKT) from oral jaw fourbar linkages in cichlid subfamilies.
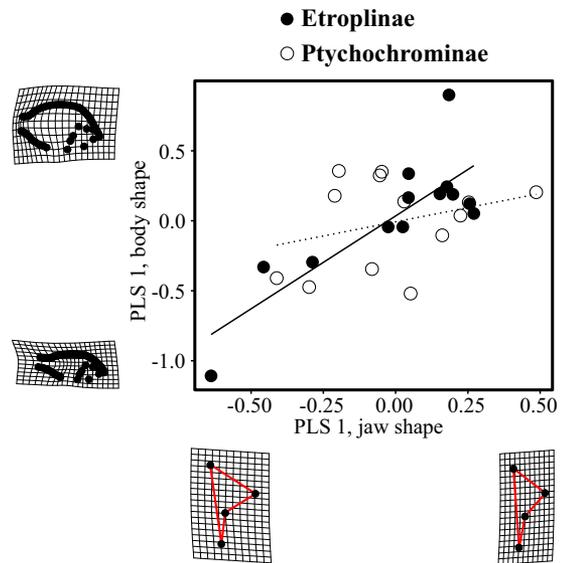

Figure 7. Axes of maximum covariation between body and oral jaw fourbar shapes from a two-block partial least-squares (2B-PLS) analysis of all species of Malagasy cichlid from this study. Shape variation is shown with warp grids. For visualization purposes only, etroplines are represented as closed circles and a solid regression line, and ptychochromines as open circles with a dotted regression line.

$P = 0.0054$). Finally, rates of shape evolution were similar for both subfamilies ($\sigma^2_{Etroplinae} = 0.022$; $\sigma^2_{Ptychochrominae} = 0.029$; $P = 0.35$).
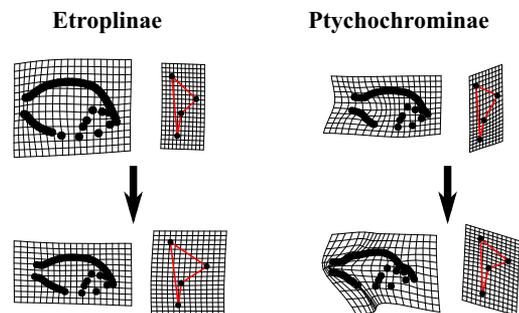
ORAL JAW MECHANICS

MKT of oral jaw fourbar linkages was approximately 8.5 times more variable in the subfamily Ptychochrominae than in Etroplinae (variance = 0.015 and 0.0017, respectively) (Fig. 6). In addition, subfamilies had significantly different MKT values ($F_{1,24} = 0.20$, $P = 0.0024$). When assessed over all species of Malagasy cichlid, MKT displayed statistically significant phylogenetic signal ($K = 0.24$, $P = 0.0045$), but this was not the case for individual subfamilies ($K_{Etroplinae} = 0.085$, $P = 0.79$; $K_{Ptychochrominae} = 0.38$, $P = 0.072$). This suggests that the significant signal observed for all species may have been partially driven by the phylogenetic difference between subfamilies. However, the lack of significance in Ptychochrominae could be related to low statistical power. Rates of evolution of MKT were similar between subfamilies ($\sigma^2_{Etroplinae} = 0.55$; $\sigma^2_{Ptychochrominae} = 0.59$; $P = 0.90$). Lastly, PGLS regression of fourbar shapes on MKT for all species was not statistically significant ($F_{1,24} = 5.35$, $P = 0.11$), with function accounting for 18% of variance in form. Results for individual subfamilies were also nonsignificant (Ptychochrominae, $F_{1,11} = 2.49$, $P = 0.24$; Etroplinae, $F_{1,11} = 3.23$, $P = 0.32$).

RELATIONSHIPS BETWEEN BODY AND JAW SHAPES

Across all species, there was a significant relationship between body and jaw shapes ($rPLS = 0.62$, $P = 0.0078$). The resulting PLS plot from this analysis confirmed that variation from deep to shallow-bodied forms was related to changes in jaw shape (warp grids in Fig. 7). However, it was clear that this particular pattern of body shape variation had a much larger impact on

Figure 8. Body shapes and associated jaw shapes from separate two-block partial least-squares (2B-PLS) analyses in Etroplinae and Ptychochrominae. Only the analysis in Etroplinae was statistically significant.

etropline jaw shapes than it did in the ptychochromines (regression lines in Fig. 7). Separate 2B-PLS analyses in each subfamily confirmed this, with a strong and statistically significant relationship between body and jaw shapes in Etroplinae ($rPLS = 0.87$, $P = 0.0039$). Here, deep-bodied species had relatively shorter jaws and shallow-bodied species had longer jaws (Fig. 8). This relationship was preserved for allometry-adjusted shape data ($rPLS = 0.86$, $P = 0.0048$). In the ptychochromines, there was a moderate PLS correlation, but it was not statistically significant ($rPLS = 0.61$, $P = 0.14$). Notably, in this subfamily, the largest axes of covariation between body and jaw shapes did not involve overall deepening and shallowing of body forms

(Fig. 8). Although allometry-adjusted shape data resulted in a slightly stronger correlation, the relationship was not significant (rPLS = 0.69, P = 0.10). Finally, the effect size from the 2B-PLS analysis was 2.56 in the etroplines and 1.11 in the ptychochromines, but the difference was not statistically significant (P = 0.087). This means that although the manner by which body shape impacted jaw shape was different in each subfamily, the absolute magnitude of the its effect did not differ statistically between them.

Comparison of cranial and postcranial body regions suggested that there was significant phylogenetic modularity in the ptychochromines (CR = 0.987, P = 0.0010), agreeing with the previous result of a nonsignificant relationship between body and jaw shapes. In the etroplines, however, there was no evidence of modularity (CR = 1.028, P = 0.99), which makes sense, as body shape variation was strongly associated with changes in the shapes of jaws in this subfamily. Observed CR values in both subfamilies, while quite similar, differed in their significance. The reason for this is likely due to the null distributions, generated from randomized permutations, having narrow ranges, such that small differences in the absolute value of CR could differentiate statistically significant modularity from not significant (Fig. S2).

Discussion

Our results showed that body shape variation in Etroplinae and Ptychochrominae was strikingly similar and was constrained along a common morphological axis, from shallow to deep-bodied forms (Fig. 4). While we did not recover a significant relationship between body shape and habitat, the observed range clearly resulted in hydrodynamically different bodies. Despite similar body shape trajectories, the two subfamilies of Malagasy cichlid displayed disparate patterns of body, head, and jaw covariation. In the ptychochromines, there was modularity between cranial and postcranial body modules, a nonsignificant relationship between body and jaw shapes, and large variance in the oral jaw mechanical measure, MKT. The result was that body shape variation had a disproportionately small impact on head and jaw shape. In contrast, etropline cichlids were characterized by an absence of modularity between cranial and postcranial morphologies, strong and significant covariation between body shape and jaw shape, and relatively small variance in MKT. Here, body shape evolution was accompanied by a concomitant change in jaw shape. These patterns point to contrasting strategies for accommodating body shape evolution while reducing impacts on jaw mechanics (head-body modularity in ptychochromines and stability of MKT in etropline jaws, via MTOM). Other studies have similarly shown disparate patterns of integration in related species (Monteiro and Nogueira 2009; Parsons et al. 2012). We suggest that integration in the etropline body plan presents a constraint on body shape evolution.

If, for instance, an etropline evolved an extreme shallow-bodied morphology, similar to the ptychochromine *O. polli* (Fig. 4), the correlated transformation of jaw form would very likely result in a mechanically different structure or render it nonfunctional altogether. Indeed, the divergent strategies observed here must each have their own sets of trade-offs, with benefits and limitations that are unique to the individual system.

INFLUENCE OF THE FORM-FUNCTION MAP

The above patterns highlight the potential of MTOM to influence trait correlations and overall morphological diversity within clades. The oral jaw fourbar linkage and its mechanical output, MKT, met our expectations for systems with MTOM. First, mapping of MKT across the fourbar morphospace confirms that there were regions over which jaw mechanics were invariable. Notably, these regions of equivalent MKT were not randomly distributed across morphospace, but occurred as continuous bands (contour lines in Fig. 5). In fact, Etropline jaw diversity was constrained within a narrow band of MKT, minimizing functional variation relative to the ptychochromines (Fig. 6). Consequently, in the etroplines, an incremental exploration of mechanically similar jaw shapes resulted in species occupying a trajectory through morphospace that loosely tracked the directionality of contours of the fourbar form-function landscape (Fig. 5). This pattern of maintaining a narrow range of MKT, resulted in fourbar morphologies of etropline species being much more similar to their immediate ancestors, compared to that of the ptychochromines. Second, regressions of fourbar shapes on MKT were relatively weak and not statistically significant, reflecting that species' jaws displayed a level of variance relative to MKT. Third, the ability to diversify with respect to jaw morphology, but maintain stability of MKT, resulted in an expected mismatch in the relative accumulation of morphological versus functional diversity, particularly in Etroplinae. This is evidenced by the observation that etropline jaw shape diversity was only two and a half times smaller than that of the ptychochromines, but its diversity of MKT was eight and a half times smaller. Lastly, the distribution of the form-function landscape allowed etroplines to achieve secondary function of the oral jaws (i.e., accommodation of changing body shape), while minimizing mechanical change. This demonstrated that it is possible for traits to be, at the same time, highly integrated and also have minimal impacts on a functional property of the other.

The capacity of MTOM systems to facilitate novelty or reduce morphological constraints has been proposed (Collar and Wainwright 2006; Vanhooydonck et al. 2006; Wainwright 2007) and tested with simulations (Alfaro et al. 2005; Parnell et al. 2008). In these studies, the concentration was primarily on the ability of MTOM systems to loosen endogenous constraints, and not on their influence on patterns of evolution for other traits that they are correlated with. However, while examples outside of this study

are exceedingly rare, there is empirical evidence that MTOM can play an important role in the evolution of related traits (Anderson et al. 2014). Given the ubiquity of morphological integration at multiple levels of body plan organization, we believe that this has profound implications for our understanding of trait diversification. It provides an additional pathway, separate from modularity, by which organisms may effectively compartmentalize functional systems.

The nonuniform distribution of MKT across morphospace also results in an additional feature of the form-function landscape that can influence evolutionary dynamics. Previous research on fourbar linkages has shown that mechanical sensitivity, or the amount of mechanical change per unit morphological change, varies with shape (Alfaro et al. 2005; Anderson and Patek 2015). For example, in Figure 5, variation in MKT along PC1 (i.e., left to right, across the plot) was 1.6 times greater at the smallest values of PC2 compared to the largest. This is demonstrated by more closely set functional contours at the bottom of the plot than the top. Under stabilizing selection for current jaw function, this pattern suggests a potential constraint in etropline species such as *P. maculatus*, *P. menarambo*, and *P. maromandia*, as they must stay within a relatively small morphological range to maintain functionality. However, selection for higher MKT would be achieved with smaller morphological transformation in these species than in other etroplines. In the ptychochromines, most jaw shapes occur in an area that is less sensitive to mechanical changes (larger PC2 values in Fig. 5), but the overall variation of the subfamily is in a direction that maximizes functional variation (roughly 90 degrees relative to MKT contours). This occupation of a region of morphospace with relatively low mechanical sensitivity to fourbar shape change may further explain why morphological distances between ptychochromine species and their immediate ancestors are greater than that of etroplines.

THE FOURBAR LINKAGE AS A MODEL FOR JAW FUNCTION

The research presented here relies on the oral jaw fourbar linkage being an accurate model for anterior jaw kinesis in cichlid fishes. Like any model, the fourbar is an abstraction from the actual system, but there is reason to believe that it captures important information that is relevant to functional and ecological diversity of teleost fishes (e.g. Westneat 1995; Hulsey and García De León 2005; Westneat et al. 2005). It is important to state that just because MKT can remain stable while fourbar morphology varies, does not mean that we interpret the functionality of the entire feeding apparatus as equivalent. Rather, it simply denotes that there is similarity in the mechanics and efficiency of the oral jaw, such that a given amount of input rotation to the system will result in a similar degree of output kinesis.

Beyond the oral jaw fourbar linkage, other components of the feeding apparatus are also important for prey capture and processing. One factor that a planar fourbar configuration does not capture is lateral movement of a fish's jaw. While lateral expansion of the posterior oral-branchial chamber is important for the generation of suction, the anterior jaw is largely constrained in fishes such as cichlids that have laterally compressed body shapes. In addition, we did not evaluate the role of pharyngeal jaws (secondary, posterior jaws formed by medial fusion of gill arch elements) in this study. However, most Malagasy cichlids have largely similar pharyngeal jaws, with distinctive, robust molariform dentition used to crush captured prey (personal observations of authors). There were two exceptions in *P. polleni*, and to a lesser extent *Katria katria*, which had less robust teeth and a more cusped, grasping morphology. Even so, there was not marked difference between subfamilies to suggest that different covariation patterns were mediated by a structural or functional difference in pharyngeal jaws.

Conclusions and Future Research

Our results have important consequences for the way we think about the evolution of correlated traits. Unless a relationship simultaneously satisfies selective pressures in each trait involved, trade-offs will constrain the degree and direction that they may vary. MTOM presents a mechanism for easing functional constraints without evolving morphological independence of traits (modularity). Similarly, a strong relationship between traits (integration) is not always diagnostic of functional dependence. Our research lends credence to the ability of MTOM to loosen trade-offs and influence the evolution of body plans. Given that MTOM is a feature of complex morphological systems and that it can result in a mismatch in the diversity of form versus function within clades, it can be difficult to identify in natural systems and is probably highly underreported. Wainwright et al. (2005) and later Wainwright (2007) suggested that MTOM is likely common at many levels of organization and may be an important factor regulating diversity of functional systems.

An immediate focus for future research is whether patterns observed in this study are seen in other groups. One natural contrast to Malagasy cichlids is the East African rift lake cichlids, where species clearly display patterns of morphological and functional diversification along trophic axes (e.g., McGee et al. 2016). It will also be important to study integration and modularity in different contexts and at multiple scales, such as developmental or genetic levels, to understand how they influence the expression of complex morphologies. Even the mechanically simple cichlid mandible is differentially regulated by genetic integration across the structure (Albertson et al. 2005). How then do molecules map to individual skeletal elements that, in combination, are capable of achieving multiple, functionally equivalent forms? Overall, it

is clear that we are just starting to understand the role and pervasiveness of MTOM in evolutionary dynamics. While the diverse teleost feeding apparatus is particularly tractable to this area of research, further exploration in other organisms and functional systems will be necessary to truly advance our knowledge of MTOM and its impact on trait covariation and evolution.

DATA ARCHIVING
Data associated with this manuscript can be found at the following https://doi.org/10.5061/dryad.k3b10.

LITERATURE CITED

Adams, D. C. 2014. A generalized K statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. Syst. Biol. 63:685–697.

———. 2016. Evaluating modularity in morphometric data: challenges with the RV coefficient and a new test measure. Methods Ecol. Evol. 7:565–572.

Adams, D. C., and E. Otarola-Castillo. 2013. Geomorph: an R package for the collection and analysis of geometric morphometric shape data. Methods Ecol. Evol. 4:393–399.

Aguilar-Medrano, R., B. Frédérich, and P. H. Barber. 2016. Modular diversification of the locomotor system in damselfishes (Pomacentridae). J. Morphol. 277:603–614.

Albertson, R. C., J. T. Streelman, T. D. Kocher, and P. C. Yelick. 2005. Integration and evolution if the cichlid mandible: the molecular basis of alternate feeding strategies. Proc. Natl. Acad. Sci. U S A 102:16287–16292.

Alfaro, M. E., D. I. Bolnick, and P. C. Wainwright 2004. Evolutionary dynamics of complex biomechanical systems: an example using the four-bar mechanism. Evolution 58:495–503.

———. 2005. Evolutionary consequences of many-to-one mapping of jaw morphology to mechanics in labrid fishes. Am. Nat. 165:E140–E154.

Anderson, P. S. L., and S. N. Patek 2015. Mechanical sensitivity reveals evolutionary dynamics of mechanical systems. Proc. R. Soc. B. 282:20143088.

Anderson, P. S. L., T. Claverie, and S. N. Patek 2014. Levers and linkages: mechanical trade-offs in a power-amplified system. Evolution 68:1919–1933.

Bookstein, F. L. 1991. Morphometric tools for landmark data: geometry and biology. Cambridge Univ. Press, New York, NY.

Clabaut, C., P. M. E. Bunje, W. Salzburger, and A. Meyer 2007. Geometric morphometric analyses provide evidence for the adaptive character of the Tanganyikan cichlid fish radiations. Evolution 61:560–578.

Claverie, T., and S. N. Patek 2013. Modularity and rates of evolutionary change in a power-amplified prey capture system. Evolution 67:3191–3207.

Collar, D. C., and P. C. Wainwright 2006. Discordance between morphological and mechanical diversity in the feeding mechanism of centrarchid fishes. Evolution 60:2575–2584.

Eschmeyer, W. N., R. Fricke, and R. van der Laan eds. 2016. Catalog of Fishes: Genera, Species, References. Available at http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp. Electronic version accessed September 28, 2016.

Friedman, M., B. P. Keck, A. Dornburg, R. I. Eytan, C. H. Martin, C. D. Hulsey, and P. C. Wainwright 2013. Molecular and fossil evidence place the origin of cichlid fishes long after Gondwanan rifting. Proc. R. Soc. B. 280. 20131733.

Goswami, A., and P. D. Polly 2010. The influence of modularity on cranial morphological disparity in Carnivora and Primates (Mammalia). PLoS One 3:e9517.

Gunz, P., and P. Mitteroecker 2013. Semilandmarks: a method for quantifying curves and surfaces. Histrix 24:103–109.

Hulsey, C. D., and F. J. García De León 2005. Cichlid jaw mechanics: linking morphology to feeding specialization. Funct. Ecol. 19:487–494.

Kassam, D. D., D. C. Adams, A. J. D. Ambali, and K. Yamaoka 2003. Body shape variation in relation to resource partitioning within cichlid trophic guilds coexisting along the rocky shore of Lake Malawi. Anim. Biol. 53:59–70.

Klingenberg, C. P. 2008. Morphological integration and developmental modularity. Annu. Rev. Ecol. Evol. Syst. 39:115–132.

———. 2014. Studying morphological integration and modularity at multiple levels: concepts and analysis. Phil. Trans. R. Soc. B. 369: 20130249.

Loiselle, P., and participants of the CBSG/ANGAP CAMP "Faune de Madagascar" workshop, Mantasoa, Madagascar 2001. 2004. Oxylapia polli. IUCN Red List of Threatened Species 2004:e.T15778A5145416. Available at https://doi.org/10.2305/IUCN.UK.2004.RLTS.T15778A5145416.en.

Martinez, C. M., J. Arroyave, and J. S. Sparks 2015. A new species of Ptychochromis from southeastern Madagascar (Teleostei: Cichlidae). Zootaxa 4044:79–92.

McCord, C. L., and M. W. Westneat 2016. Evolutionary patterns of shape and functional diversification in the skull and jaw musculature of triggerfishes (Teleostei: Balistidae). J. Morphol. 277:737–752.

McGee, M. D., B. C. Faircloth, S. R. Borstein, J. Zheng, C. D. Hulsey, P. C. Wainwright, and M. E. Alfaro 2016. Replicated divergence in cichlid radiations mirrors a major vertebrate innovation. Proc. R. Soc. B. 283:20151413.

McMahan, C. D., P. Chakrabarty, J. S. Sparks, W. L. Smith, and M. P. Davis. 2013. Temporal patterns of diversification across global cichlid biodiversity (Acanthomorpha: Cichlidae). PLoS One 8:e71162.

Monteiro, L. R., and M. R. Nogueira 2009. Adaptive radiations, ecological specialization, and the evolutionary integration of complex morphological structures. Evolution 64:724–744.

Muschick, M., A. Indermaur, and W. Salzburger 2012. Convergent evolution within an adaptive radiation of cichlid fishes. Curr. Biol. 22:2362–2368.

Olsen, A. M. 2016. linkR: 3D lever and linkage mechanism modeling. Version 1.1.1. Available at https://CRAN.R-project.org/package=linkR.

Parnell, N. F., C. D. Hulsey, and J. T. Streelman 2008. Hybridization produces novelty when the mapping of form to function is many to one. BMC Evol. Biol. 8:122.

Parsons, K. J., E. Márquez, and R. C. Albertson 2012. Constraint and opportunity: the genetic basis and evolution of modularity in the cichlid mandible. Am. Nat. 179:64–78.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org/.

Rohlf, F. J. 2015. The tps series of software. Histrix 26:9–12.

Rohlf, F. J., and M. Corti 2000. Use of two-block partial least-squares to study covariation in shape. Syst. Biol. 49:740–753.

Rüber, L., and D. C. Adams 2001. Evolutionary convergence of body shape and trophic morphology in cichlids form Lake Tanganyika. J. Evol. Biol. 14:325–332.

Sabaj Pérez, M. H. (Ed.). 2014. Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an online reference, version 5.0 (22 September 2014). American Society of Ichthyologists and Herpetologists, Washington, D.C. Available at http://www.asih.org/resources.

Sallan, L. C., and M. Friedman 2011. Heads or tails: staged diversification in vertebrate evolutionary radiations. Proc. R. Soc. B. 20112454.

Sanger, T. J., D. L. Mahler, A. Abzhanov, and J. B. Losos 2011. Roles for modularity and constraint in the evolution of cranial diversity among *Anolis* lizards. Evolution 66:1525–1542.

Sparks, J. S. 2004. Molecular phylogeny and biogeography of the Malagasy and South Asian cichlids (Teleostei: Perciformes: Cichlidae). Mol. Phylogenet. Evol. 30:599–614.

———. 2008. Etroplinae and taxonomic revision of the Malagasy cichlid genus *Paretroplus* (Teleostei: Cichlidae). B. Am. Mus. Nat. Hist. 314:1–151.

Sparks, J. S., and R. C. Schelly 2011. A new species of *Paretroplus* (Teleostei: Cichlidae: Etroplinae) from northeastern Madagascar, with a phylogeny and revised diagnosis for the *P. damii* clade. Zootaxa 2768:55–68.

Sparks, J. S., and W. L. Smith 2004. Phylogeny and biogeography of cichlid fishes (Teleostei: Perciformes: Cichlidae). Cladistics 20:501–517.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. https://doi.org/10.1093/bioinformatics/btu033.

Stiassny, M. L. J., and C. L. Gerstner 1992. The parental care behaviour of *Paratilapia polleni* (Perciformes, Labroidei), a phylogenetically primitive cichlid from Madagascar, with a discussion of the evolution of maternal care in the family Cichlidae. Environ. Biol. Fish. 34:219–233.

Stiassny, M. L. J., and J. S. Sparks 2006. Phylogeny and taxonomic revision of the endemic Malagasy genus *Ptychochromis* (Teleostei: Cichlidae), with the description of five new species and a diagnosis for *Katria*, new genus. Am. Mus. Novit. 3535:1–55.

Streelman, J. T., and P. D. Danley 2003. The stages of vertebrate evolutionary radiation. Trends Ecol. Evol. 18:126–131.

Trapani, J. 2003. Geometric morphometric analysis of body-form variability in *Cichlasoma minckleyi*, the Cuatro Cienegas cichlid. Environ. Biol. Fish. 68:357–369.

Turner, G. F. 2007. Adaptive radiation of cichlid fish. Curr. Biol. 17:R827–R831.

Vanhooydonck, B., A. Herrel, R. Van Damme, and D. J. Irschick 2006. The quick and the fast: the evolution of acceleration capacity in *Anolis* lizards. Evolution 60:2137–2147.

Wainwright, P. C. 2007. Functional versus morphological diversity in macroevolution. Annu. Rev. Ecol. Evol. Syst. 38:381–401.

Wainwright, P. C., M. E. Alfaro, D. I. Bolnick, C. D. Hulsey 2005. Many-to-one mapping of form to function: a general principle in organismal design? Integr. Comp. Biol. 45:256–262.

Wainwright, P. C., D. R. Bellwood, M. W. Westneat, J. R. Grubich, A. S. Hoey 2004. Biol. J. Linnean Soc. 82:1–25.

Westneat, M. W. 1990. Feeding mechanics of teleost fishes (Labridae; Perciformes): a test of four-bar linkage models. J. Morphol. 205:269–295.

———. 1994. Transmission of force and velocity in the feeding mechanisms of labrid fishes. Zoomorph. 114:103–118.

———. 1995. Feeding, function, and phylogeny: analysis of historical biomechanics in labrid fishes using comparative methods. Syst. Biol. 44:361–383.

———. 2006. Skull biomechanics and suction feeding in fishes. Pp. 29–75 *in* Shadwick, R. E. and G. V. Lauder, eds. Fish biomechanics: fish physiology, Vol. 23. Elsevier Academic Press, San Diego, CA.

Westneat, M. W., M. E. Alfaro, P. C. Wainwright, D. R. Bellwood, J. R. Grubich, J. L. Fessler, K. D. Clements, and L. L. Smith 2005. Local phylogenetic divergence and global evolutionary convergence of skull function in reef fishes of the family Labridae. Proc. R. Soc. B. 272:993–1000.

Young, R. L., T. S. Haselkorn, and A. V. Badyaev 2007. Functional equivalence of morphologies enables morphological and ecological diversity. Evolution 61:2480–2492.

Zelditch, M. L. 1988. Ontogenetic variation in patterns of phenotypic integration in the laboratory rat. Evolution 42:28–41.

Zelditch, M. L., and A. C. Carmichael 1989. Ontogenetic variation in patterns of developmental and functional integration in skulls of Sigmodon fulviventer. Evolution 43:814–824.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Sampling regime for estimates of oral jaw fourbar shapes (purple points) for calculations of MKT (gray squares) across morphospace.
Figure S2. Results of phylogenetic modularity analyses in Etroplinae (top) and Ptychochrominae (bottom).
Table S1. Specimens used for body shape analyses and associated catalog numbers.
Table S2. Specimens used for jaw shape analyses and associated catalog numbers.
Table S3. Habitat information for Malagasy cichlids of subfamilies Etroplinae and Ptychochrominae.