

Macroevolutionary analyses indicate that repeated adaptive shifts towards predatory diets affect functional diversity in Neotropical cichlids

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During adaptive radiation, diversification within clades is limited by adaptation to the available ecological niches, and this may drive patterns of both trait and species diversity. However, adaptation to disparate niches may result in varied impacts on the timing, pattern and rate of morphological evolution. In this study, we examined the relationship between feeding ecology and functional diversification across a diverse clade of freshwater fishes, the Neotropical cichlids. Species dietary niches were ordinated via multivariate analysis of stomach content data. We investigated changes in the rate and pattern of morphological diversification associated with feeding, including dietary niche and degree of dietary specialization. A major division in dietary niche space was observed between predators that consume fish and macroinvertebrates vs. other groups with diets dominated by small invertebrates, detritus or vegetation. These trophic niches were strongly associated with groupings defined by functional morphospace. Clades within the piscivore/macroinvertevore group rarely transitioned to other dietary niches. Comparatively, high dietary specialization enhanced functional diversification, driving the evolution of more extreme morphologies. Divergent patterns of trophic diversification among Neotropical cichlids appear to derive from different performance demands in regional abiotic and biotic environments associated with biogeographical history.

ADDITIONAL KEYWORDS: adaptation – Cichlinae – comparative phylogenetics – feeding ecology – functional morphology – Ornstein–Uhlenbeck – specialization.

INTRODUCTION

Adaptive radiation is often characterized as a process of diversification to occupy vacant niche space created by environmental change or encountered via dispersal (Schluter, 2000; Gavrilets & Losos, 2009; Losos, 2010; Bolnick *et al.*, 2010; Yoder *et al.*, 2010; Mahler *et al.*,

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2010). Environmental change, extinction, dispersal and evolutionary innovations can create ecological opportunities that set the stage for lineages to undergo adaptive radiation (Schluter, 2000; Gavrilets & Losos, 2009; Losos, 2010; Mahler *et al.*, 2010). Some traits may be key innovations that enhance the diversification of other traits, trait complexes and lineages (Hulsey *et al.*, 2006; Alfaro *et al.*, 2009; Wainwright & Price, 2016). Certain traits may have a disproportionate effect on the rate of overall phenotypic change during adaptive radiation (Streelman & Danley, 2003; Sallan & Friedman, 2011), whereas other traits and their associated functions may represent evolutionary dead ends, limiting further trait evolution and lineage diversification (Collar *et al.*, 2009; Burin *et al.*, 2016; Egan *et al.*, 2018). Examining the macroevolutionary consequences of ecological adaptation on the timing, pattern and rate of trait diversification is therefore essential to understand a process such as adaptive radiation.

Trait diversity within regional species assemblages is influenced by rates of diversification in response to selection (Streelman & Danley, 2003; Ingram *et al.*, 2012; Moen & Morlon, 2014; Wainwright & Price, 2016). Variations in the rates of trait diversification have been described for several vertebrate families (Collar *et al.*, 2009; Holzman *et al.*, 2012; Davis *et al.*, 2014, 2016). However, the relationship between morphological adaptation and ecological diversification may exhibit complex relationships between form and function, such as ‘many-to-one mapping’, whereby multiple morphological phenotypes may yield similar ecological performance (Alfaro *et al.*, 2005; Wainwright *et al.*, 2005; Parnell *et al.*, 2008). This makes it particularly important to incorporate functional traits into comparative studies of evolutionary diversification (e.g. traits with an explicit link to ecological performance; Wainwright, 2007). Fundamentally, the ability to interpret phenotypic variation as a reliable indicator of ecological performance and adaptive diversification relies on the assumption that morphology and ecology are linked functionally (Schluter, 2000; Feilich & López-Fernández, 2019).

To explore ecological correlates of morphological diversification, we investigated the relationship between functional morphology and diet in Neotropical cichlids. Cichlids are a diverse family of freshwater fishes in Africa and the Neotropics, with comparatively few species native to India and Madagascar (e.g. Stiassny, 1991; Smith *et al.*, 2008; McMahan *et al.*, 2013). Repeated adaptive radiations characterize cichlid diversification, especially among certain lineages in the East African Rift Lakes (Seehausen, 2006; Wagner *et al.*, 2012). Neotropical cichlids (subfamily Cichlinae) are sister to the African cichlid subfamily Pseudocrenilabrinae (Irisarri *et al.*, 2018;

Schedel *et al.*, 2019). Variation in traits associated with feeding ecology is a fundamental axis of diversification across cichlids, with different lineages demonstrating a remarkable array of specializations (Sturmbauer *et al.*, 1992; Norton & Brainerd, 1993; Hulsey & García de León, 2005; Montaña & Winemiller, 2013; López-Fernández *et al.*, 2014; Burress, 2016). Cichlinae form a continentally dispersed clade, with > 600 species distributed in seven tribes (Geophagini, Heroini, Cichlasomatini, Cichlini, Chaetobranchini, Retroculini and Astronotini, in decreasing order of species richness). All Cichlinae tribes are widespread in South America, but only Heroini has diversified extensively throughout Central America (López-Fernández *et al.*, 2013; Říčan *et al.*, 2013, 2016). South American cichlid evolution followed a pattern consistent with an early burst of trait diversification (López-Fernández *et al.*, 2013; Arbour & López-Fernández, 2014). Comparatively, colonization of new habitats and niches in Central America by the tribe Heroini was associated with increased rates of divergence in functional morphological traits associated with feeding (Arbour & López-Fernández, 2016) and molecular evolution of vision (Hauser *et al.*, 2017). The South American genus *Crenicichla* (tribe Geophagini) has been identified as containing smaller, more recent radiations (Burress *et al.*, 2018; Piálek *et al.*, 2019). Dietary specialization is common among both South and Central American cichlids (e.g. piscivores, substrate-sifting invertivores, molluscivores, planktivores, periphyton grazers, detritivores and frugivores), including convergent adaptations observed between species of the two regions (e.g. Winemiller *et al.*, 1995; Montaña & Winemiller, 2013; López-Fernández *et al.*, 2014). In cichlids, functional morphology associated with feeding appears to evolve under selection associated with mechanical/anatomical constraints (Arbour & López-Fernández, 2014, 2018; Hulsey *et al.*, 2018). Recent comparative phylogenetic analyses of Neotropical cichlids suggest that functional trait diversification has varied in response to ecological opportunity, both during the initial diversification within South America and during the later invasion of Central America, consistent with a major prediction of the ecological theory of adaptive radiation (Mahler *et al.*, 2010; Glor, 2010; Arbour & López-Fernández, 2016). Thus, Neotropical cichlids provide a fertile system in which to study the relationship between functional diversification and ecological adaptations.

Here, we evaluate the proposed link between phenotypic and ecological diversification of Neotropical cichlids, using comprehensive datasets on dietary ecology and function across a broad taxonomic sampling of Neotropical cichlids, as a way to validate assumptions made in previous analyses in which morphology has been assumed to reflect ecological

variation (Arbour & López-Fernández, 2013, 2014, 2016; López-Fernández *et al.*, 2013). In that framework, we combine previously published quantitative descriptions of feeding biomechanics (Arbour & López-Fernández, 2013, 2014) with dietary data from wild populations (e.g. Winemiller *et al.*, 1995; López-Fernández *et al.*, 2012; Montaña & Winemiller, 2013) in a phylogenetic context. We investigate changes in rates and patterns of diversification associated with variation in morphology, biomechanics and diet. We use multivariate analysis (canonical correspondence analysis) and macroevolutionary modelling (OUwie) to correlate dietary data with functional traits and evolution across 44 species of Neotropical cichlids. We test whether transitions in dietary niches correspond to changes in functional trait variation or diversification.

MATERIAL AND METHODS

FUNCTIONAL MORPHOLOGY AND PHYLOGENETICS OF CICHLINAE

We obtained morphological data from a previously published analysis of the functional diversity of feeding in 75 species of Neotropical cichlids (44 were selected for this study because dietary data quantified with the same methods were available, i.e. volume of stomach contents), representing all major South and Central American lineages and most feeding functional configurations (Arbour & López-Fernández, 2014). This dataset included ten functional morphological and biomechanical variables, as follows: (1) adductor mandibulae mass (AM), indicative of jaw closing force production (Wainwright *et al.*, 2004); (2) sternohyoideus mass (ST), indicative of jaw opening force production (Lauder & Shaffer, 1993; Wainwright *et al.*, 2004); (3) lower pharyngeal jaw/fifth ceratobranchial mass (CB5), indicating pharyngeal jaw crushing potential (Liem, 1973; Currey, 1984; Hulsey *et al.*, 2006); (4) maximal jaw protrusion distance (Waltzek & Wainwright, 2003); (5) lower jaw opening mechanical advantage (MA), describing the transmission of force and velocity during jaw movement (Wainwright & Richard, 1995); (6) lower jaw closing MA; (7) quadrate offset, which relates jaw shape to bite occlusion patterns (Anderson, 2009; Arbour & López-Fernández, 2013); (8) oral jaw four-bar linkage kinematic transmission coefficient (KT), describing the transmission of force and velocity during mouth opening (Westneat, 1990); (9) hyoid–neurocranium four-bar KT (transmission of force and velocity during hyoid depression and buccal expansion); and (10) suction index (Carroll *et al.*, 2004). See Arbour & López-Fernández (2014) for more details on the measurements and additional references. All size-dependent variables were

phylogenetically size corrected using the residuals of a log–log regression on the cube root of body mass (all muscle and bone masses were also cube rooted) before further analysis (Arbour & López-Fernández, 2014) using the R function `phyl.resid` from package `phytools` (Revell, 2012).

For all phylogenetic corrections or comparative analyses, we used an evolutionary tree for Neotropical cichlids from a recently published, comprehensive next-generation molecular phylogenetic analysis of 415 exons from 139 species (Ilves *et al.*, 2018). Given that this phylogeny has not been dated, owing to the practical challenges of approaches for age estimation for large phylogenomic datasets (Ilves *et al.*, 2018), we used a congruification approach (Eastman *et al.*, 2013) to map dates from a previous analysis of teleosts (Matschiner *et al.*, 2017) onto nodes within the cichlid phylogeny. We used the R functions `congruify` and `chronos` in the package `geiger` to map ages from overlapping Neotropical cichlid clades to the maximum likelihood phylogeny from Ilves *et al.* (2018). Additionally, we summarized results over 100 trees from the posterior distribution of a divergence time analysis from López-Fernández *et al.* (2013) (based on five loci and several fossil calibrations). The trees in both studies were nearly identical, ensuring that the generation of a posterior distribution did not introduce topological artefacts into our analyses (for further details, see Ilves *et al.* 2018). All dated phylogenies were scaled to a relative length of one before analysis, in order to make the results more directly comparable across chronograms.

NEOTROPICAL CICHLID FEEDING ECOLOGY

Dietary composition was determined by direct analysis of the gut contents and quantified using volumetric measures of the relative contribution of each dietary item (Winemiller, 1990 and see descriptions of items on next page). Stomach content data were compiled from previous studies of Neotropical cichlids, with the constraint that methods and units for quantification were equivalent among studies (Winemiller, 1991; Arcifa & Meschiatti, 1993; Winemiller *et al.*, 1995; Meschiatti & Arcifa, 2002; Moreira & Zuanon, 2002; de Moraes & Barbola, 2004; Gonzales & Vispo, 2004; Cochran-Biederman & Winemiller, 2010; López-Fernández *et al.*, 2012; Montaña & Winemiller, 2013; Pease *et al.*, 2018; Soria-Barreto *et al.*, 2019). Although other analyses of dietary composition in Neotropical cichlids are available in the literature, they were not amenable to incorporation into our dataset because quantification was made in different units (e.g. relative frequency of occurrence, relative importance of dietary items).

Data on stomach contents expressed as a percentage of the volume were obtained from 4877

adult specimens representing 44 species. Items from the analysis of stomach contents were grouped into 11 major categories from previous analyses of Neotropical cichlid feeding ecology, and the mean (proportional) volumetric contribution was determined per dietary category (for data and full references, see Supporting Information). The 11 major categories were as follows: (1) fish (including bones, fins and flesh); (2) macrocrustacea (decapods, especially palaemonid shrimp); (3) microcrustacea (amphipods, branchiopods, cladocerans, copepods, isopods and ostracods); (4) meiofauna/microfauna (small benthic/epibenthic invertebrates, including mites, nematodes, annelids, rotifers, bryozoans, tardigrades, protozoans and horsehair worms); (5) molluscs; (6) aquatic insects (largely larvae from Diptera, Ephemeroptera and Trichoptera); (7) terrestrial arthropods; (8) terrestrial plants (fresh leaves, fruits, seeds and flowers); (9) aquatic vegetation (filamentous algae, diatoms and aquatic plants); (10) vegetative detritus (leaf litter, woody debris and fine and coarse organic detritus); and (11) animal detritus (scales and arthropod fragments). Dietary data from different studies were averaged for each species across the 11 dietary categories and weighted by the number of specimens per analysis. The resulting dietary dataset included most trophic regimens within Neotropical cichlids, with the exception of frugivores (*Tomocichla*), and included some of the most specialized feeders in Cichlinae (e.g. piscivorous *Cichla*). Various genera and species of both sifting (e.g. *Geophagus*, *Satanoperca*, *Thorichthys*) and non-sifting lineages from across the phylogeny (e.g. *Crenicichla*, *Mayaheros*, *Cichlasoma*) were included. We examined the major groupings of dietary variation across Neotropical cichlids with a hierarchical cluster analysis using the R function `hclust` using the unweighted pair group method with arithmetic mean (UPGMA) agglomeration method.

To test whether any lineages in our dataset have undergone adaptive evolutionary shifts in diet, we summarized the variation in cichlid dietary data through correspondence analysis (CA) using the R function `cca` in the package `vegan` (Legendre *et al.*, 1983; Ter Braak, 1986; Oksanen *et al.*, 2015). Scores from the first three axes (chosen based on scree plots) were used in evolutionary model-fitting analyses (Spalink *et al.*, 2016). The evolution of continuous traits is often described by a Brownian motion (BM) model, which is governed by the rate parameter (σ^2) (O'Meara *et al.*, 2006). However, selection or adaptation can be incorporated into the evolution of traits through models describing an Ornstein–Uhlenbeck (OU) process, which is governed by the rate of evolution, the location of one or more adaptive optima (θ) and the strength of selection (α) (Hansen & Martins, 1996; Hansen, 1997; Ingram & Mahler, 2013).

The impact of different OU peaks can be mapped onto different branches of a phylogeny to represent changes in adaptive constraints on the evolution of a trait. We consider adaptive evolutionary shifts to be transitions between different selective regimens as reflected in a phylogeny. We used 'l1ou' (Khabbazian *et al.*, 2016), an approach that uses a lasso algorithm to search for the best-fitting positions of shifts to new OU optima along the branches of the phylogeny, to detect adaptive shifts in the evolution of diet (as described across the CA scores). The best-fitting model of adaptive shifts was selected using a phylogenetic Bayesian information criterion, as proposed by Khabbazian *et al.* (2016) and implemented in the R function `estimate_shift_configuration`. We used the function `l1ou_bootstrap_support` from the `l1ou` R package to calculate the bootstrap support for each inferred adaptive shift in dietary evolution within Cichlinae. To determine the likelihood of the number and configuration of shifts occurring under a non-adaptive process, we compared the observed number of shifts, and their bootstrap support, with the number and support of shifts generated from 100 datasets simulated under BM evolution (Supporting Information, Figs S1, S2), using the R functions `sim.char` and `ratematrix` from the package `geiger` (Harmon *et al.*, 2008).

RELATIONSHIPS BETWEEN FUNCTIONAL MORPHOLOGY AND DIET

We used canonical correspondence analysis (CCA) to test for relationships between functional morphology and diet in the 44 species of Neotropical cichlids examined, using the function `cca` from the R package `vegan` (Oksanen *et al.*, 2015). Permutation tests were used to assess the significance of each CCA axis using the function `anova.cca` in the R package `vegan` (Legendre *et al.*, 1983, 2011; Oksanen *et al.*, 2015). These analyses do not account for the impact of phylogenetic relatedness on diet–morphology correlations. Evolutionary relatedness may bias correlations between traits, but there are no direct phylogenetic corrections available for CCA. These patterns are therefore contrasted with more explicit macroevolutionary analyses below.

FUNCTIONAL DIVERSIFICATION AND FEEDING ROLES

Evolutionary model fitting was used to test whether diet in Neotropical cichlids was associated with changes in the diversification rate or optimal values of functional traits. Variation in Cichlinae functional morphology was analysed using a phylogenetically corrected principal components analysis, using the function `phyl.pca` from the R package `phytools` (Revell, 2012), and parallel analysis was used to

select a number of critical axes of variation. We used a maximum likelihood approach to fit a series of models differing in adaptive constraints and evolutionary rates on the principal component (PC) scores of all critical axes of functional morphological variables simultaneously, using the R function 'OUwie.joint' (Beaulieu & O'Meara, 2015). Null models of evolution for functional morphology included a single-rate BM model of character evolution and a single-rate/single-peak OU model. We also fitted BM and OU models allowing for varying rates (V), varying adaptive peaks (M) or both (MV) between dietary niches based on previous cluster analysis of dietary variation across all 44 species (see Fig. 1 and dietary results).

We fitted models with two or three dietary niches based on the major divisions identified in Figure 1. The two-group models tested whether rates or adaptive optima differed between piscivores/macroinverteviores and species primarily relying on other resources, which represented the first and major division in a cluster analysis of dietary composition (Fig. 1, groups A and B). The three-group models split the latter

group into those species primarily consuming small benthic invertebrates and those consuming vegetation and detritus within the non-piscivore group, which represented the second major division in the cluster analysis (for more details, see diet results; Fig. 1, three groups: A, C and D). The evolutionary history of the two and three dietary niches were reconstructed for model fitting using stochastic character mapping (Huelsenbeck *et al.*, 2003; Bollback, 2006) and the function `make.simmap` in the R package `phytools` (Revell, 2012; Supporting Information, Fig. S3). Evolutionary models were compared using sample-size-corrected Akaike information criteria (AICc), following Burnham & Anderson (2002). We calculated the Δ AICc for each model both over the Ilves *et al.* (2018) topology and over a distribution of 100 trees from the posterior distribution López-Fernández *et al.* (2013). Preferred models of evolution were those with a Δ AICc of less than two (Burnham & Anderson, 2002).

Given the relatively small sample size for our model fitting (44 species), we examined the performance of OUwie.joint to identify the best-fitting model of trait

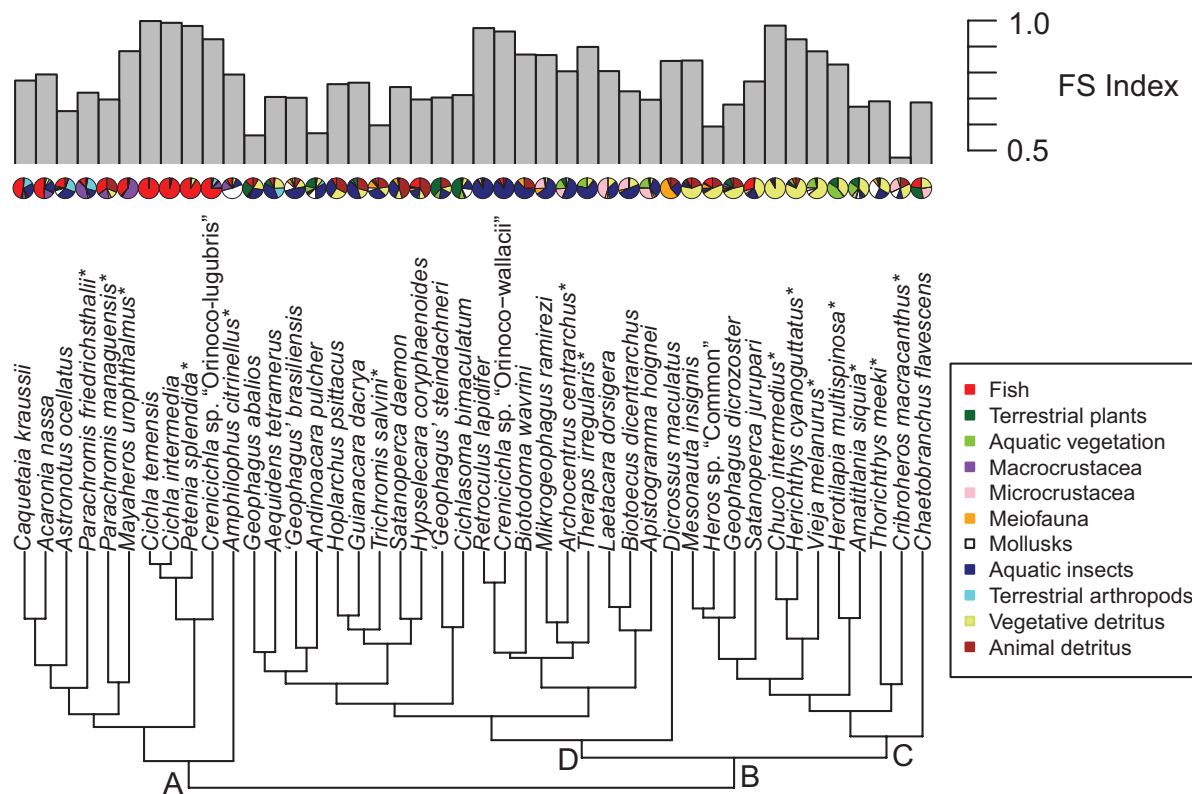


Figure 1. Dietary diversity of 44 species of Neotropical cichlids. Bottom panel, cluster analysis of dietary composition. Pie charts show the average proportional volumetric composition of stomach contents (key in box on the right). Species with asterisk (*) have Central American distributions; all others are from South America. Top panel, bar plot showing the feeding specialization (FS) index for each species. Letters denote major divisions in dietary variation: A, fish, macrocrustaceans, terrestrial arthropods and mollusks; B, detritus, vegetation and small invertebrates; C, animal/vegetative detritus, aquatic vegetation and terrestrial plants; and D, aquatic insects, microcrustaceans and meiofauna.

evolution. For both dietary niche and relative dietary specialization, we simulated data using OUwie.sim under the best-fitting model (see Results) and under a BM1 model of trait evolution, across 100 SIMMAP reconstructions using the dated phylogeny of Ives *et al.* (2018).

FUNCTIONAL DIVERSIFICATION AND SPECIALIZATION

Although cluster analysis revealed several major divisions among feeding roles in Cichlinae (see Results), there was still considerable overlap in diets among more generalized feeders from each of the major dietary niches. For example, although *Astronotus ocellatus* was grouped within the piscivores–macroinverteviores, it also consumed a high proportion of aquatic insects (31%, the single largest contribution to its diet), which also represent a major component of the diet of microinverteviores (Fig. 1; Supporting Information, Table S7). Therefore, in addition to examining dietary niches, we also explored the relationship between functional morphology and dietary specialization in Cichlinae. An index of feeding specialization (FS; ranging from zero to one) was calculated based on Levin's index of niche breadth (equation below, where p is the proportional food volume of item i ; $N = 11$ dietary categories; Krebs, 1999; Belmaker *et al.*, 2012). A feeding specialization (FS) index of zero represents a perfect generalist, feeding equally on all resources, and one represents taxa completely specialized on a single resource.

$$FS = 1 - \left(\frac{\sum_i \frac{1}{p_i^2} - 1}{N - 1} \right)$$

We explored whether specialization was associated with differences in evolutionary rates or adaptive optima. A score of zero would represent a truly random feeder (all categories have equal weight), based on a broken-stick distribution (which described random apportionment of niches) for 11 diet categories, whereas a randomly feeding taxon (hypothetical non-selective generalist) would obtain a score of FS ~0.462. However, no species were found to have FS values close to this cut-off (see Results), with all species feeding non-randomly (0.532–0.998, mean = 0.781). Therefore, we assigned each species to one of two categories of relative specialization (relative generalist vs. specialist), based on the ancestral value of feeding specialization (FS = 0.79, as determined using the R function ace from the package ape, under an assumption of BM evolution), to illustrate lineages that have increased or decreased in FS index from their ancestral condition. Evolutionary model fitting (as described for the feeding categories above) was then used to test whether relative specialist vs. generalist taxa differed in functional adaptations or rates of diversification. The evolutionary history of specialization categories

was reconstructed for model fitting using stochastic character mapping (Huelsenbeck *et al.*, 2003; Bollback, 2006) and the function make.simmap in the R package phytools (Revell, 2012; Supporting Information, Fig. S4). Using maximum likelihood model fitting in the R function OUwie (Beaulieu & O'Meara, 2015), we fitted BM and OU models differing in the number of feeding selective regimens (zero, no selection; one, general selection across taxa; or two, different selection for each category) and the number of evolutionary rates (one, no differences in rates between categories; or two, different rates) for the low- and high-specialization categories. An additional model including a single feeding regimen but different rates of evolution per category (OUV) was also included (R script provided as Supporting Information, supplementary file).

RESULTS

VARIATION IN NEOTROPICAL CICHLID DIET

On average, the most frequent food items of the 44 cichlid species examined were aquatic insects (24.1%), vegetative detritus (18.5%) and fish (16.6%). Cluster analysis of dietary variation in Cichlinae showed a primary division between species feeding primarily on fish (51.9%) and macrocrustacea (15.0%), in addition to those consuming molluscs and terrestrial arthropods to a lesser extent (5.9 and 7.2%, respectively), vs. those species consuming microinvertebrates, detritus and vegetation (Fig. 1). Within the latter group, species were divided into two groups: (1) those feeding primarily on small, benthic invertebrates (aquatic insects, 33.8%; microcrustacea, 10.7%); and (2) those feeding primarily on detritus (vegetative, 29.3%; animal, 12.4%) or vegetation (aquatic, 5.5%; terrestrial, 5.2%). Species varied along the first axis from a correspondence analysis of diet between piscivorous species (–CA1) to taxa feeding on a mixture of fish and large invertebrates (–CA2), and the second axis separated taxa that consume large fractions of plant matter from those feeding on small benthic invertebrates (microcrustaceans, aquatic insects and meiofauna). The third axis separated species feeding on molluscs and macrocrustacea from those feeding on meiofauna and animal detritus (Supporting Information, Tables S1).

Using l1ou, we found nine adaptive shifts in diet (as summarized by CA scores), all of which had moderate to strong bootstrap support (0.82–1.00; Fig. 2, left panel). Adaptive shifts in diet were found within both South and Central American species and, notably, all occurred among lineages moving from feeding on small benthic invertebrates (positive CA1 scores), detritus or vegetation to preying upon fish and large or hard-bodied invertebrates (negative CA1 scores; Fig. 2, right panels). The species that shifted towards negative CA1 scores (left side of CA plots; Fig. 2) were nearly

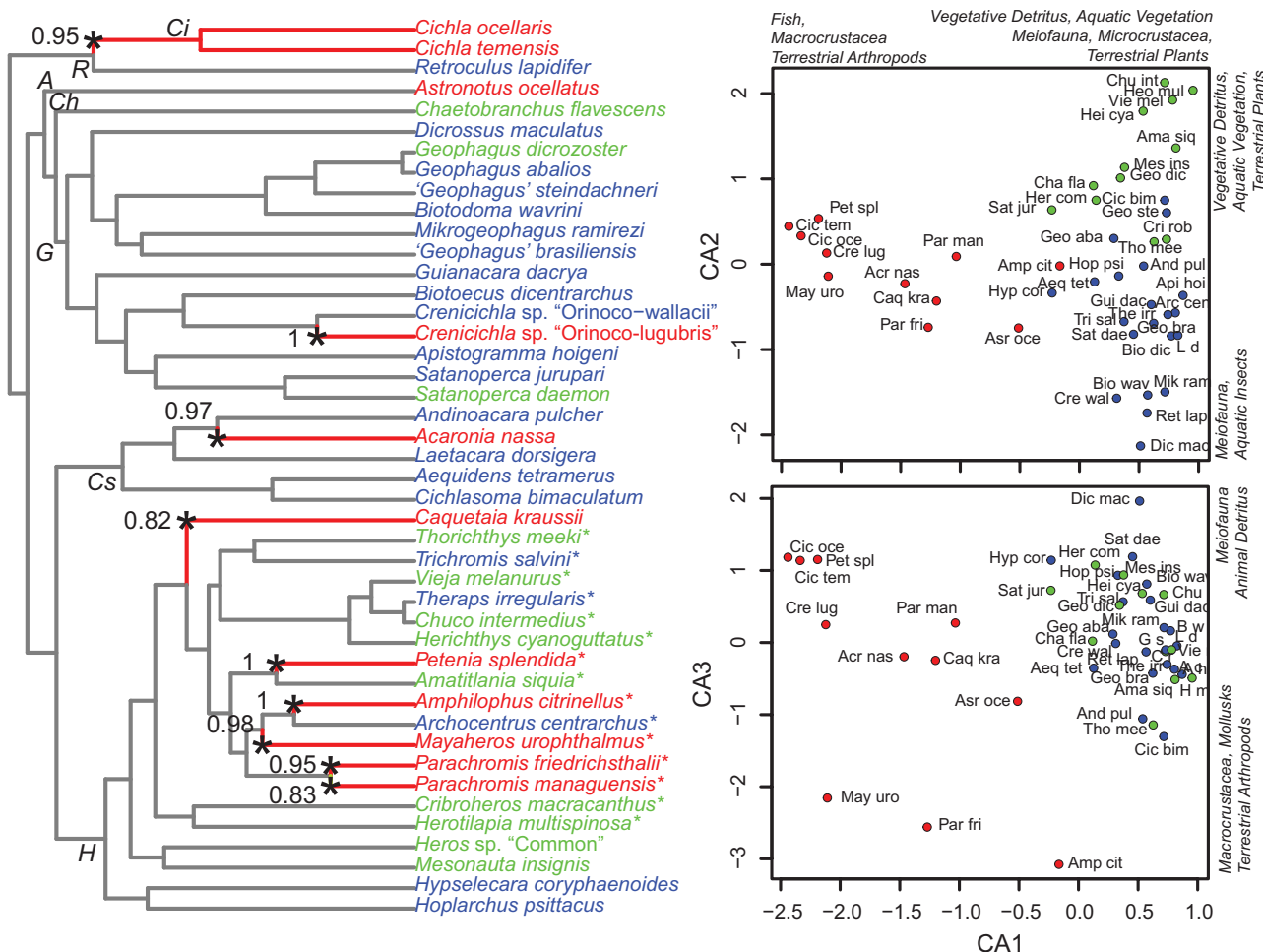


Figure 2. Results of adaptive shift analysis on dietary composition in Neotropical cichlids. Left panel, phylogeny of 44 species of Neotropical cichlids. Asterisks on branches denote the inferred location of an adaptive evolutionary shift; adjacent numbers provide the bootstrap support for each shift. Species names are coloured by dietary group as in Figure 1. Species names with asterisk (*) have Central American distributions. Right panel, scores from a correspondence analysis of dietary composition. Colours indicate feeding group. Species name abbreviations in Table S9.

identical to one of the major two groups of dietary variation (see paragraph above), with the exception of *Astronotus ocellatus*, the most generalized feeder from the piscivore/macroinvertevore group (Figs 1, 2) and showing more moderate CA1 scores. Interestingly, no shifts represented transitions between species feeding on small benthic invertebrates to detritus/vegetation or vice versa (Fig. 1, groups C and D).

The number of shifts in diet was significantly higher than could be generated under BM evolution ($P < 0.01$; Supporting Information, Fig. S1). This was also the case for shifts in simulated datasets that received high bootstrap support (Supporting Information, Fig. S1), suggesting that such shifts can be interpreted as adaptive. Lineages showing adaptive shifts in our data

were also not more likely to have experienced a shift under BM evolution (Supporting Information, Fig. S2), indicating that our results were not driven by branch lengths or phylogenetic relationships alone. Overall, the adaptive evolution of highly predatory diets, specializing on evasive prey, such as fish, was unlikely to have been driven by a random-walk process alone.

RELATIONSHIPS BETWEEN DIET AND FUNCTIONAL MORPHOLOGY

Canonical correspondence analysis of dietary composition and functional morphology revealed four significant relationships, explaining a moderate amount of variation (~30%) in the diet of these 44 species of

Neotropical cichlids (Supporting Information, Table S2). Canonical correspondence analysis associated the consumption of fish with high-velocity transmission in the lower jaw (low lower jaw MA) and buccal cavity (high hyoid KT) and poor suction (Fig. 3). Canonical correspondence analysis also associated the consumption of vegetation and vegetative detritus with unevenly occluding oral jaws (QO), strong suction feeding (SI; especially for detritus) and high force transmission in the lower jaws (MA; Fig. 3). Species consuming aquatic insects and other microinvertebrate prey (meiofauna or microcrustacea) also possessed fast oral jaw kinematics (oral KT) and larger ST and AM mass.

FUNCTIONAL DIVERSIFICATION WITHIN DIETARY NICHES

Principal components analysis revealed two critical axes of jaw functional morphology, which largely corresponded to patterns found across analyses of these and other species in a previous study (Arbour & López-Fernández, 2014). In particular, PC1 represented a gradient between efficient velocity transmission (but poor force transmission), evenly occluding jaws, larger lower jaw adductors and poor suction ability (ram feeders; e.g. *Crenicichla* and *Cichla* species) vs. those with efficient force transmission (but poor velocity transmission), smaller jaw adductors, unevenly occluding jaws and high suction capability (suction feeders/biters Supporting Information, Table S3).

The best-fitting model of functional evolution in Cichlinae was one that involved multiple OU optima for dietary niches as defined in previous cluster analyses of stomach content data (Tables 1 and 2; Supporting Information, Tables S4 and S5). Both the two- and three-dietary-niche models showed similar support, with nearly all well-supported models ($\Delta\text{AICc} < 2$) including a separate adaptive optimum for fish/macroinvertebrate feeders. Comparatively, separate optima for microinvertebrate and detritus–vegetation feeders showed more mixed support (Table 1). Divisions between adaptive optima were more prominent along PC1 (Table 2), with fish–macroinvertebrate feeders showing more strongly ram-optimized traits, small-invertebrate feeders showing moderate PC1 values and detritus–vegetation feeders being strongly suction optimized (Fig. 4; Table 2). The adaptive optima of fish–macroinvertebrate feeders on PC2 favoured larger ST and CB5 mass and less mobile oral jaws (lower oral jaw KT); however, this group included species spanning nearly the entire range of PC2 scores (Fig. 4).

EVOLUTIONARY CONSEQUENCES OF SPECIALIZATION

Neotropical cichlids were found to be moderately to strongly specialized feeders; nevertheless, feeding

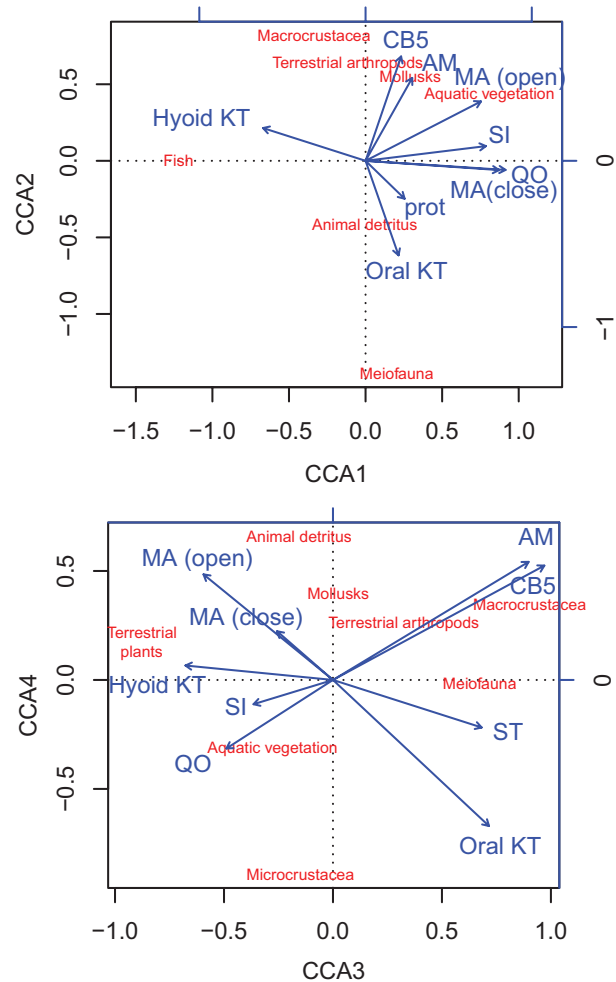


Figure 3. Results of a canonical correspondence analysis of dietary composition (red) and feeding functional morphology (blue) from 44 species of Neotropical cichlids. Abbreviations: AM, adductor mandibulae mass; CB5, lower pharyngeal jaw mass; KT, kinematic transmission coefficient; MA, mechanical advantage; prot, maximum oral jaw protrusion; QO, quadrate offset; SI, suction index; ST, sternohyoideus mass. The length of vectors denoting morphology is proportional to the strength of correlation between a morphological variable and the dietary elements associated with it. For instance, in both panels, macrocrustaceans are strongly correlated with the weight of the AM and CB5s.

specialization varied substantially across taxa (FS ~0.6–1.0; Fig. 1). More specialized feeders exhibited a wide phylogenetic distribution (Supporting Information, Fig. S4) and consumed a variety of dietary resources (Fig. 1), including fish (e.g. *Cichla* species), aquatic insects (e.g. *Retroculus lapidifer*) and detritus (e.g. *Herichthys cyanoguttatus*). The least specialized feeders included substrate sifters (*Cribroheros robertsoni* and *Geophagus abalios*) and detritus plus

Table 1. Evolutionary model fitting of functional morphology based on two or three dietary groups across 100 SIMMAP character reconstructions

Model	<i>k</i>	lik	AICc	ΔAICc	Frequency of best fit	Frequency of poor support
BM	2	−331.1	668.8	16.47	0	1
OU	4	−323.5	658.7	6.300	0	1
BMV, 2 groups	4	−325.9	663.4	11.74	0	1
OUM, 2 groups	6	−319.6	653.8	0.3160	0.46	0.38
OUMV, 2 groups	8	−317.8	656.2	3.514	0.13	0.71
BMV, 3 groups	6	−322.7	663.3	10.56	0.03	0.97
OUM, 3 groups	8	−318.6	654.6	1.366	0.36	0.43
OUMV, 3 groups	10	−316.1	662.5	10.64	0.02	0.95

Values of *k*, lik and AICc are given as the mean, ΔAICc as the median. Models included Brownian motion (BM) and Ornstein–Uhlenbeck (OU) processes with varying rates (V) and varying adaptive optima (M). The frequency of best fit is the proportion of SIMMAP reconstructions with ΔAICc = 0. The frequency of poor support is the proportion of SIMMAP reconstructions with ΔAICc > 2. Abbreviations: AICc, sample-size-corrected Akaike information criterion; *k*, number of parameters; lik, likelihood.

invertebrate feeders (*Trichromis salvini*, *Aequidens tetramerus* and *Heros liberifer*).

The best-fitting model of functional morphological evolution with respect to feeding specialization was one that incorporated selective constraint towards a single adaptive optimum, but with different evolutionary rates between specialists and generalists (Table 3; Supporting Information, Table S6). The specialist feeding category was associated with a substantial increase in evolutionary rates across both axes of functional morphology. On average, specialized feeders evolved 4.3 and 2.2 times faster than their more generalized counterparts across PC1 and PC2, respectively (Table 4; Supporting Information, Table S7). Specialized feeders also appeared to be more likely to possess extreme PC scores across both axes (e.g. *Cichla*, *Crenicichla*, *Dicrossus* and *Herotilapia*), whereas generalized feeders tended to exhibit moderate morphologies (e.g. *Biotocetus*, *Geophagus abalios* and *Satanoperca daemon*; Fig. 4). Simulated scores were found to be evolving under the best-fitting model of evolution in both cases, when that was the generating model (Supporting Information, Table S8). When traits were simulated under BM1, the best fitting models (OUM and OUV; see Results) were rarely found to have the lowest AICc values (< 5% of SIMMAP reconstructions). Thus, the results detailed below are unlikely to be an artefact of small taxonomic sample size or of the model fitting approach.

DISCUSSION

DIETARY NICHE EVOLUTION IN CICHLINAE

The ecological theory of adaptive radiation links the diversification of species and traits to ecological opportunities associated with changing environmental

conditions, including the availability of new resources and biotic interactions, such as competition and predation (Simpson, 1953; Schluter, 2000; Glor, 2010; Losos & Mahler, 2010; Mahler *et al.*, 2010; Yoder *et al.*, 2010). The specific predictions commonly provided for adaptive radiations include high initial rates of trait and lineage diversification (e.g. an early burst of evolution) and a demonstration of trait utility in the context of ecological performance (Schluter, 1996, 2000; Glor, 2010). Phylogenetic comparative analyses in Cichlinae have suggested that trait and lineage diversification are associated with ecological opportunity in both South and Central American clades (López-Fernández *et al.*, 2013; Arbour & López-Fernández, 2016; Piálek *et al.*, 2019). In the present study, we also found a significant relationship between variation in functional morphology and diet that accompanies ecological diversification within Cichlinae.

Functional morphology explained only a moderate amount of dietary variation overall (Supporting Information, Table S2), whereas strong relationships were obtained for several traits and dietary components. The consumption of fish plus macrocrustacea plus terrestrial arthropods vs. detritus plus vegetation plus small invertebrates was correlated with traits describing ram–suction feeding trade-offs (Fig. 3; Supporting Information, Table S2), such as lower jaw lever MAs and ‘suction index’. This same suite of functional traits has been associated with the major axis of functional diversity within a broader analysis of Cichlinae (Arbour & López-Fernández, 2014) and within the more limited morphological analyses described here (Fig. 4). Macroevolutionary model fitting showed strong support for divergent adaptive regimens between piscivores plus macroinvertebrates and other

Table 2. Evolutionary rates and optima from evolutionary model fitting of functional morphology based on two or three dietary groups

Model	Evolutionary rate (σ^2)						Adaptive optimum (θ)					
	PC1			PC2			PC1			PC2		
	F-Ma	Mi	D-V	F-Ma	Mi	D-V	F-Ma	Mi	D-V	F-Ma	Mi	D-V
BM	212.9			154.0			-	-	-	-	-	-
OU	333.7			401.2			0.7348	-	-	-0.8591	-	-
BMV, 2 groups	88.31	217.2		36.3	178.0		-	-	-	-	-	-
OUM, 2 groups	544.5			826.4			-23.97	3.869		-2.182	-0.8452	
OUMV, 2 groups	244.7	799.0		193.3	399.4		-21.87	3.340		-0.9472	-0.5507	
BMV, 3 groups	99.78	146.0	359.9	34.8	149.8	272.6	-	-	-	-	-	-
OUM, 3 groups	470.8			994.2			-18.09	2.284	8.863	-5.356	0.6163	-2.558
OUMV, 3 groups	449.9	902.8	1102.7	301.5	515.1	529.0	-17.84	2.008	8.844	-4.810	0.9136	-1.553

Dietary categories: D, detritus; F, fish; Ma, macroinvertebrates; Mi, microinvertebrates; V, vegetation. Models included Brownian motion (BM) and Ornstein-Uhlenbeck (OU) processes with varying rates (V) and varying adaptive optima (M). Values are given as the mean across 100 SIMMAP character reconstructions. The best-supported model is indicated in bold. Abbreviation: PC, principal component.

feeders along this major axis of functional variation (Table 1). Furthermore, evolutionary rates within this ram-suction functional gradient have been shown to be consistent with predictions based on changing ecological opportunity; declining through time within the South American radiation and increasing with renewed ecological opportunities upon colonization of Central America by heroin cichlids (Arbour & López-Fernández, 2016). Our results provide a direct link between changes in evolutionary rates in functional and biomechanical traits and quantitative diet analyses.

Although we identified several significant trait-diet correlations, a substantial amount of dietary variation was not explained by our set of morphological traits (Supporting Information, Table S3). One potential factor that could contribute to this low correspondence is error in dietary estimates based on the analysis of stomach contents. Also, species may possess adaptations for certain food resources that normally comprise a small volumetric proportion of their diet or are only available seasonally (Liem, 1973; Robinson & Wilson, 1998; Binning *et al.*, 2009; Collar *et al.*, 2009). In addition, behaviours unrelated to feeding, such as mouth brooding, which is observed in some Neotropical cichlids, might impose other constraints on the evolution of the feeding apparatus (López-Fernández *et al.*, 2012). Furthermore, many aspects of functional morphology (especially along our first PC axis) vary with body shape, particularly the degree of elongation (Arbour & López-Fernández, 2014), associated with other aspects of ecological performance, such as locomotion and habitat use (Claverie & Wainwright, 2014; Astudillo-Clavijo *et al.*, 2015; Feilich, 2016). Functional constraints associated with body shape may indirectly affect the potential to acquire feeding-related adaptations and thereby limit the strength of association between morphology and diet. Additionally, intraspecific variation in feeding and morphological traits, such as through polymorphisms or phenotypic plasticity, may also influence the relationship between ecology and phenotype (Meyer, 1987, 1990; Swanson *et al.*, 2003; Muschick *et al.*, 2011). However, despite these potential sources of variation, the relationship between functional morphology and dietary composition was significant, indicating that patterns of functional morphological evolution provide meaningful insight into the evolution of trophic diversity in Neotropical cichlids.

ADAPTIVE SHIFTS TOWARDS PREDATORY DIETS AND FUNCTIONAL DIVERSITY IN CICHLINAE

Interspecific dietary variation revealed a clear pattern of macroevolutionary bias. Analyses with no a priori assignment to diet guilds revealed dietary

Table 3. Evolutionary model fitting of functional morphology based on two categories of feeding specialization across 100 SIMMAP character reconstructions

Model	<i>k</i>	lik	AICc	ΔAICc	Frequency of best fit	Frequency of poor support
BM	2	-331.1	668.8	15.71	0	1
OU	4	-323.5	658.7	5.538	0.05	0.93
BMV	4	-327.4	666.4	13.60	0	1
OUM	6	-321.3	656.9	3.942	0.18	0.68
OUV	6	-319.9	654.0	0	0.56	0.21
OUMV	8	-317.8	655.6	2.047	0.21	0.51

Models included Brownian motion (BM) and Ornstein–Uhlenbeck (OU) processes with varying rates (V) and varying adaptive optima (M). The frequency of best fit is the proportion of SIMMAP reconstructions with ΔAICc = 0. The frequency of poor support is the proportion of SIMMAP reconstructions with ΔAICc > 2.

Abbreviations: AICc, sample-size-corrected Akaike information criterion; *k*, number of parameters; lik, likelihood.

Table 4. Evolutionary rates and optima from evolutionary model fitting of functional morphology based on two categories of feeding specialization

Model	Evolutionary rate (σ^2)				Adaptive optimum (θ)			
	PC1		PC2		PC1		PC2	
	Generalist	Specialist	Generalist	Specialist	Generalist	Specialist	Generalist	Specialist
BM	212.9		154.004		–	–	–	–
OU	333.7		401.1844		0.7348		-0.8591	
BMV	105.5	384.3	159.3	144.7	–	–	–	–
OUM	367.7		1022		2.640	-5.849	-2.516	4.054
OUV	208.1	887.9	5243	1.175e4	2.059		-2.079	
OUMV	230.9	897.3	5785	1.169e4	2.891	-4.710	-3.257	3.040

Models included Brownian motion (BM) and Ornstein–Uhlenbeck (OU) processes with varying rates (V) and varying adaptive optima (M). Values are given as the mean across 100 SIMMAP character reconstructions. The best-supported model is indicated in bold.

Abbreviation: PC, principal component.

transitions towards piscivory and macroinvertevory. The 11ou analyses estimated that all transitions corresponded to adaptive shifts between the ancestral dietary regimens (dominated by microinvertebrates, vegetation and detritus) towards diets comprising fish and macroinvertebrates (Fig. 2, red branches). The only species that differed in classification between adaptive shift analyses and non-phylogenetically informed cluster analyses of dietary variation (Fig. 2, red branches vs. black branches, and Fig. 1, A vs. B, respectively) was *Astronotus ocellatus*, a trophic generalist that consumes insects, spiders, crustaceans and fish (and see 11ou results). Therefore, variation across the primary axis of dietary variation (Fig. 2, CA1) was driven largely by (asymmetrical) diversification towards piscivory and macroinvertevory rather than a random-walk evolutionary process.

Taxa represented by the piscivore–macroinvertevory group (Fig. 1A) also encompassed a substantial proportion of morphological variation (López-Fernández *et al.*, 2013) and species diversity within Cichlinae,

including one of the largest genera (*Crenicichla*, > 90 described species). Species within this dietary niche were strongly convergent in functional morphology, possessing low lower jaw MA, evenly occluding jaws, low suction ability, large jaw muscles (Fig. 4) and elongate bodies. This suite of traits is consistent with the pursuit of fast, manoeuvrable prey, requiring the ability to accelerate both the body and the mouthparts rapidly to engulf prey (Norton & Brainerd, 1993; Wainwright *et al.*, 2001; Waltzek & Wainwright, 2003). These traits were also associated with the major axis of functional diversity across Neotropical cichlids (Arbour & López-Fernández, 2014).

Consistent with the results obtained in this analysis, López-Fernández *et al.* (2012) observed a trade-off in the consumption of benthic invertebrates and fish by South American cichlids. This trade-off might be reinforced by the asymmetrical transitions between fish–macroinvertebrate feeders and other dietary roles (Fig. 2). The consumption of large, evasive prey, such as fish and shrimp, imposes distinct functional demands that are

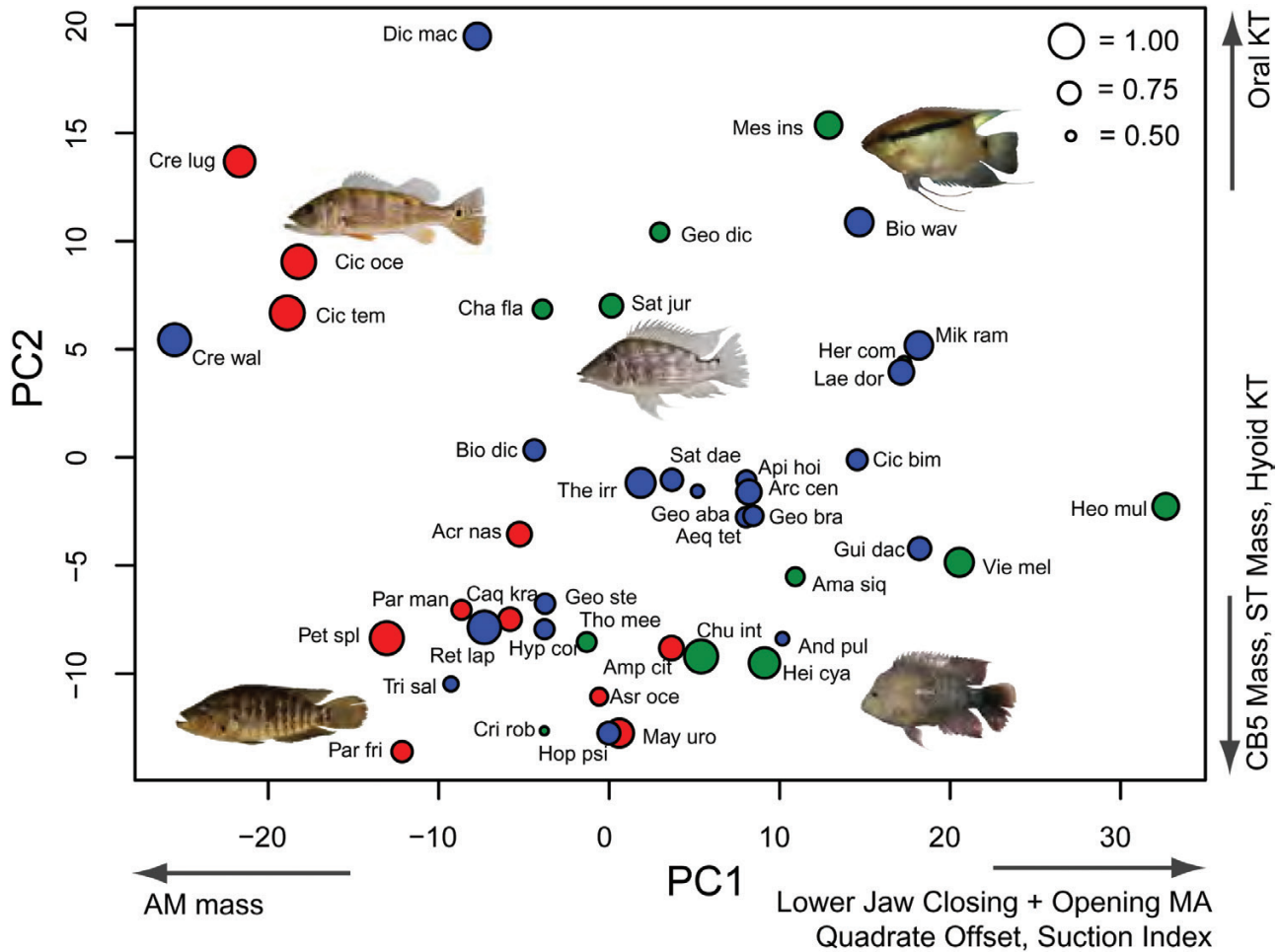


Figure 4. Results of a principal components analysis of ten functional morphological traits in Neotropical cichlids. Points are coloured by feeding group as in Figure 1; size is proportional to feeding specialization index. Arrows indicate increasing importance of specific functional traits along a gradient of morphological variation. Full species names are provided in Table S9.

likely to represent a crucial innovation in Neotropical cichlid dietary and morphological diversity. Among the ecomorphologically convergent North American centrarchids (Montaña & Winemiller, 2013), piscivory was associated with both a shift in adaptive optimum and evolutionary rates, and these differences were attributed to the high performance demands of consuming evasive prey (Collar *et al.*, 2009). Correspondingly, reversals from a piscivorous–macroinvertevorous diet towards diets comprising microinvertebrates, detritus or vegetation were rare, suggesting a strong selective constraint/advantage for these strongly predatory diets.

BIOGEOGRAPHY AND ECOLOGICAL OPPORTUNITY IN TROPHIC DIVERSIFICATION OF CICHLINAE

Biogeographical history appears to have a strong influence on ecological and functional diversification

in Cichlinae. Transitions between dietary niches in South America were predominantly from invertivores consuming small aquatic insects and crustaceans to species consuming larger invertebrates and fish. Central American lineages contained more species that included large fractions of detritus and vegetation in their diets (Fig. 2, green species names; Supporting Information, Fig. S3, right side). Separation between microinvertevore and detritivore/herbivore lineages showed only moderate support for distinct adaptive optima in functional morphology (Table 1). Cichlids in Central America might have filled what has been referred to as the ‘Ostariophysan gap’, i.e. a relative dearth of (frequently) detritivorous species from groups including Siluriformes and Characiformes (Winemiller *et al.*, 1995). Indeed, specialized detritivory and herbivory are much more common among the Central American radiation and their

sister clade comprising the South American genera *Heros*, *Mesonauta*, *Uaru* and *Symphysodon* (Goulding *et al.*, 1988; Crampton, 2008).

The adaptive relationship between detritus/vegetation feeding and functional morphology might be more complex than observed for piscivory plus macroinvertevory. In a previous analysis of the functional morphology of Cichlinae, a larger number of adaptive shifts occurred towards optima in suction-optimized morphospace compared with ram-optimized space based on an adaptive landscape with no a priori ecological hypotheses (Arbour & López-Fernández, 2014). These shifts corresponded predominantly to taxa with detritus- or vegetation-based diets (e.g. *Vieja*, *Herotilapia*, *Heros*, *Mesonauta* and *Symphysodon*). The selective constraints on functional morphology for these feeders might be lower than for species feeding on fish and large invertebrates, or there might be more functional configurations and morphologies that facilitate consumption of small invertebrates and detritus (Wainwright *et al.*, 2005). We should also note that subsequent evaluations of the method ‘surface’ used in the previous analysis have found it frequently to detect more peaks than appropriate (Khabbazian *et al.*, 2016; Adams & Collyer, 2018).

In a similar manner, the relationships between the consumption of small benthic invertebrates and functional traits are likely to be shaped strongly by body size. The so-called dwarf cichlids (e.g. *Apistogramma*, *Dicrossus*, *Biotoecus* and *Mikrogeophagus*) feature prominently among the microinvertevore group and have been shown to be morphologically distinct and significantly smaller than specialized substrate sifters with more generalized diets, such as *Geophagus* and *Satanoperca* (López-Fernández *et al.*, 2014; Steele & López-Fernández, 2014). Most dwarf cichlids inhabit dense beds of aquatic vegetation or leaf litter, where they prey on aquatic insect larvae and other benthic invertebrates living in small interstitial spaces (Lowe-McConnell, 1969; Keenleyside, 1991). Even within the genus *Crenicichla*, a lineage dominated by piscivores, there are a number of small-bodied species, including *Crenicichla* sp. ‘Orinoco-wallacii’ in our dataset, that feed on small aquatic invertebrates (Montaña & Winemiller, 2009; Burress *et al.*, 2013).

DIETARY SPECIALIZATION IS ASSOCIATED WITH ACCELERATED FUNCTIONAL DIVERSIFICATION

Although adaptation to dietary niches constrained the diversification of cichlids across their functional morphospace, greater dietary specialization was associated with higher rates of functional morphological evolution. Likewise, dietary specialists were more

likely to have colonized the extremes of Neotropical cichlid ram–suction morphospace, resulting in higher functional disparity. The low functional disparity and rate of diversification of generalist taxa might be driven by biomechanical constraints on being a ‘jack of all trades’, whereas taxa specializing on fewer dietary resources require less flexibility in the use of the feeding apparatus. The phenotypic evolution of specialists and generalists might therefore be represented better by a model of released selective constraint in specialists (Slater, 2013) rather than simply by changes in the rate of evolution. Given that OU models varying in selective constraint are particularly complex, we were not able to optimize these parameters successfully, but such a model might explain our data better than simply increased rates of evolution.

Martin & Wainwright (2011) found increased rates of diversification in feeding morphology in two ecologically diverse radiations of pupfish and suggested that trophic novelty is a driving factor in adaptive radiation. It has been postulated that during an adaptive radiation, ecological specialists arise from generalist ancestors, although this pattern may not be supported in all cases (Losos & De Queiroz, 1997; Glor, 2010). The rapid phenotypic evolution of specialist lineages compared with generalists observed in our study could facilitate the ‘early burst’ patterns of diversification characteristic of adaptive radiation and similar processes. Ingram (2012) proposed that ‘early bursts’ might be less likely when communities are dominated by omnivores rather than trophic specialists. Our empirical observation of trophic specialists capable of the rapid exploitation of disparate regions of morphospace is consistent with this theoretical prediction.

CONCLUSIONS

Selection favouring specialized dietary niches is prominent in macroevolutionary explanations for adaptive radiations, such as those observed among African Rift Lake cichlids, Caribbean anoles and Hawaiian honeycreepers. We used dietary ecology and functional morphology of Neotropical cichlids to test the relationship between patterns of phylogenetic, morphological and ecological diversity. We found significant relationships between the variation and diversification of cichlid diets and functional morphology. Evolutionary shifts in the trophic ecology of Neotropical cichlids appear to be biased towards predatory regimens favouring consumption of fish and macroinvertebrates. Adaptive divergence appears to have restricted morphological diversification both through selection towards particular functional trait combinations and by constraining certain transitions between fish–macroinvertebrate feeders and other dietary

niches. Altogether, we demonstrated that ecological specialization enhanced morphological diversification within this large clade of Neotropical fishes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. Summary of correspondence analysis of dietary composition in 44 species of Neotropical cichlids.

Table S2. Canonical correspondence of Neotropical cichlid dietary composition and feeding functional morphology.

Table S3. Loading factor coefficients from phylogenetic principal components analyses of functional morphology in 44 species of Neotropical cichlids.

Table S4. Evolutionary model fitting of functional morphology based on two or three dietary groups reconstructed using SIMMAP across 100 chronograms from López-Fernández *et al.* (2013).

Table S5. Evolutionary rate and optima from evolutionary model fitting of functional morphology based on reconstruction of two or three dietary groups using SIMMAP across 100 chronograms from López-Fernández *et al.* (2013).

Table S6. Evolutionary model fitting of functional morphology based on two categories of specialization reconstructed using SIMMAP across 100 chronograms from López-Fernández *et al.* (2013).

Table S7. Evolutionary rate and optima from evolutionary model fitting of functional morphology based on two categories of feeding specialization, with reconstruction using SIMMAP across 100 chronograms from López-Fernández *et al.* (2013).

Table S8. Performance of model fitting of pPC1 (phylogenetic Principal Component) and 2 scores based on either: (1) two categories of dietary niche; or (2) two categories of relative dietary specialization. Data were simulated under the parameters of the best-fitting models (OUM and OUV, respectively) and under the parameters for BM1. BM, Brownian Motion; M, multiple optima; OU, Ornstein-Uhlenbeck; V, multiple rates.

Table S9. Mean volumetric proportional contribution of 11 prey categories to the stomach contents of 44 species of cichlid.

Figure S1. Comparison of the number of shifts and their bootstrap support from the observed dietary data of 44 species of Cichlinae.

Figure S2. Frequency and location of adaptive shifts generated under Brownian motion (BM)-simulated characters.

Figure S3. Reconstruction of dietary niches using SIMMAP based on two (left) or three (right) groups.

Figure S4. Reconstruction of feeding specialization groups using SIMMAP.

SHARED DATA

All chronograms from López-Fernández *et al.* (2013) and all functional trait data are available through Dryad (<https://doi.org/10.5061/dryad.34621> and <https://doi.org/10.5061/dryad.j04r6>). The dated tree (derived from the phylogeny of Ilves *et al.*, 2018) and all dietary data (Table S9) are available in the Supporting Information.