Ecological diversification associated with the pharyngeal jaw diversity of Neotropical cichlid fishes

Edward D. Burress*

Department of Biological Sciences and Auburn University Museum of Natural History, Auburn University, Auburn, AL 36849, USA

Summary

1. Innovations can facilitate bursts of diversification by increasing access to novel resources and the attainment of novel functional designs. Pharyngognathy, exhibited by highly diverse groups such as wrasses and cichlid fishes, is hypothesized to increase foraging capacity and efficiency.

2. Here, I test the hypothesis that pharyngeal jaw shape and tooth morphology are adaptive in an ecologically diverse radiation of Neotropical cichlid fishes that spans North, Central and South America.

3. I partitioned species into generalized trophic guilds using published stomach content analyses and quantified shape variation of the lower pharyngeal jaw (LPJ) using geometric morphometrics. Additionally, I tested for convergence in LPJ shape and trophic guild by mapping the phylogeny onto the principal components and testing for shifts towards similar evolutionary regimes.

4. Major LPJ shape variation included the length and orientation (i.e. narrow or wide) of the lateral processes and length of the medial process, which varied based on the proportion of fishes and plants consumed. Pharyngeal tooth number, diversity and the frequency of tooth types were not evenly distributed among trophic guilds. There were seven distinct evolutionary regimes that converged upon four optima.

5. Pharyngeal jaw diversification is associated with the exploitation of novel resources among Neotropical cichlids such that pharyngeal specialization has increased access to otherwise poorly accessible resources, such as resources that are difficult to crush (e.g. hard-shelled organisms) and assimilate (e.g. algae).

Key-words: adaptive radiation, Cichlidae, co-evolution, dentition, functional morphology, guild, niche, pharyngognathy

Introduction

Throughout the history of life, innovations have periodically generated bursts of diversification. Many bony fishes exhibit gill arches that are modified to assist with prey processing (Liem 1973). In some cases, the pharyngeal arches have been modified into a functional set of jaws (i.e. pharyngeal jaw apparatus). A series of modifications to the pharyngeal apparatus have been hypothesized to increase foraging capacity, versatility and efficiency (Liem 1973; Hulsey 2006; Wainwright *et al*. 2012) and are associated with some of the most species-rich and functionally diverse lineages of fishes, perhaps most notably marine

wrasses and freshwater cichlids (Wainwright *et al*. 2012). Oral and pharyngeal jaws are developmentally decoupled such that they are derived from the first (in part) and seventh pharyngeal arches, respectively (Fraser *et al*. 2009). However, both sets of jaws operate in tandem such that the oral jaws are involved in food acquisition (i.e. grasping prey) while the pharyngeal jaws process food (i.e. tearing and/or crushing prey; Liem 1973). This functional decoupling is hypothesized to represent a major release of the oral jaws from demands associated with processing food (Liem 1973) and may have facilitated the trophic diversification of cichlid fishes.

Among cichlids, the pharyngeal apparatus consists of two independent upper bones and two lower bones that are sutured together into a single structure (Fig. 1). These bones have various degrees of unicuspid and bicuspid

*Correspondence author. E-mail: edb0014@auburn.edu
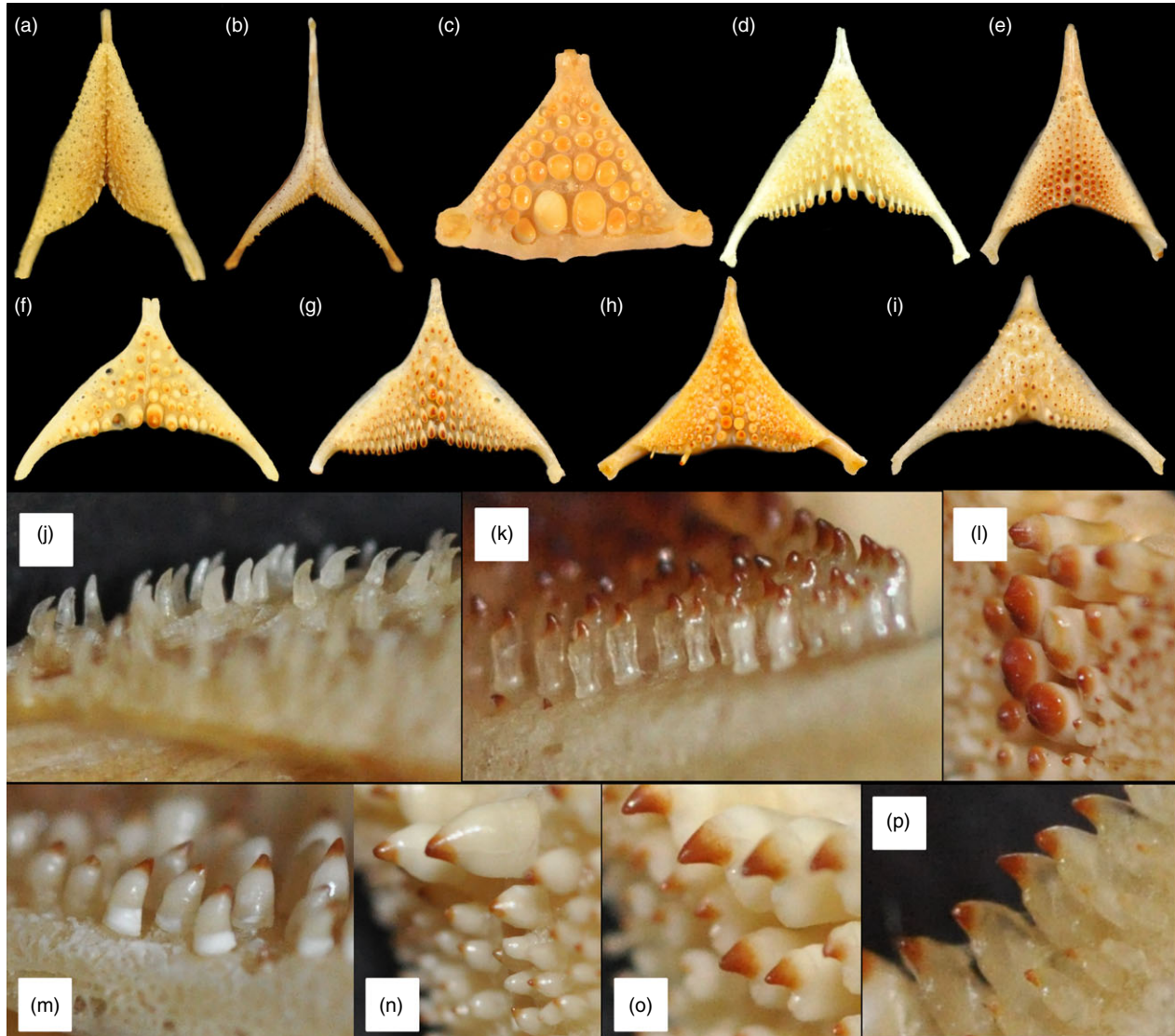
Fig. 1. Representative lower pharyngeal jaw diversity among Neotropical cichlids: (a) *Cichla intermedia*, (b) *C. flavescens*, (c) *Crenicichla minuano*, (d) *Parachromis dovii*, (e) *Geophagus abalios*, (f) *Crenicichla reticulata*, (g) *Tomocichla tuba*, (h) *Paraneetroplus maculicauda* and (i) *Aequidens diadema*. Pharyngeal tooth types observed among Neotropical cichlids (adapted from Casciotta & Arratia 1993): (j) unicuspid, recurved: *Cichla temensis*; (k) generic bicuspid: *Geophagus abalios*; (l) molariform: *Herichthys cyanoguttatus*; (m) bevelled: *Crenicichla scottii*; (n) generic unicuspid, conical: *Crenicichla scottii*; (o) bicuspid, recurved: *Parachromis dovii*; and (p) bicuspid, crenulate: *Crenicichla lugubris*. Images are not to scale.

teeth that may be conical, flattened or molariform (Fig 1; Casciotta & Arratia 1993; Hulsey 2006). Well-developed musculature modulates these bones to allow for considerable manipulation of prey, ranging from crushing hard-shelled organisms (i.e. snails and bivalves) to sifting minute edible items from mouthfuls of sediment, which would not be feasible tasks for oral jaws (Drucker & Jensen 1991; Hulsey 2006). A muscular sling contracts, pulling the lower pharyngeal jaw (LPJ) dorsally against the upper jaws, which are stabilized by and articulate with the neurocranium (Wainwright *et al.* 2012). LPJ shape and dentition are plastic (Huysseune 1995; Muschick *et al.* 2011; Gunter *et al.* 2013) and continuous pharyngeal tooth replacement allows for maintenance of tooth size, shape

and number (Huysseune 1995). Thus, LPJ shape and dentition may respond to functional demands imposed by specific prey items (Hulsey *et al.* 2008).

The shape and dentition of the LPJ have been associated with the trophic characteristics of species. For example, using comparisons among species pairs, reinforcement of the LPJ and molariform teeth is associated with durophagy (i.e. specialization on hard-shelled organisms; Hulsey 2006). Hypertrophied LPJs enable more crushing force (Meyer 1989), thereby reducing handling time associated with manipulating hard-shelled prey (Hulsey, Hendrickson & de León 2005). Robust, molariform teeth are often located along the posterior midline where most structural stress is concentrated during mastication of

difficult to crush prey (Hulsey *et al.* 2008). Durophagy has been a key axis of divergence during speciation of some river- and lake-dwelling cichlids (Kidd, Kidd & Kocher 2006; Burress *et al.* 2013a) and is a frequent source of polymorphism (Hulsey *et al.* 2008). Among other guilds, associations between form and function of the pharyngeal jaws are less established. Many types of pharyngeal dentition are adapted for piscivory, such as recurved unicuspid, bevelled and bicuspid crenulate teeth (Witte & Barel 1976). Among piscivores, where prey does not require crushing force, but is instead consumed whole, the LPJ is often reduced and the teeth are adapted for merely grasping prey (Hellig *et al.* 2010; Burress *et al.* 2013a). Reduced LPJ morphology has also been linked to shifts towards utilization of pelagic resources (Barluenga *et al.* 2006). Links between pharyngeal jaw morphology and ecological guilds, such as omnivory, herbivory and zooplanktivory, are hypothetical, but many potential associations have recently been proposed (Burress 2014).

Cichlids exhibit fast rates of speciation and phenotypic diversification among fishes (Near *et al.* 2013; Rabosky *et al.* 2013). Pharyngognathy is hypothesized to represent one of a few key innovations that facilitated the ecological diversification and subsequent proliferation of cichlids (Liem 1973; reviewed in Burress 2014). This dynamic apparatus emerged via functional and morphological reorganization and subsequent integration of existing muscles and bones; thus, functional specialization may occur without altering the modularity of the various components (Liem 1973). Therefore, small structural modifications in shape or dentition may be sufficient for dramatic and rapid movement across regions of the adaptive landscape (Liem 1973). The adaptive value of pharyngeal shape and dentition is well documented among specific lineages or populations cichlids (Hulsey 2006; Hulsey *et al.* 2008; Burress *et al.* 2013a); however, this has not been explicitly tested using a large clade within a single analytical framework (Burress 2014). Here, I formally test this facet of the hypothesized utility of pharyngeal diversification: that LPJ shape and tooth morphology are associated with resource exploitation across trophic guilds in a transcontinental adaptive radiation of Neotropical cichlid fishes.

Materials and methods

I dissected the LPJ of 146 individuals from 45 Neotropical cichlid species accessioned in the Auburn University Museum of Natural History. The LPJ was photographed in occlusal view using a mounted Nikon D5100 digital camera (Nikon Corporation, Tokyo, Japan). I used 17 landmarks that describe the orientation and length of the medial and lateral processes, size of the dentigerous area and curvature of the LPJ (Fig. S1, Supporting information). Sliding landmarks were equally spaced along the lateral and posterior margins (Fig. S1). Sliding landmarks are not associated with a homologous structure, but are used to quantify the curvature of an object. Photographs were digitized and land-marked using TPSUTIL (Rohlf 2004) and TPSDIG2 (Rohlf 2006), respectively. I used Procrustes fit to generate a consensus shape

and remove variation associated with size and rotation using tpsRelw (Rohlf 2007). I then used these coordinates to generate phylogenetic principal components using the phyl.pca command, which is part of the phytools package in R (Revell 2009). Phylogenetic PCA generates variables that describe major axes of shape variation after the removal of phylogenetic correlation (Revell 2009). See below for details of the phylogeny used in this analysis. Accession numbers and additional information about the material examined are listed in Table S1.

I partitioned species into generalized trophic guilds using existing literature (Table 1): obligate and facultative piscivores, invertivore (i.e. soft bodied), sifting and non-sifting omnivores, molluskivores and herbivores. Obligate piscivores consume almost exclusively fishes, whereas facultative piscivores consume primarily fishes (i.e. >55% by volume) but may consume lesser fractions of invertebrates. Sifting and non-sifting omnivores were separated based on descriptions of their feeding behaviour and morphologies (López-Fernández *et al.* 2012, 2014; Arbour & López-Fernández 2013). Invertivores, molluskivores and herbivores were coded based on invertebrates, mollusks and plant material representing their primary prey item, respectively. The use of categories to define trophic patterns that are continuous is admittedly limited and subjective. For example, omnivory is ubiquitous and varies widely among species (Burress 2014). Thus, owing to this limitation, I also used continuous variables (i.e. proportions of prey items) based on detailed stomach content analyses (Table S2). Itemized proportions were pooled into three generalized categories: plants, invertebrates and fishes that were conserved across most published analyses. Values were taken directly from text, tables or estimated from figures if necessary. If results were partitioned across age classes, seasons or habitats, all such categories were pooled, except with respect to age classes of piscivores due to the well-established ontogeny of piscivory (Winemiller 1989; Burress *et al.* 2013b). In such cases, only adult classes were considered. I used linear regressions to test whether the proportion of these prey items could predict LPJ shape (i.e. pPCs). Pharyngeal dentition was quantified by counting the number of teeth using a dissecting microscope. Intact, broken and missing teeth were included in counts. I compared the number of pharyngeal teeth among trophic guilds using Tukey's Honestly Significant Difference (HSD) *post hoc* tests. All statistics were performed in R. I also recorded the presence of specialized dentition types based on those described by Casciotta & Arratia (1993): (i) posteriorly recurved unicuspid, (ii) anteriorly recurved unicuspid, (iii) anteriorly recurved bicuspid, (iv) crenulate bicuspid, (v) bevelled and (vi) molariform. All species had (at least) generic conical unicuspid or bicuspid dentition and were thus not considered specialized, unless they were enlarged, recurved and positioned along the posterior margin. Terms used in reference to the different regions and aspects of the LPJ are illustrated in Fig. S2.

To evaluate LPJ shape and dietary convergence (or divergence) among Neotropical cichlids, I estimated the phylogeny and divergence times using BEAST (Drummond *et al.* 2012) based on the input file from López-Fernández *et al.* (2013a; doi: 10.5061/dryad.34621), which included five loci (cytb, 16S, ND4, RAG2 and S7 intron 1) for 166 species of cichlids (originally from López-Fernández, Winemiller & Honeycutt, 2010). Some additional sequences (*Crenicichla minuano* and *C. scottii*) were also added (originally from Piálek *et al.* 2012). Settings were unchanged such as clades were constrained to be monophyletic, speciation priors, models of sequence evolution across gene

Table 1. Forty-five Neotropical cichlid species examined in this study and references used to categorize species into generalized trophic guilds. Subguilds consist of obligate (O) and facultative (F) piscivores and sifting (S) and non-sifting (NS) omnivores. Abbreviations correspond to Fig. 2

Species	N	Abbreviation	Guild	References
Acarichthys heckelii	5	Ac_he	Omnivore-NS	Hawlitschek, Yamamoto & Carvalho-Neto (2013)
Acaronia nassa	4	Ac_na	Piscivore-F	de Mérona & Rankin de Mérona (2004)
Amphilophus citrinellus	2	Am_ci	Omnivore-NS	Elmer *et al.* (2010)
Andinoacara pulcher	4	An_pu	Omnivore-NS	Winemiller, Kelso-Winemiller & Brenkert (1995)
Apistogramma hoignei	2	Ap_ho	Omnivore-NS	López-Fernández *et al.* (2012)
Archocentrus centrarchus	1	Ar_ce	Omnivore-NS	Winemiller, Kelso-Winemiller & Brenkert (1995)
Astatheros robertsoni	2	As_ro	Omnivore-S	Winemiller, Kelso-Winemiller & Brenkert (1995)
Astronotus ocellatus	5	As_oc	Invertivore	López-Fernández *et al.* (2012)
Australoheros facetus	2	Au_fa	Omnivore-NS	Yafe *et al.* (2002)
Biotodoma wavrini	4	Bi_wa	Omnivore-NS	López-Fernández *et al.* (2012)
Caquetaia kraussii	3	Ca_kr	Piscivore-F	Winemiller (1989)
Chaetobranchus flavescens	5	Ch_fl	Zooplanktivore	de Mérona & Rankin de Mérona (2004)
Cichla monoculus	2	Ci_mo	Piscivore-O	de Mérona & Rankin de Mérona (2004)
Cichla orinocensis	1	Ci_or	Piscivore-O	López-Fernández *et al.* (2012)
Cichla temensis	5	Ci_te	Piscivore-O	López-Fernández *et al.* (2012)
Cichlasoma orinocense	4	Ci_on	Invertivore	López-Fernández *et al.* (2012)
Cichlasoma urophthalmus	2	Ci_ur	Omnivore-NS	Hinojosa-Garro *et al.* (2013)
Crenicichla lugubris	5	Cr_lu	Piscivore-F	López-Fernández *et al.* (2012)
Crenicichla minuano	6	Cr_mi	Molluskivore	Burress *et al.* (2013a)
Crenicichla scottii	5	Cr_sc	Piscivore-F	Burress *et al.* (2013b)
Crenicichla wallacii	1	Cr_wa	Invertivore	López-Fernández *et al.* (2012)
Cryptoheros nigrofasciatus	3	Cr_ni	Herbivore	Winemiller, Kelso-Winemiller & Brenkert (1995)
Cryptoheros spilurus	4	Cr_sp	Herbivore	Cochran-Biederman & Winemiller (2010)
Dicrossus filamentosus	3	Di_fi	Invertivore	López-Fernández *et al.* (2012)
Geophagus abalios	5	Ge_ab	Omnivore-S	López-Fernández *et al.* (2012)
Geophagus dicrozoster	4	Ge_di	Omnivore-S	López-Fernández *et al.* (2012)
Geophagus steindachneri	3	Ge_st	Omnivore-S	López-Fernández *et al.* (2012)
Geophagus surinamensis	2	Ge_su	Omnivore-S	Winemiller, Kelso-Winemiller & Brenkert (1995)
Guianacara stergiosi	3	Gu_st	Omnivore-NS	López-Fernández *et al.* (2012)
Gymnogeophagus rhabdotus	2	Gy_rh	Omnivore-S	Yafe *et al.* (2002)
Herichthys cyanoguttatus	2	He_cy	Molluskivore	Hulsey *et al.* (2006)
Heros severus	5	He_se	Omnivore-NS	López-Fernández *et al.* (2012)
Hoplarchus psittacus	5	Ho_ps	Omnivore-NS	López-Fernández *et al.* (2012)
Hypsophrys nicaraguensis	1	Hy_ni	Herbivore	Higham *et al.* (2006)
Hypsophrys nematopus	1	Ne_ne	Herbivore	Burcham (1988)
Mesonauta festivum	4	Me_fe	Invertivore	Winemiller *et al.* (2011)
Mikrogeophagus ramirezi	4	Mi_ra	Invertivore	López-Fernández *et al.* (2012)
Parachromis dovii	1	Pa_do	Piscivore-F	Winemiller, Kelso-Winemiller & Brenkert (1995)
Paraneetroplus maculicauda	1	Pa_ma	Herbivore	Cochran-Biederman & Winemiller (2010)
Petenia splendida	3	Pe_sp	Piscivore-F	Cochran-Biederman & Winemiller (2010)
Satanoperca daemon	5	Sa_da	Omnivore-S	López-Fernández *et al.* (2012)
Satanoperca mapiritensis	3	Sa_ma	Omnivore-S	López-Fernández *et al.* (2012)
Symphysodon discus	1	Sy_di	Omnivore-NS	Crampton (2008)
Thorichthys meeki	7	Th_me	Molluskivore	Cochran-Biederman & Winemiller (2010)
Tomocichla tuba	4	To_tu	Herbivore	Winemiller, Kelso-Winemiller & Brenkert (1995)

partitions, divergence time calibrations, settings of the uncorrelated relaxed clock model (Drummond *et al.* 2012) and MCMC settings. Analysis was performed using BEAST v1.8.0 and BEAGLE library v2.1 on the CIPRES Web Portal (Miller, Pfeiffer & Schwartz 2010; Ayres *et al.* 2011; Drummond *et al.* 2012). Convergence was assessed with TRACER v1.5 (Rambaut & Drummond 2009), and the maximum clade credibility tree was computed from the posterior distribution of trees with 10% burnin using TREEANNOTATOR v1.7.5 (Rambaut & Drummond 2007). The tree was then pruned using the ape package in R.

To estimate ancestral states, I mapped the phylogeny onto pPC1 and pPC2 (i.e. phylomorphospace) using MESQUITE v2.75 (Maddison & Maddison 2011). In this procedure, internal (i.e.

ancestral) node values are estimated using weighted squared-change parsimony (Maddison 1991; Revell *et al.* 2007). The resulting phylomorphospace depicts both the magnitude and the direction of shape change along each branch. Lastly, I estimated the evolutionary history of trophic guild diversification using maximum-likelihood (mk1 model) character state reconstruction in Mesquite. Maximum-likelihood analyses find the ancestral states (e.g. internal nodes) that maximize the probability that the observed character states (e.g. terminal nodes) would evolve under a stochastic model of evolution (Schluter *et al.* 1997; Pagel 1999). The data matrix was coded using the previously described trophic guilds (Table 1). This reconstruction was then overlaid onto phylomorphospace so that the
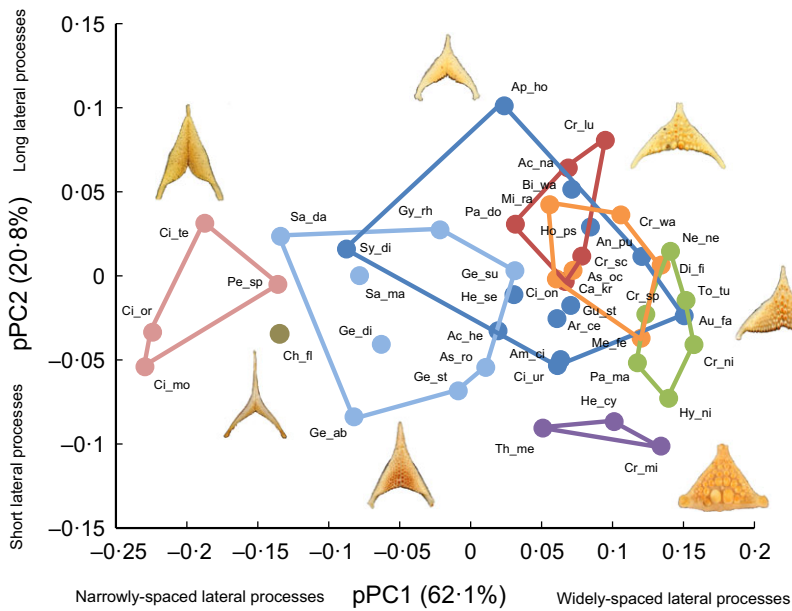
Fig. 2. Size and phylogeny corrected shape variation of the lower pharyngeal jaw among 45 Neotropical cichlid species. Colours correspond to generalized trophic guilds: obligate piscivore (pink), facultative piscivore (red), invertivore (orange), sifting omnivore (light blue), non-sifting omnivore (dark blue), molluskivore (purple), herbivore (green) and zooplanktivore (brown). Images are meant to serve as examples of jaws associated with the extremes of morphospace. For references to colour see the online version.

evolution of LPJ shape and trophic guild could be interpreted simultaneously.

To access the adaptive landscape, and quantify convergence, I used SURFACE analysis (Ingram & Mahler 2013), which uses stepwise Akaike Information Criterion to locate regime shifts (k) on the phylogeny and then identify whether these shifts are towards convergent regimes. This process involves iteratively adding regime shifts to a Hansen model, then iteratively removing shifts to identify convergent regimes (k'). The reduction in complexity ($k-k'$) corresponds to the number of regimes that can be collapsed into an existing regime (i.e. convergence; Δk). Distinct LPJ shape evolutionary regimes were visualized by overlaying the convergent and non-convergent regimes onto the phylogeny. Additionally, these regimes, and estimated optima, were projected onto LPJ morphospace to visualize the adaptive landscape associated with Neotropical cichlid LPJ diversity. To test whether convergence was significantly different than could result from a null expectation, I generated 100 simulated data sets under a 'null' model using the surfaceSimulate function (Ingram & Mahler 2013). I then tested for convergence (Δk) using these simulated data and calculated the probability of the observed data based on the null distribution.

Results

PHARYNGEAL SHAPE

Shape analysis resulted in two biologically meaningful pPCs: pPC1 described 62·1% of the total variation and represents variation between narrow to broadly spaced lateral processes; and pPC2 described 20·8% of the total variation and represents variation between long and short lateral processes with small and large dentigerous areas, respectively. Invertivores and herbivores are associated with the extremes of positive pPC1 values (i.e. widely spaced lateral processes; Fig. 2a). Invertivores, herbivores and non-sifting omnivores have converged in this region

of morphospace. Molluskivores occupy a unique region of morphospace along the extremes of both positive pPC1 and negative pPC2 values (e.g. *Thorichthys* and *Herichthys*; Fig. 2a), reflecting their short, widely spaced lateral processes and large dentigerous areas. Omnivores occupy a large region of morphospace that overlaps all other guilds except molluskivores (Fig. 2a). Piscivores exhibit two discrete LPJ morphologies, those associated with negative pPC1 and near-zero pPC2 values (e.g. *Cichla* and *Petenia*) and those associated with positive pPC1 and pPC2 values (e.g. *Crenicichla*, *Acaronia*, *Caquetaia* and *Parachromis*; Fig. 2a). The zooplanktivore (e.g. *Chaetobranchus flavescens*) had a LPJ shape similar to that of some piscivores (Fig. 2a). The proportion of fishes consumed significantly predicted pPC1 ($R^2 = 0·198$; $F_{1,44} = 10·61$; $P = 0·0022$) and exhibited a non-significant trend with pPC2 ($R^2 = 0·078$; $F_{1,44} = 3·66$; $P = 0·062$) such that piscivory tends to increase with longer lateral processes and small dentigerous areas, respectively (Fig. 3). The proportion of plants consumed also predicted pPC1 ($R^2 = 0·158$; $F_{1,44} = 8·08$; $P = 0·0069$), but did not predict pPC2 ($R^2 = 0·047$; $F_{1,44} = 2·12$; $P = 0·15$) such that herbivory is associated with short, widely spaced lateral processes (Fig. 3). However, the proportion of invertebrates consumed did not predict pPC1 ($R^2 = 0·002$; $F_{1,44} = 0·082$; $P = 0·776$) or pPC2 ($R^2 = 0·006$; $F_{1,44} = 0·246$; $P = 0·622$) (Fig. 3). PC1 and PC2 likely represent continua between high and low degrees of processing potential (i.e. tearing) before ingestion and high and low potential for crushing force, respectively.

PHARYNGEAL DENTITION

The number of pharyngeal teeth varied among trophic guilds, with obligate piscivores (e.g. *Cichla*) having significantly more teeth than other guilds (Fig. 4a). Sifting

omnivores had significantly more pharyngeal teeth than non-sifting species (Fig. 4a). Invertivores and molluskivores had the least numbers of teeth (Fig. 4a). I observed all pharyngeal tooth types discussed by Casciotta & Arratia (1993) (Fig. 1). Pharyngeal tooth diversity (Fig. 4b) varied among trophic guilds, such that dentition types were not evenly distributed among guilds (Fig. 4c). Facultative piscivores had the most diverse pharyngeal dentition. Bevelled unicuspid and bicuspid crenulate teeth were exclusively associated with facultative piscivores (Fig. 4c) and were positioned along the lateral and posterior margins, respectively (Fig. 1). Obligate piscivores (e.g. *Cichla*) and a zooplanktivore (e.g. *Chaetobranchus flavenscens*) had poor pharyngeal tooth diversity, possessing exclusively posteriorly recurved unicuspid and anteriorly recurved bicuspid dentition, respectively (Fig. 4b,c). Invertivores, molluskivores, omnivores and herbivores had similar diversity (Fig. 4b), but differed in the frequency of tooth types (Fig. 4c). Many species of all guilds had anteriorly recurred teeth along the posterior margin. Among piscivores, these were typically unicuspid and conical or weakly laterally compressed. These teeth among omnivores and herbivores were bicuspid and strongly laterally compressed into blade-like structures.

EVOLUTIONARY REGIMES AND CONVERGENCE

The evolutionary relationships recovered in the MMC tree (Fig. S3) were consistent with those of López-Fernández *et al.* (2013a,b) and Říčan *et al.* (2013) and indicate several cases of convergence in both trophic guild and LPJ morphology. Omnivory (i.e. sifting or non-sifting) was estimated as the ancestral trophic state of all major clades (Fig S3). Molluskivory evolved independently at least twice, within *Crenicichla* and the *Thorichthys+Herichthys* clade (Fig. S3). Piscivory evolved independently at least six times (Fig. S3). Invertivory and herbivory also appear to have evolved multiple times among disparate lineages (Fig. S3). Ancestral state reconstruction of LPJ shape suggests that the ancestral LPJ shape is associated with extant omnivorous (e.g. sifting and non-sifting) species and ancestral nodes estimated to be omnivorous (Fig. 5). Additionally, shifts in dietary patterns, such as towards piscivory, herbivory and molluskivory, appear to coincide with shifts towards LPJ shapes along the periphery of phylomorphospace (Fig. 5). There were seven evolutionary regimes in LPJ shape diversification (Table S3; Fig. 6), which resulted in shifts towards four optima estimated using SURFACE analysis (Table S3; Fig 6b,c). Most species exhibited a non-convergent regime associated with widely spaced lateral processes (i.e. positive pPC1 values; Fig. 6a, b). A second non-convergent regime occurred within the clade containing *Geophagus* and *Gymnogeophagus* (Fig. 6a). There were also two cases of convergent regimes. First, *Petenia splendida*, *C. flavescens* and three *Cichla* species exhibit shifts towards convergent regimes, namely LPJ morphologies characterized by elongate, narrowly spaced

lateral processes (Fig. 6a,b,d). Secondly, *Symphysodon discus*, *Apistogramma hoignei*, *Satanoperca daemon* and *Satanoperca mapiritensis* exhibit shifts towards convergent regimes associated with relatively short, widely spaced lateral processes and convex lateral margins (Fig. 6b,d). The null distribution resulted in 0–4 cases of convergence (Δk), with a mean of one case of convergence ($\Delta k = 1.0$; Fig. 6c). Five (of 100) simulated data sets resulted in Δk values equal to or larger than that of the observed data. Therefore, the observed data resulted in convergence that exceeds expectations under the null model ($P = 0.05$).

Discussion

MORPHOLOGY-TO-ECOLOGY LINKAGES

Throughout the history of life, structural or functional innovations have periodically facilitated a burst of diversification. Pharyngognathy has been hypothesized as one such crucial innovation (Liem 1973; Wainwright *et al.* 2012; Burress 2014). Here, variation in LPJ morphology and dentition was associated with trophic function at broad evolutionary and ecological scales. The proportion of fishes and plant material consumed predicted important shape variation in the LPJ, suggesting they may elicit functional demands that have led to selection on LPJ shape. The capacity to generate crushing force was also a major source of variation among Neotropical cichlids. Many trophic functions require intermediate degrees of crushing force and prey manipulation prior to ingestion. For example, molluskivores, herbivores, invertivores and facultative piscivores formed a continuum along pPC2 representing the transition from high to low functional demands associated with pre-ingestion prey processing. On one extreme, molluskivores possessed a hypertrophied LPJ with short lateral and medial processes, and robust molariform teeth necessary for processing hard-shelled organisms (Hulsey 2006). These features allow the pharyngeal apparatus to tolerate the stress incurred during shell crushing (Hulsey *et al.* 2008). The musculature that articulates the pharyngeal bones is also well developed among these species (Liem 1973). In contrast, herbivory requires minimal crushing force but may have functional demands associated with prolonged pharyngeal processing necessary to facilitate assimilation of nutrient-poor plant material (Burress 2014). Densely packed dentition may facilitate this process among herbivores, which consume large fractions of algae, diatoms and/or vegetative detritus (e.g. leaves and twig fragments). For example, *Hypsophrys nematopus* grazes large fractions of algae and diatoms from rock surfaces (Burcham 1988; Winemiller, Kelso-Winemiller & Brenkert 1995). Many tightly spaced conical teeth may facilitate rupturing algae and diatom cells by acting as a mill that grinds material (Hulsey, Hendrickson & de León 2005) and subsequently allow better assimilation (Xie 2001). In contrast, many herbivorous Afrotropical cichlids are physiologically specialized and equipped
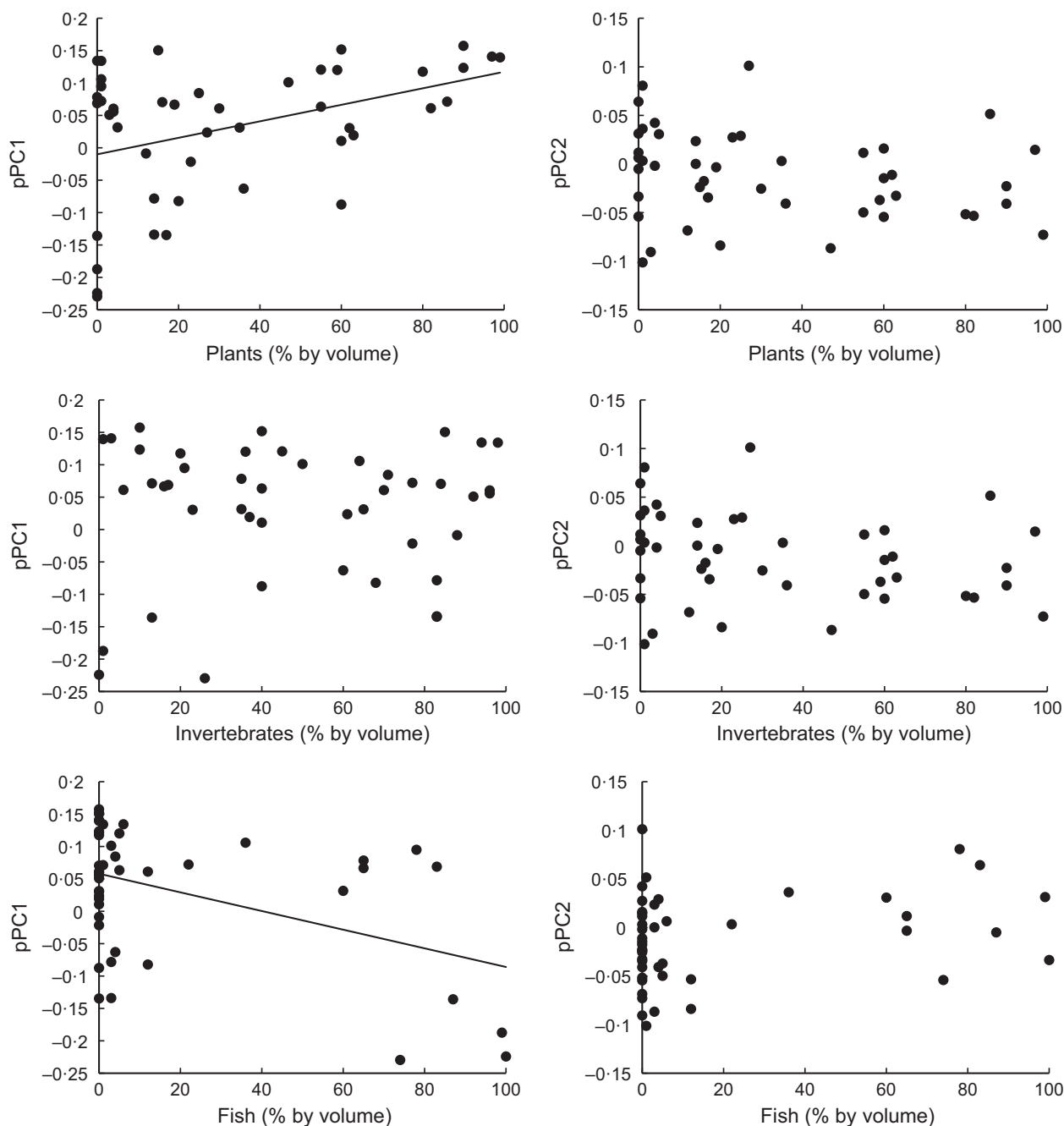
Fig. 3. Relationships between diet and phylogenetic principal components (pPC) that reflect major shape variation among the lower pharyngeal jaws of Neotropical cichlid fishes. Lines denote significant relationships. Positive and negative values of pPC1 are associated with widely and narrowly spaced lateral processes, respectively. Positive and negative values of pPC2 are associated with long and short lateral processes, respectively.

for algivory such that they have long digestive tracts and specific digestive enzymes (Sturmbaurer, Mark & Dallinger 1992) and often possess flattened LPJ teeth to merely pack material before ingestion (Liem 1973). Thus, modification of pharyngeal dentition and the digestive system may represent different solutions to managing poor quality food resources exploited by herbivorous Neotropical and Afrotropical cichlids, respectively.

Omnivory is ecologically complex and functionally demanding because an organism feeds at multiple trophic levels and consumes prey that vary greatly in nutrient content and digestibility (Rudnick & Resh 2005). Indeed, omnivores had highly variable LPJ morphologies, of which the non-sifting species partially overlapped with facultative piscivores, invertivores and herbivores in morphospace. Thus, this LPJ shape may represent a generalized shape suitable for many trophic functions such that different trophic niches may be utilized by merely modifying the types and number of pharyngeal teeth. For example, evolutionary shifts from non-sifting omnivory to
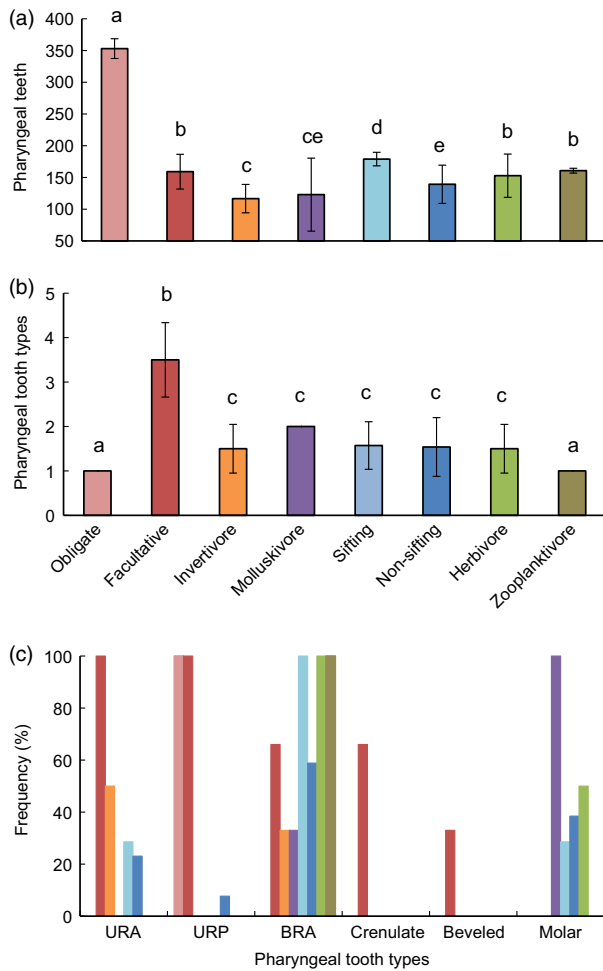
Fig. 4. Mean (±SD) pharyngeal tooth number (a), tooth types (b) and frequency (c) among generalized trophic guilds. Tooth types are adapted from Casciotta & Arratia (1993) and include (i) unicuspid, recurved anteriorly (URA), (ii) unicuspid, recurved posteriorly (URP), (iii) bicuspid, recurved anteriorly (BRA), (iv) bicuspid crenulate, (v) bevelled and (vi) molariform. See text for discussion of tooth types. Letters denote significant comparisons based on Tukey's Honestly Significant Difference *post hoc* tests. Colour codes are consistent among panels. For references to colour see the online version.

herbivory consists of increasing tooth number frequency of tooth types, rather than significant modification of LPJ shape. Indeed, this region of morphospace (i.e. positive pPC1 values) represents an adaptive optimum. In contrast, sifting omnivores occupy a unique region of morphospace with narrowly spaced lateral processes and long medial processes. This LPJ morphology may have co-evolved with these species' laterally compressed bodies (i.e. narrowly spaced lateral processes) and large buccal cavities (i.e. long medial processes) associated with sifting behaviour and mouth brooding, respectively (López-Fernández et al. 2012; Arbour & López-Fernandez 2013). Because foraging via benthic sifting and mouth brooding co-occur in many groups such as *Geophagus* and *Satanoperca*, LPJ shape may be largely the result of integration
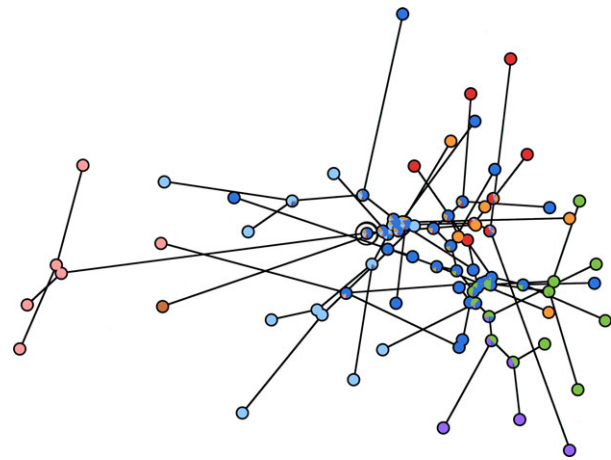

Fig. 5. Phylomorphospace depicting the ancestral state reconstruction of lower pharyngeal jaw (LPJ) shape and trophic guild. Branches reflect the magnitude and direction of changes in LPJ shape. Values of internal nodes were calculated using weighted square-change parsimony (Maddison 1991; Revell *et al.* 2007). Pie diagrams depict the likelihood of the ancestral trophic state based on maximum-likelihood (mk1 model) character state reconstruction (Maddison & Maddison 2011). Colour codes correspond to those in Fig. 2. For references to colour see the online version.

with other phenotypic traits associated with foraging and reproductive behaviour. If this is the case, selection favoured LPJ phenotypes that maximize the space available for sifting food and brooding young. Many of these sifting species (i.e. *Gymnogeophagus* and *Geophagus*) exhibit regime shifts towards an optima associated with narrowly spaced lateral processes of intermediate length with slightly concave lateral margins. The remaining sifting omnivores (e.g. *Satanoperca*), along with some non-sifting omnivores (i.e., *Apistogramma* and *Symphysodon*), exhibit regime shifts that converge upon an optimum associated with narrowly spaced lateral processes of intermediate length with slightly convex lateral margins.

Sifting omnivores also possessed more pharyngeal teeth than non-sifters and also tended to have bicuspid dentition. Benthic sifting is often considered an adaptive quality among Neotropical cichlids (López-Fernández *et al.* 2013a, 2014), and may encompass species that intentionally or inadvertently ingest plant material while targeting animal prey buried underneath sediments and detritus (López-Fernández *et al.* 2014). López-Fernández *et al.* (2014) found that sifting species were not more effective at physically unearthing buried prey, thus the functional advantages of LPJ specialization may be manifested after prey is taken into the mouth. The pharyngeal jaw functions much like a rake, separating edible materials from sediment (i.e. winnowing), which are expelled through the gill openings or the mouth (Drucker & Jensen 1991). Densely packed pharyngeal teeth may facilitate this process by increasing winnowing efficiency or versatility by allowing smaller items to be separated from sediment. The benthivory adaptive peak (López-Fernández *et al.* 2013a, 2014) is further refined here considering the existence of
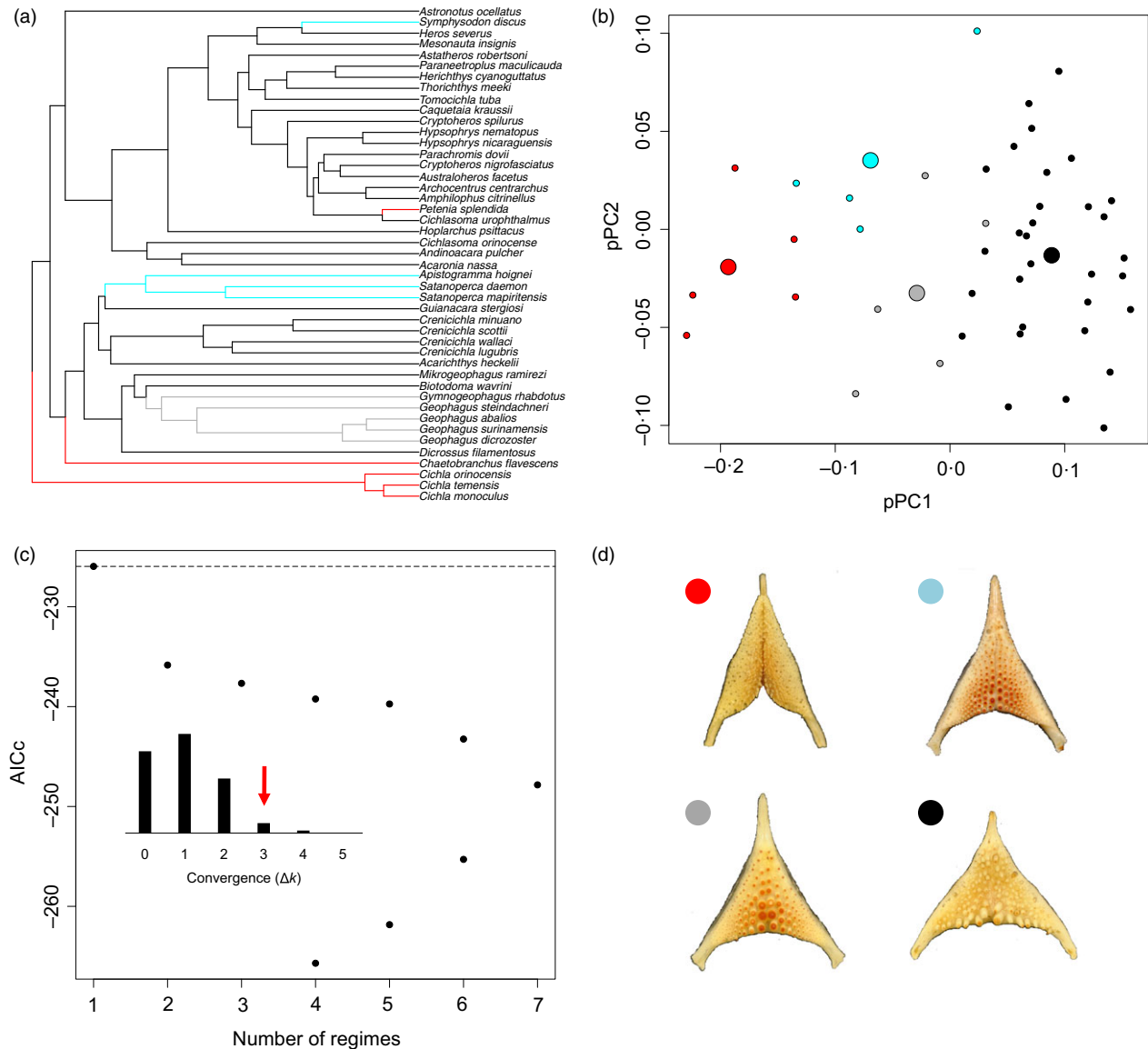
Fig. 6. Results from SURFACE analysis of lower pharyngeal jaw (LPJ) shape among Neotropical cichlid fishes. (a) Phylogeny, with convergent (coloured) and non-convergent (greyscale) regimes estimated from the best-fit model. (b) Trait values (phylogenetic principle components) that describe LPJ shape for each species (small circles) and estimated optima (large circles). Regime colours match those in the phylogeny. (c) Change in AICc during the forward and backward phases of the analysis. Dashed line represents the AICc value for an Ornstein–Uhlenbeck model of evolution. The frequency distribution of null models is inset. The red arrow indicates the position of the observed data ($P = 0.05$). (d) Representative LPJs near each optima. For references to colour see the online version.

two distinct LPJ shape optima among these species. As described above, these optima can be distinguished by the concave or convex curvature of the lateral margins and highlight the complexity of the adaptive landscape such that a previously identified adaptive peak based on behaviour and external morphology (López-Fernández *et al.* 2013a, 2014) may encompass two distinct trophic optima as revealed by regimes of LPJ evolution.

Piscivory is often considered an adaptive peak among fishes (Collar *et al.* 2009) and specifically among Neotropical cichlids (López-Fernández *et al.* 2013a). Among Neotropical cichlids, piscivory is associated with two discrete LPJ morphologies: long, narrowly spaced lateral

processes (e.g. *Cichla* and *Petenia*) and long, widely spaced lateral processes (e.g. *Acaronia*, *Caquetaia*, *Crenicichla* and *Parachromis*). These groups represent obligate and facultative piscivores, respectively. *Cichla* have poorly developed musculature associated with the pharyngeal apparatus (Liem 1973) and pharyngeal dentition that is adapted purely for food transport (i.e. grasping), with no provisions for processing prey prior to swallowing (i.e. tearing or crushing). *Cichla*, along with another obligate piscivore (*Petenia*) and a zooplanktivore (*Chateobranchus*), exhibit regime shifts that converge upon a LPJ optima. Piscivory and zooplanktivory likely share a reduced need for pharyngeal processing considering they

swallow prey whole (Winemiller 1989; Burress *et al.* 2015). In contrast, facultative piscivores consume important fractions of secondary prey items and have fewer and more diversely shaped and sized pharyngeal teeth that may facilitate processing this wider variety of prey items (i.e. insects and crustaceans). For example, these species often possess large conical teeth along the LPJ midline, particularly medially at the posterior margin. Functionally, such enlarged dentition may limit pharyngeal gape (Wainwright 1991; Burress *et al.* 2015), but may facilitate exploitation of various prey types that require processing such as tearing or crushing actions (Burress 2014). One such prey item are large littoral crustaceans, which supplement the diets of many of these species (Winemiller, Kelso-Winemiller & Brenkert 1995; Montaña & Winemiller 2009; Burress 2012). Having the functional plasticity necessary to feed on different prey types would be particularly favourable when resource availability fluctuates or is unpredictable (Winemiller 1990; Burress *et al.* 2015).

Phenotypic plasticity can be observed and experimentally induced in cichlid fishes (Huysseune 1995; Hulsey, Hendrickson & de León 2005; Hulsey *et al.* 2008; Muschick *et al.* 2011). Phenotypic plasticity is observed at a scale (i.e. population) that is finer than considered in this study (i.e. species); nonetheless, plasticity in LPJ shape may confound some of the aforementioned interpretations, particularly those associated with foraging along the soft-to-hard-shelled prey axis. Most literature dealing with phenotypic plasticity in cichlid LPJs pertains to exploitation of soft- and hard-bodied prey (Huysseune 1995; Hulsey, Hendrickson & de León 2005; Hulsey 2006); thus, phenotypic plasticity could be particularly confounding in the conclusions drawn about molluskivory. However, there were no obvious mismatches between the dietary literature and LPJ morphologies of the species examined. Nonetheless, plasticity in LPJ shape and dietary patterns likely contribute some of the variation observed in this study and may explain some of the overlap among trophic guilds (Witte, Barel & Van Oijen 1997).

PHYLOGENETIC CONSIDERATIONS

The sister group of Neotropical cichlids, African cichlids (Pseudocrenilabrinae), also exhibit diverse LPJ shapes that appear to be associated with their diet and diversification (Muschick, Indermaur & Salzburger 2012; Muschick *et al.* 2014). Although LPJ diversification has been studied at broad scales (Muschick, Indermaur & Salzburger 2012; Muschick *et al.* 2014), diet has not been explicitly linked to LPJ morphology. Nevertheless, African cichlids exhibit many of the same LPJ modifications as Neotropical cichlids. For example, piscivores have reduced LPJs with elongate lateral and medial processes and recurved teeth (Liem 1973; Hellig *et al.* 2010), whereas molluskivores have hypertrophied LPJs with short processes and molari-

form teeth (Huysseune 1995). Pharyngeal dentition among African cichlids is also characterized by myriad tooth types including many that are observed in Neotropical cichlids, such as unicuspid and bicuspid teeth that can be molariform, flattened, recurved and/or blade-like in shape (Liem 1973). Thus, many of the morphology-to-trophic linkages described herein are likely generalizable across cichlids (Burress 2014).

The sister group of cichlids, the engineer blennies (*Pholidichthys*), have modified pharyngeal arches, but do not exhibit pharyngognathy (Wainwright *et al.* 2012; Near *et al.* 2013). Similar to cichlids, they have two independent upper pharyngeal jaw bones and the 5th ceratobranchials are fused into a single structure (i.e. the LPJ; Springer & Friehofer 1976). However, in contrast, their pharyngeal dentition is simple and non-specialized, consisting of conical recurved teeth (Springer & Friehofer 1976). The Pholidichthyidae consists of only two species, suggesting that merely exhibiting components of phayngognathy is not sufficient for impressive diversification. More disparately related groups, such as the Labridae (wrasses), Embiotocidae (surfperches) and Pomacentridae (damselfishes) exhibit more diverse pharyngeal jaw morphologies and correspondingly more species diversity (Wainwright *et al.* 2012). Surfperches and damselfishes have perhaps the most similar pharyngeal jaw arrangement to cichlids, such that they have muscular slings that allow for a biting mechanism and a fused lower jaw element (Wainwright *et al.* 2012). However, pharyngognathy likely evolved independently among these groups (Wainwright *et al.* 2012).

CONCLUSIONS

Modification of LPJ shape and dentition results in numerous trophic adaptations, most notably associated with the evolution of piscivory, benthic sifting, herbivory and molluskivory, thus, increasing access to nutrient sources, and subsequently ecological niches, that would not otherwise be available. For example, nutrients from hard-shelled organisms such as mollusks or nutrient-poor materials such as algae are exploitable due to specializations of LPJ shape and dentition. LPJ shape (Hulsey *et al.* 2008) and dentition (Hulsey, Hendrickson & de León 2005) may affect the efficiency (i.e. handling time) associated with exploitation of specific prey items or may preclude exploitation altogether (Burress *et al.* 2015). Similar LPJ morphologies and dentition have evolved independently in association with piscivory and sifting omnivory. Thus, LPJ shape and tooth morphology may have an adaptive value within an ecologically diverse and speciose radiation of Neotropical cichlids. Indeed, ecological diversification has played a key role in cichlid speciation. For example, divergence in LPJ shape has been associated with ecological speciation events in Neotropical lakes (Barluenga *et al.* 2006; Elmer *et al.* 2010) and rivers (Burress *et al.* 2013a) and adaptive radiation in the East African Great

Lakes (Muschick, Indermaur & Salzburger 2012). Furthermore, pharyngeal dentition is more diverse than that of the premaxilla and dentary (i.e. oral dentition) among Neotropical cichlids (Casciotta & Arratia 1993) and may have played an important role in their proliferation (Burress 2014), much like craniofacial diversification among Afrotropical cichlids (Hulsey *et al.* 2010). Here, I have extrapolated existing knowledge of the form to function linkage of the LPJ from intraspecific (Hulsey 2006; Hellig *et al.* 2010), species pair (Kidd, Kidd & Kocher 2006; Hulsey *et al.* 2008) and congeneric (Burress *et al.* 2013a, 2015) contexts to a lineage that represents a major teleost adaptive radiation (López-Fernández *et al.* 2013a; McMahan *et al.* 2013) that spans North, Central and South America. Pharyngognathy has likely facilitated the exploitation of novel resources during the rapid and extensive diversification of cichlid fishes.

Acknowledgements

Data accessibility

Data are presented in the manuscript and its Supporting information or are already available in the literature from López-Fernández *et al.* (2013b) http://dx.doi.org/10.5061/dryad.34621.

References

Arbour, J.H. & López-Fernandez, H. (2013) Ecological variation in South American geophagine cichlids arose during an early burst of adaptive morphological and functional evolution. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **280**, 20130849.

Ayres, D.L., Darling, A., Zwickl, D.J., Beerli, P., Holder, M.T., Lewis, P.O. *et al.* (2011) BEAGLE: an application programming interface and high-performance computing library for statistical phylogenetics. *Systematic Biology*, **61**, 170–173.

Barluenga, M., Stölting, K.N., Salzburger, W., Muschick, M. & Meyer, A. (2006) Sympatric speciation in Nicaraguan Crater Lake cichlid fish. *Nature*, **439**, 719–723.

Burcham, J. (1988) Fish communities and environmental characteristics of two lowland streams in Costa Rica. *Revista de Biologia Tropical*, **36**, 273–285.

Burress, E.D. (2012) *Food web structure of a subtropical South American stream with particular focus on the co-evolution of form and function in an endemic species flock*. MSc Thesis, Appalachian State University, Boone, NC, USA. Available at http://libres.uncg.edu/ir/f/Burress,%20Edward_2010_Thesis.pdf/.

Burress, E.D. (2014) Cichlid fishes as models of ecological diversification: patterns, mechanisms, and consequences. *Hydrobiologia*, **2014**, 1–21.

Burress, E.D., Duarte, A., Serra, W.S., Loureiro, M., Gangloff, M.M. & Siefferman, L. (2013a) Functional diversification within a predatory species flock. *PLoS ONE*, **8**, e80929.

Burress, E.D., Duarte, A., Serra, W.S., Gangloff, M.M. & Siefferman, L. (2013b) Species-specific ontogenetic diet shifts among Neotropical *Crenicichla*: using stable siotopes and tissue stoichiometry. *Journal of Fish Biology*, **82**, 1904–1915.

Burress, E.D., Duarte, A., Serra, W.S. & Loureiro, M. (2015) Rates of piscivory predict pharyngeal jaw morphology in a piscivorous lineage of

cichlid fishes. *Ecology of Freshwater Fish*, doi: 10.1111/eff.12236. [Epub ahead of print].

Casciotta, J.R. & Arratia, G. (1993) Jaws and teeth of American cichlids (Pisces: Labroidei). *Journal of Morphology*, **217**, 1–36.

Cochran-Biederman, J.L. & Winemiller, K.O. (2010) Relationships among habitat, ecomorphology and diets of cichlids in the Bladen River, Belize. *Environmental Biology of Fishes*, **88**, 143–152.

Collar, D.C., O'Meara, B.C., Wainwright, P.C. & Near, T.J. (2009) Piscivory limits diversification of feeding morphology in centrarchid fishes. *Evolution*, **63**, 1557–1573.

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

Drucker, E.G. & Jensen, J.S. (1991) Functional analysis of a specialized prey processing behavior: winnowing by surfperches (Teleostei: Embiotocidae). *Journal of Morphology*, **210**, 267–287.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.

Elmer, K.R., Lehtonen, T.K., Kautt, A.F., Harrod, C. & Meyer, A. (2010) Rapid sympatric ecological differentiation of Crater Lake cichlid fishes within historic times. *BMC Biology*, **8**, 60.

Fraser, G.J., Hulsey, C.D., Bloomquist, R.F., Uyesugi, K., Manley, N.R. & Streelman, J.T. (2009) An ancient gene network is co-opted for teeth on old and new jaws. *PLOS Biology*, **7**, e1000031.

Gunter, H.M., Fan, S., Xiong, F., Franchini, P., Fruciano, C. & Meyer, A. (2013) Shaping development through mechanical strain: the transcriptional basis of diet-induced phenotypic plasticity in cichlid fish. *Molecular Ecology*, **22**, 4516–4531.

Hawlitschek, O., Yamamoto, K.C. & Carvalho-Neto, F.G.M.R. (2013) Diet composition of fish assemblage of Lake Tupe, Amazonas, Brazil. *Revista Colombiana de Ciencia Animal*, **5**, 313–326.

Hellig, C.J., Kerschbaumer, M., Defc, K.M. & Koblmüller, S. (2010) Allometric shape change of the lower pharyngeal jaw correlates with a dietary shift to piscivory in a cichlid fish. *Naturwissenschaften*, **97**, 663–672.

Higham, T.E., Hulsey, C.D., Říčan, O. & Carroll, A.M. (2006) Feeding with speed: prey capture evolution in cichlids. *Journal of Evolutionary Biology*, **20**, 70–78.

Hinojosa-Garro, D., Arceo-Gómez, J., Zamrano, L. & Escalera-Vázquez, L.H. (2013) Fish diet composition in permanent and semi-permanent pools in tropical wetlands of the Yucatan Peninsula. *Neotropical Ichthyology*, **11**, 881–890.

Hulsey, C.D. (2006) Function of a key morphological innovation: fusion of the cichlid pharyngeal jaw. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **273**, 669–675.

Hulsey, C.D., Hendrickson, D.A. & de León, F.J.G. (2005) Trophic morphology, feeding performance and prey use in the polymorphic fish *Herichthys minckleyi*. *Evolutionary Ecology Research*, **7**, 303–324.

Hulsey, C.D., Marks, J., Hendrickson, D.A., Williamson, C.A., Cohen, A.E. & Stephens, M.J. (2006) Feeding specialization in *Herichthys minckleyi*: a trophically polymorphic fish. *Journal of Fish Biology*, **68**, 1399–1410.

Hulsey, C.D., Roberts, R.J., Lin, A.S.P., Guldberg, R. & Streelman, T.J. (2008) Convergence in a mechanically complex phenotype: detecting structural adaptations for crushing in cichlid fish. *Evolution*, **62**, 1587–1599.

Hulsey, C.D., Mims, M.C., Parnell, N.F. & Streelman, J.T. (2010) Comparative rates of lower jaw diversification in cichlid adaptive radiations. *Journal of Evolutionary Biology*, **23**, 1456–1467.

Huysseune, A. (1995) Phenotypic plasticity in the lower pharyngeal jaw dentition of *Astatoreochromis alluaudi* (Teleostei: Cichlidae). *Archives of Oral Biology*, **40**, 1005–1014.

Ingram, T. & Mahler, D.L. (2013) SURFACE: detecting convergent evolution from comparative data by fitting Ornstein-Uhlenbeck models with stepwise Akaike Information Criterion. *Methods in Ecology and Evolution*, **4**, 416–425.

Kidd, M.R., Kidd, C.E. & Kocher, T.D. (2006) Axes of differentiation in the bower-building cichlids of Lake Malawi. *Molecular Ecology*, **15**, 459–478.

Liem, K.F. (1973) Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. *Systematic Zoology*, **22**, 425–441.

López-Fernández, H., Winemiller, K.O. & Honeycutt, R.L. (2010) Multilocus phylogeny and rapid radiations in Neotropical cichlid fishes (Percifromes: Cichlidae: Cichlinae). *Molecular Phylogenetics and Evolution*, **55**, 1070–1086.

López-Fernández, H., Winemiller, K.O., Montaña, C. & Honeycutt, R.L. (2012) Diet-morphology correlations in the radiation of South American Geophagine cichlids (Perciformes: Cichlidae: Cichlinae). *PLoS ONE*, **7**, e33997.

López-Fernández, H., Arbour, J.H., Winemiller, K.O. & Honeycutt, R.L. (2013a) Testing for ancient adaptive radiations in Neotropical cichlid fishes. *Evolution*, **67**, 1321–1337.

López-Fernández, H., Arbour, J.H., Winemiller, K.O. & Honeycutt, R.L. (2013b) Data from: Testing for ancient adaptive radiations in Neotropical cichlid fishes. *Dryad Digital Repository*, doi: 10.5061/dryad.34621.

López-Fernández, H., Arbour, J.H., Willis, S., Watkins, C., Honeycutt, R.L. & Winemiller, K.O. (2014) Morphology and efficiency of a specialized foraging behavior, sediment sifting, in Neotropical cichlid fishes. *PLoS ONE*, **9**, e89832.

Maddison, W.P. (1991) Squared-change parsimony reconstructions of ancestral states for continuous-valued characters on a phylogenetic tree. *Systematic Zoology*, **40**, 304–314.

Maddison, W.P. & Maddison, D.R. (2011) Mesquite: a modular system for evolutionary analysis. Version 2.75.

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

de Mérona, B. & Rankin de Mérona, J. (2004) Food resource partitioning in a fish community of the central Amazon floodplain. *Neotropical Ichthyology*, **2**, 75–84.

Meyer, A. (1989) Cost of morphological specialization: feeding performance of the two morphs in the trophically polymorphic cichlid fish, *Cichlasoma citrinellum*. *Oecologia*, **80**, 431–436.

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop, 2010, doi: 10.1109/GCE.2010.5676129.

Montaña, C.G. & Winemiller, K.O. (2009) Comparative feeding ecology and habitats use of *Crenicichla* species (Perciformes: Cichlidae) in a Venezuelan floodplain river. *Neotropical Ichthyology*, **7**, 267–274.

Muschick, M., Indermaur, A. & Salzburger, W. (2012) Convergent evolution within an adaptive radiation of cichlid fishes. *Current Biology*, **22**, 1–7.

Muschick, M., Barluenga, M., Salzburger, W. & Meyer, A. (2011) Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. *BMC Evolutionary Biology*, **11**, 116.

Muschick, M., Nosil, P., Roesti, M., Dittman, M.T., Harmon, L. & Salzburger, W. (2014) Testing the stages model in the adaptive radiation of cichlid fishes in East African Lake Tanganyika. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **281**, 20140605.

Near, T.J., Dornburg, A., Eytan, R.I., Keck, B.P., Smith, W.L., Kuhn, K.L. *et al.* (2013) Phylogeny and tempo of diversification in the superradiation of spiny-rayed fishes. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 12738–12743.

Pagel, M. (1999) The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology*, **48**, 612–622.

Piálek, L., Říčan, O., Casciotta, J., Almirón, A. & Zrzavý, J. (2012) Multilocus phylogeny of *Crenicichla* (Teleostei: Cichlidae), with biogeography of the *C. lacustris* group: species flocks as a model for sympatric speciation in rivers. *Molecular Phylogenetics and Evolution*, **62**, 46–61.

Rabosky, D.L., Santini, F., Eastman, J., Smith, S.A., Sidlauskas, B., Chang, J. *et al.* (2013) Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nature Communications*, **4**, 1958.

Rambaut, A. & Drummond, A.J. (2007) *TreeAnnotator Version 1.7.5*. University of Edinburgh, Edinburgh, UK.

Rambaut, A. & Drummond, A.J. (2009) *Tracer Version 1.5.0*. University of Edinburgh, Edinburgh, UK.

Revell, L.J., Johnson, M.A., Schulte, J.A., Kolbe, J.J. & Losos, J.B. (2007) A phylogenetic test for adaptive convergence in rock-dwelling lizards. *Evolution*, **61**, 2898–2912.

Revell, L.J. (2009) Size-correction and principal components for interspecific comparative studies. *Evolution*, **63**, 3258–3268.

Říčan, O., Piálek, L., Zardoya, R., Doadrio, I. & Zrzavy, J. (2013) Biogeography of the Mesoamerican Cichlidae (Teleostei: Heroini): colonization through the GAARlandia land bridge and early diversification. *Journal of Biogeography*, **40**, 579–593.

Rohlf, F.J. (2004) *TpsUtil, Version 1.44*. Department of ecology and Evolution, State University of New York at Stony Brook, New York, USA.

Rohlf, F.J. (2006) *TpsDig2, Version 2.10*. Department of Ecology and Evolution, State University of New York at Stony Brook, New York, USA.

Rudnick, D. & Resh, V. (2005) Stable isotope, mesocosm and gut contents analysis demonstrate trophic differences in two invasive decapod crustacea. *Freshwater Biology*, **50**, 1323–1336.

Schluter, D., Price, T., Mooers, A.Ø. & Ludwig, D. (1997) Likelihood of ancestor states in adaptive radiation. *Evolution*, **51**, 1699–1711.

Springer, V.G. & Friehofer, W.C. (1976) *Study of the Monophylic Fich Family Pholidichthyidae (Perciformes)*. Smithsonian Institution Press, Washington, DC, pp. 216–243.

Sturmbauer, C., Mark, W. & Dallinger, R. (1992) Ecophysiology of aufwuchs-eating cichlids in Lake Tanganyika: niche separation by trophic specialization. *Environmental Biology of Fishes*, **35**, 283–290.

Wainwright, P.C. (1991) Ecomorphology: experimental functional anatomy for ecological problems. *American Zoologist*, **31**, 680–693.

Wainwright, P.C., Smith, W.L., Price, A., Tang, K.L., Sparks, J.S., Ferry, L.A. *et al.* (2012) The evolution of pharyngognathy: a phylogenetic and functional appraisal of the pharyngeal jaw key innovation in labroid fishes and beyond. *Systematic Biology*, **61**, 1001–1027.

Winemiller, K.O. (1989) Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan llanos. *Environmental Biology of Fishes*, **26**, 177–199.

Winemiller, K.O. (1990) Spatial and temporal variation in tropical fish trophic networks. *Ecological Monographs*, **60**, 331–367.

Winemiller, K.O., Kelso-Winemiller, L.C. & Brenkert, A.L. (1995) Ecomorphological diversification in fluvial cichlid fishes. *Environmental Biology of Fishes*, **44**, 235–261.

Winemiller, K.O., Hoeinghaus, D.J., Pease, A.A., Esselman, P.C., Honeycutt, R.L., Gbanaador, D. *et al.* (2011) Stable isotope analysis reveals food web structure and watershed impacts along the fluvial gradient of a Mesoamerican coastal river. *River Research and Applications*, **27**, 791–803.

Witte, F. & Barel, C.D.N. (1976) The comparative functional morphology of the jaw apparatus of the piscivorous and intrapharyngeal mollusk-crushing *Haplochromis* species. *Revue des Travaux de L'Institut des Peches Maritimes*, **40**, 739–796.

Witte, F., Barel, K.D.N. & Van Oijen, J.P. (1997) Intraspecific variation of haplochromine cichlids from Lake Victoria and its taxonomic implication. *South African Journal of Science*, **93**, 585–594.

Xie, P. (2001) Gut contents of bighead carp *Aristichthys nobilis* and the processing and digestion of algal cells in the alimentary canal. *Aquaculture*, **195**, 149–161.

Yafe, A. *et al.* (2002) Feeding of two cichlidae species (Perciformes) in an hypertrophic urban lake. *Iheringia. Série Zoology*, **92**, 73–79.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Material examined.

Table S2. Data matrix used to test for correlations between lower pharyngeal jaw morphology and dietary traits.

Table S3. Summary of regime shifts and convergence in lower pharyngeal jaw morphology of Neotropical cichlids from the best-supported Hansen model from SURFACE analysis.

Figure S1. Landmark scheme used to quantify the shape of the lower pharyngeal jaw.

Figure S2. Depictions of the regions and aspects of the lower pharyngeal jaw referenced in the manuscript text.

Figure S3. Maximum likelihood ancestral state reconstruction showing the evolution of trophic guild among Neotropical cichlids.