SYMPOSIUM

Convergent Evolution of Cichlid Fish Pharyngeal Jaw Dentitions in Mollusk-Crushing Predators: Comparative X-Ray Computed Tomography of Tooth Sizes, Numbers, and Replacement

C. Darrin Hulsey [1],* Axel Meyer* and J. Todd Streelman[†]

*Department of Biology, University of Konstanz, Konstanz, Germany; [†]School of Biological Sciences, Institute of Technology, Atlanta, GA 30332, USA

[1]E-mail: darrin.hulsey@uni-konstanz.de

Synopsis Dental convergence is a hallmark of cichlid fish adaptive radiations. This type of repeated evolution characterizes both the oral jaws of these fishes as well as their pharyngeal jaws that are modified gill arches used to functionally process prey like hard-shelled mollusks. To test several hypotheses regarding the evolution of cichlid crushing pharyngeal dentitions, we used X-ray computed tomography scans to comparatively examine dental evolution in the pharyngeal jaw of a diversity of New World Heroine cichlid lineages. The substantial variation in erupted tooth sizes and numbers as well as replacement teeth found in these fishes showed several general patterns. Larger toothed species tended to have fewer teeth suggesting a potential role of spatial constraints in cichlid dental divergence. Species with larger numbers of erupted pharyngeal teeth also had larger numbers of replacement teeth. Replacement tooth size is almost exactly predicted ($r = 0.99$) from the size of erupted teeth across all of the species. Mollusk crushing was, therefore, highly associated with not only larger pharyngeal teeth, but also larger replacement teeth. Whether dental divergence arises as a result of environmental induced plasticity or originates via trophic polymorphism as found in the species *Herichthys minckleyi*, there appear to be general rules that structure interspecific divergence in cichlid pharyngeal erupted and replacement dentitions.

Introduction

The repeated evolution of the same phenotypes often characterizes cichlid fish adaptive radiations (Rüber et al. 1999; Stiassny and Meyer 1999). For instance, convergence in trophic morphology is a ubiquitous component of cichlid ecological specialization from the East African rift lakes to the Neotropics (Fryer and Iles 1972; Winemiller et al. 1995; Hulsey et al. 2019; Arbour et al. 2020). However, many patterns of cichlid trophic convergence have not been explicitly examined. For instance, cichlids that are specialized to exploit hard-shelled prey could commonly exhibit the repeated evolution of particular dental traits like larger teeth, fewer teeth, greater rates of tooth replacement, and larger replacement tooth sizes (Herring 1993; Didier et al. 1994; Evans and Sanson 2006; Santana et al. 2011; Dumont et al. 2012; Bemis and Bemis 2015; Burress 2016; Bemis et al. 2019; Soria-Barreto et al. 2019; Huang et al. 2020). Larger teeth could be advantageous because they provide a greater surface area over which crushing forces can be applied, but might commonly tradeoff with having fewer tooth numbers (Crompton and Hiiemae 1969; Eastman 1977; Estes and Williams 1984; Underwood et al. 2015; Bemis et al. 2017). Crushing predators could also lose their teeth more frequently and therefore have to invest more in constantly developing additional replacement teeth as well as producing exceptionally large replacement teeth (Vermeij 1987; Hung et al. 2015;

Johanson et al. 2020). Alternatively, larger teeth may be more resilient to breakage or may be buttressed by neighboring teeth and need to be replaced less frequently (Bemis and Bemis 2015; Leuzinger et al. 2020). To test several hypotheses regarding the evolution of cichlid crushing dentitions, we used X-ray computed tomography (CT) scans to comparatively examine dental evolution in the pharyngeal jaw of a diversity of New World cichlid lineages.

Like most teleost fishes, cichlids possess two toothed jaws, the oral and the pharyngeal jaws (Fig. 1A), and tend to replace their teeth on both jaws approximately once every 100 days throughout their lives (Streelman et al. 2003; Mehta and Wainwright 2007; Hulsey 2009; Fraser et al. 2013; Ellis et al. 2016; Hulsey et al. 2017; Karagic et al. 2020a, 2020b). Cichlids' oral jaws are primarily used to capture prey and are largely homologous to mammalian jaws (Wainwright et al. 2001). Their toothed pharyngeal jaws are modified gill arches that are used to break-down or process prey (Liem 1973). In fact, the enhanced ability to efficiently crush hard-shelled prey may be a key functional innovation of the cichlid pharyngeal jaw (Liem 1973; Hulsey 2006). In contrast to many types of ecological specialization such as piscivory (Wainwright et al. 2001; Arbour et al. 2020), the ability to break down durable prey is likely accompanied by the evolution of highly predictable mechanical consequences and dentitions (Wainwright 2006; Hulsey et al. 2008; Van Casteren and Crofts 2019). Durophagous organisms must produce and resist exceptional compressive forces when processing their heavily defended prey (Wainwright 1987; Grubich 2003). Hard-shelled mollusks also likely require the greatest amounts of compressive force to breakdown of any freshwater prey type that cichlids exploit (Vermeij 1987). Therefore, when comparing cichlids that use their pharyngeal jaws to crush mollusks to other non-molluscivorous species, we would expect that the dentition of the pharyngeal jaw (Fig. 1) should exhibit the evolution of highly predictable phenotypes (Wainwright 1988; Meyer A 1989; Hulsey et al. 2005).

The importance of dental specialization to cichlid diversification is vividly demonstrated by trophically polymorphic species such as *Herichthys minckleyi* that exhibit clear within species divergence in their pharyngeal jaws (Fig. 1B–G). In *H. minckleyi*, there are two co-occurring and interbreeding pharyngeal jaw morphotypes (Kornfield and Taylor 1983; Trapani 2003, 2004) that differ to the extent that they exploit snails (Hulsey et al. 2005; Chaves-Campos et al. 2011). Papilliform individuals exhibit
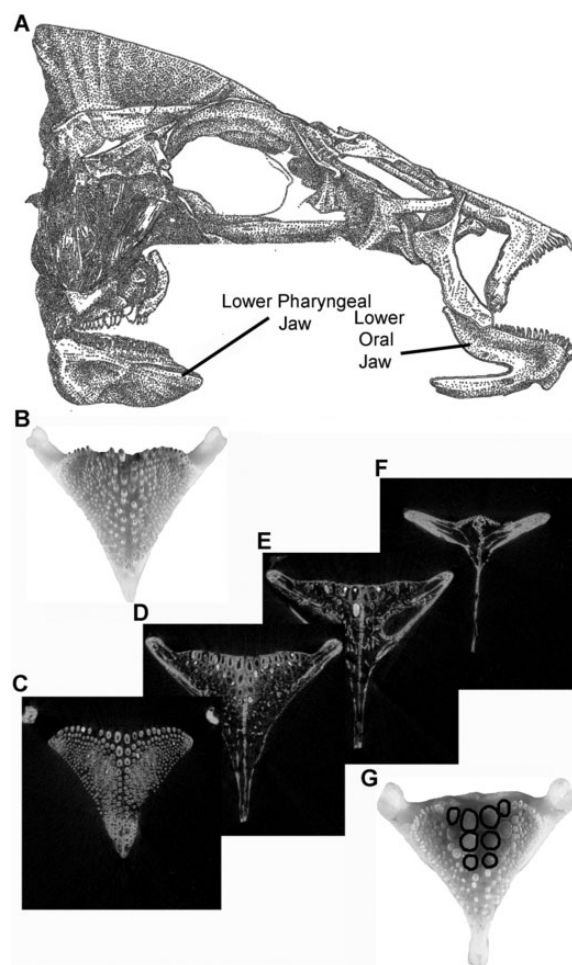

Fig. 1 The jaws of cichlids have been key to their diversification. The pharyngeal and oral jaws are shown in a diagrammatic lateral view (**A**). The dorsal view of a non-molluskivorous papilliform *H. minckleyi* jaw is shown (**B**). CT scans layers produced of this jaw (**C**) allow the visualization of the number of erupted teeth as well as the number of replacement teeth as one toggles through the stacks of CT images (**D–E**) to the ventral region of the jaw (**F**). A molluskivorous molariform *H. minckleyi* jaw is shown (**G**), and the areas encircled on the measured eight teeth on all jaws are depicted.

more gracile pharyngeal jaws and small pointed teeth that are specialized to masticate less durable prey while molariform *H. minckleyi* have robust jaws and enlarged molar-like teeth putatively adapted to crush mollusks (Hulsey et al. 2006a, 2006b; Fig. 1B and G). Discrete trophic polymorphism as found in the pharyngeal jaws of *H. minckleyi* is rare in extant taxa, but may be an intermediate step in the diversification of many cichlid species (Meyer 1990; Wainwright et al. 1991; Barluenga et al. 2006). For instance, other relatively closely related cichlids in the tribe Heroini that dominate the aquatic fauna of Central America could provide an ideal group to examine the repeated evolution of dental

modifications associated with durophagous pharyngeal jaws because of the large number of times this trophic specialization has putatively evolved (Hulsey 2006; Hulsey et al. 2008; Burress et al. 2020). Comparisons among members of this group could be used to examine questions such as whether the size of replacement teeth is fairly fixed so that morphotypes and species having larger teeth always replace their teeth with large molar-like teeth and species or morphotypes with smaller teeth tend to replace theirs with characteristically small teeth. Alternatively, it could be that plasticity in how cichlid dentitions are replaced could readily lead to switching between tooth types (Huysseune 1995). In general, the relationship between intraspecific polymorphism and patterns of interspecific divergence remains unclear. However, for trophic polymorphism to represent a transient stage through which species diverge, we would expect the patterns of dental divergence found in *H. minckleyi* to be common among closely related cichlid species that similarly differ in the extent to which they feed on mollusks.

We used CT scans of lower pharyngeal jaws (LPJs) to address several questions about the pharyngeal dentition of Heroine cichlids in general and *H. minckleyi* morphotypes in particular. First, we asked if there was an association between erupted tooth numbers and either tooth size or replacement tooth numbers. Then, we determined whether replacement tooth size showed consistent patterns of divergence with respect to erupted tooth size across the fish jaws examined. Finally, we asked if any of these dental traits were evolutionarily associated with the repeated evolution of crushing hard-shelled mollusks.

Materials and Methods

The LPJ of both morphotypes of *H. minckleyi* and 12 other species of New World cichlids (Fig. 2) were dissected from the buccal cavity of individuals caught from the wild. These jaw elements are readily dissected, and the dentition of the upper pharyngeal elements, that are more difficult to remove, often reflect the dentition on the lower pharyngeal element (C.D. Hulsey, personal observation). All fish were
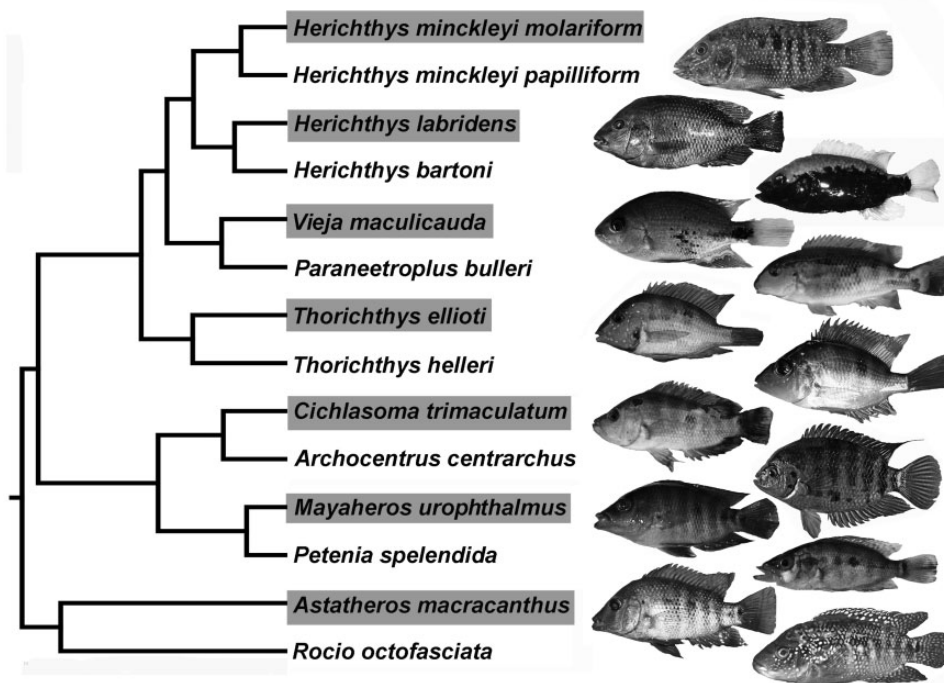
Fig. 2 The general evolutionary relationships among the species examined are shown according to the recent Ilves et al. (2018) phylogeny. Five taxa we examined in this study were not included in this more recent phylogeny and so their relationships are depicted as inferred in Hulsey et al. (2010). The topologies of these two phylogenies differ in two ways: (1) *Astatheros macracanthus* and *Rocio octofasciata* are now thought to be placed outside of the other lineages examined and (2) *P. splendida* is reconstructed as an outgroup to *Mayaheros urophthalmus* and the clade containing *Archocentrus centrarchus* and *Cichlasoma trimaculatum* (Ilves et al. 2018). Branch lengths reflect relative divergence times as inferred in Hulsey et al. (2010). The names of the mollusk-crushing species are highlighted with gray and the images of all the cichlid species are shown to the right of their names in the phylogeny to highlight their general phenotypic diversity.

preserved in formalin and transferred to 70% ethanol for long-term storage prior to dissection. The standard lengths (SLs) of the fish were determined using dial calipers and measured to the nearest 0.1 mm. Once dissected, the LPJs were cleaned of all muscle and the jaws were allowed to dry. Species were originally chosen because they likely represent independent evolutionary origins of closely related molluskivorous and non-molluskivorous species in the tribe Heroini (Winemiller et al. 1995; Hulsey et al. 2004, 2008; Hulsey 2006). The LPJs examined were obtained from adult individuals ranging from 65 mm to 146 mm SL. The jaws were dried carefully in order to avoid artifacts in future analyses. The LPJs of approximately three individuals per species (Table 1) were examined to provide an improved estimate of taxon-specific dental traits.

Micro-CT scans were obtained previously (Hulsey et al. 2008) using a Scanco Viva/Micro-CT X-ray scanner and workstation (Scanco Medical, Southeastern, PA) that implemented sampling intervals ranging from 16 μm to 60 μm. The scanner was set to a voltage of 55 kVp and a current of 145 μA. Noise was removed using a low-pass Gaussian filter ($\sigma = 1.2$, support $= 2$). The Scanco 3D viewer software was used to reconstruct the scans. Following scanning, it was noticed that the scans contained structures with a clear bimodality in their X-ray attenuation. This bimodality likely reflects differences in structural density (Stock 1999). The high-attenuation structures were confined to the high-density tooth caps that could contain sequestered metals and/or enameloid, which is analogous to enamel in mammalian teeth (Suga et al. 1992).

The use of CT scans here largely followed the methods that have now been implemented in a number of studies to examine tooth morphology and replacement (Bemis and Bemis 2015; Bemis et al. 2017, 2019; Kolman et al. 2019; Huang et al. 2020; Leuzinger et al. 2020). The resulting digital image layers generated in each CT scan allowed us to visualize using transverse sections the entire LPJ structure from the dorsal tooth surface containing erupted teeth to the interior containing the intraosseous replacement teeth, and ultimately down to the ventral side of the LPJ. The digital images of each jaw were imported into ImageJ 2.0.0-rc-2 (Schneider et al. 2012) and several measurements of the erupted and replacement teeth were made. First, the number of erupted teeth as indicated by the areas of high density was counted. This could have resulted in the undercounting of a few teeth lacking tooth caps of high density. But, the number of teeth likely undercounted seemed negligible (C.D. Hulsey, personal observation). Then, the dorsal-most area of eight putatively homologous teeth was measured on each jaw using the freehand tool in ImageJ. The teeth measured included the three most posterior

Table 1 The contrasting *H. minckleyi* morphotypes and molluskivorous species followed by its non-molluskivorous closely related morphotype/species are listed

Taxon	Diet	*n*	SL	Erupted tooth numbers	Replacement tooth numbers	Erupted tooth size (mm²)	Replacement Tooth Size(mm²)
Herichthys minckleyi molariform	M (28.0)	8	86.4 ± 14.2	226 ± 53	52 ± 13	0.78 ± 0.61	0.87 ± 0.61
vs. *H. minckleyi* papilliform	P (0.5)	11	89.6 ± 21.5	399 ± 73	90 ± 21	0.14 ± 0.10	0.17 ± 0.10
Herichthys labridens	M (46.1)	5	70.4 ± 5.6	115 ± 35	29 ± 10	0.65 ± 0.36	0.89 ± 0.38
vs. *H. bartoni*	D (0.1)	5	82.3 ± 6.9	203 ± 48	40 ± 14	0.17 ± 0.09	0.23 ± 0.12
Vieja maculicauda	M (20.0)	6	86.4 ± 7.0	130 ± 9	41 ± 12	0.24 ± 0.09	0.31 ± 0.15
vs. *Paraneetroplus bulleri*	I (0.0)	1	106.0	106	49	0.17 ± 0.05	0.19 ± 0.09
Thorichthys ellioti	M (26.3)	1	96.4	184	53	0.15 ± 0.06	0.23 ± 0.04
vs. *Thorichthys helleri*	I (0.7)	3	82.2 ± 2.7	222 ± 15	35 ± 1	0.15 ± 0.06	0.18 ± 0.09
Cichlasoma trimaculatum	M (11.7)	5	73.8 ± 9.0	133 ± 18	41 ± 8	0.23 ± 0.12	0.29 ± 0.15
vs. *Archocentrus centrarchus*	A (0.0)	7	73.7 ± 10.7	238 ± 25	49 ± 9	0.12 ± 0.05	0.14 ± 0.06
Mayaheros urophthalmus	M (33.8)	5	84.2 ± 6.3	138 ± 8	44 ± 6	0.26 ± 0.12	0.34 ± 0.17
vs. *P. splendida*	PV (0.0)	2	97.1 ± 10.8	99 ± 26	26 ± 1	0.10 ± 0.03	0.12 ± 0.06
Astatheros macracanthus	M (62.7)	3	83.9 ± 8.1	208 ± 18	65 ± 13	0.34 ± 0.13	0.41 ± 0.15
vs. *Rocio octofasciata*	P (0.0)	4	78.5 ± 6.9	140 ± 8	40 ± 8	0.16 ± 0.09	0.19 ± 0.09

The prey type that contributes most to the diet of the taxa are taken from Bergman Winemiller et al. (1995), Bergmann and Motta (2005), and Hulsey (2006): M, molluscivore; P, plant feeder; D, detritus feeder; A, arthropods; PV, piscivore. The percentage of mollusks included in the diet is also given. For each species, the sample size (*n*), the mean ± SE of the SL of erupted and replacement tooth numbers, as well as erupted and replacement area of the eight posterior homologous teeth on the pharyngeal jaw are given.

teeth on each side of the midline of the sutured jaw as well as a second tooth along the posterior tooth row on each side of the central-most tooth along the midline (Fig. 1G). Based on their positions in the jaw, these teeth are likely to be homologous, tend to be the largest on the pharyngeal jaws, and should facilitate the most accurate comparisons across species (Hulsey et al. 2015, 2017). Following the measurements of the erupted teeth, the number of developing replacement teeth was determined by counting the high-density tooth caps present in the interior of the jaw. To measure the dorsal area of replacement teeth, only replacement teeth forming underneath the previously measured eight erupted teeth were measured. Not all teeth had replacement teeth forming and only the area of those with intact tooth caps, determined by the presence of fully circular high-density material, were measured.

To perform the comparative analyses, we square-root transformed the species erupted and replacement tooth areas and divided them by the linear measurements of fish SL. Tooth numbers were not adjusted by SL as erupted tooth numbers have been shown in an ontogenetic series of *H. minckleyi* to have a non-significant association with SL in both morphotypes (Hulsey et al. 2015). For analyses, we used the phylogenetic reconstructions and branch lengths in Hulsey et al. (2010). This phylogeny differs only slightly in its basic topology from the recent Ilves et al. (2018) phylogeny that utilized a large number of loci but did not include five taxa examined here. Within this comparative framework, the "ape" package in R 3.6.2 (R Core Team 2017) was used to generate phylogenetically controlled correlations among tooth traits (Paradis and Schliep 2019). We also performed phylogenetic Analyses of Variance (ANOVAs) implemented in the R package "phytools" (Revell 2012) with 1000 simulations to determine if there was an evolutionary association between a more mollusk-crushing feeding habit and the cichlid tooth phenotypes. Because it increased our statistical power and mollusks often represent one of the most durable prey types in freshwater (Vermeij 1987), we effectively contrasted the more molluscivorous species with all other non-molluscivorous feeding species. For our evolutionary analyses, we also chose to treat the species' diet types as categorical variables because of the preponderance of 0% mollusks in the diet that would lead to zero inflation if treated as continuous variables. Therefore, if the species diet contained >10% mollusks they were categorized as a molluscivore and <2% mollusks they were categorized as a non-molluscivore for the phylogenetic ANOVAs.

Results

The LPJ tooth phenotypes varied substantially across the diversity of cichlids examined (Table 1). Erupted tooth numbers ranged from an average of 99 in *Petenia splendida* to a high of 399 in the papilliform morphotype of *H. minckleyi*. The number of replacement teeth ranged from an average low of 26 in *P. splendida* to a high of 90 in the papilliform morphotype of *H. minckleyi*. The average size of both erupted teeth and replacement teeth in some species like *H. labridens* and the molariform *H. minckleyi* approached almost 1.0 mm^2, but in species like *P. splendida* and the papilliform *H. minckleyi*, teeth were approximately one tenth this size at only about 0.1 mm^2.

There were several evolutionary correlations among the tooth phenotypes. As one might expect based on spatial constraints, cichlids with larger teeth had fewer teeth. Tooth numbers were evolutionarily negatively correlated with tooth areas ($r = -0.79$; $P = 0.001$). Additionally, the number of replacement teeth was highly correlated with erupted tooth number across these fishes ($r = 0.87$; $P < 0.001$). Furthermore, the dorsal tooth areas of the replacement teeth were always larger than the erupted teeth in every cichlid (Table 1), and there was an exceptionally strong correlation between the two measures of tooth size ($r = 0.99$; $P < 0.001$). Although neither erupted ($P = 0.178$) nor replacement ($P = 0.917$) tooth numbers had a clear association with a molluskivorous feeding habit, erupted ($P = 0.002$) and replacement ($P = 0.001$) tooth areas were highly associated with crushing hard-shelled mollusks.

Discussion

There is substantial variation in both the cichlid pharyngeal jaw erupted and replacement teeth (Table 1). However, the extensive variation in erupted tooth sizes and numbers as well as the replacement teeth both within and among species do appear to show some general patterns of variation. As has been found for numerous lineages (Herring 1993; Didier et al. 1994; Evans and Sanson 2006; Santana et al. 2011; Dumont et al. 2012; Bemis and Bemis 2015; Burress 2016; Soria-Barreto et al. 2019; Bemis et al. 2019; Huang 2020), molluscivorous species tended to have larger teeth. One possibility that was not explored here that could be examined in the future is that some of these non-molluscivorous species could become molluscivorous at larger body sizes if their teeth and muscles become sufficiently large and strong (Wainwright 1988). We also found that larger toothed taxa tended to have fewer

numbers of teeth suggesting a potentially important role for constructional constraints in cichlid dental divergence. These types of spatial constraints have been increasingly shown to play a role in the evolution of the considerable phenotypic diversity found in groups like cichlids (Hulsey et al. 2007, 2018). Species with larger numbers of erupted pharyngeal teeth also have larger numbers of replacement teeth. Finally, perhaps the most robust pattern observed was the finding that replacement tooth size could be almost exactly predicted from the size of erupted teeth. These general patterns of dental evolution are likely to characterize all cichlid fishes.

The erupted and replacement dental phenotypes found in the LPJ of the trophically polymorphic fish *H. minckleyi* do seem to generally characterize adaptation in numerous other cichlid fish species. Importantly, it does not appear that the *H. minckleyi* morphotypes are ever replacing one type of tooth, large molariform, or small papilliform, with the other morphotype's size of teeth. Like all of the cichlids examined, pharyngeal replacement teeth appeared to show a universal and highly similar increase in tooth areas. Replacement teeth were never smaller in any species suggesting that, for instance, molariform *H. minckleyi* is not likely to switch to being papilliform *H. minckleyi*. As the cichlid pharyngeal jaw and its associated teeth have often been suggested to exhibit adaptive phenotypic plasticity (Huysseune 1995; Muschick et al. 2011; Gunter and Meyer 2014; Schneider et al. 2014; Gunter et al. 2017; Schneider and Meyer 2017; Karagic et al. 2020a), our analyses likely also provides general parameters for how teeth can be replaced in these fishes and therefore how their pharyngeal jaw phenotypes can respond plastically to more durable prey. However, our inferences are limited to a single cycle of tooth replacement which could last for as little as 50 days in some of these fishes (Fraser et al. 2013; Bemis and Bemis 2015; Ellis et al. 2016). Future analyses that experimentally manipulate force production during prey processing especially over longer periods in both morphotypes of *H. minckleyi* would provide a more definitive test of this tooth type switching. Although it is often assumed that the adaptations found in trophically polymorphic species are present in other species within a clade, this assumption is rarely tested (Hulsey et al. 2008). This generality is critical to the notion that plasticity or polymorphic species could commonly represent the initial stages of diversification in reproductively isolated species (Meyer 1990; Smith and Skúlason 1996; Barluenga et al. 2006).

The type of comparative analyses presented here for the sympatric morphotypes of *H. minckleyi* and other cichlid species could help us move beyond traditional simplistic geographic analyses of distributional patterns to the evaluation of the underlying assumptions necessary for evolutionary processes such as sympatric speciation (Bolnick and Fitzpatrick 2007). Advantageously, the generality of patterns uncovered here with our relatively small sample sizes of the immense interspecific and intraspecific diversity of cichlids could be further tested in additional lineages. This is important because the pharyngeal jaw of cichlids has long been implicated as a major driver of the diversification of this incredibly species-rich and morphologically diverse group of fish (Fryer and Iles 1972; Liem 1973; Meyer 1993; Hulsey et al. 2006a). The type of analyses presented here could also be expanded to examine additional trophic guilds other than molluscivory such as planktivory or herbivory that have repeatedly evolved in cichlids (Fryer and Iles 1972; Winemiller et al. 1995; Hulsey et al. 2019; Arbour et al. 2020). Adaptation to these other types of food resources could readily explain additional patterns in the evolution of pharyngeal tooth phenotypes (Burress 2016). Nevertheless, the apparently repeated evolution of dental adaptations for molluscivory in clearly divergent cichlid species lends support to the idea that polymorphic differences that initially arise in the trophic apparatus could play a critical role in the general diversification of cichlids.

Our knowledge of tooth replacement in vertebrates is greatly increasing especially as we broaden the examination of these patterns outside of mammalian systems (Edmund 1960; Huysseune and Sire 1998; Smith et al. 2009; Wu et al. 2013; Tucker and Fraser 2014; Bemis and Bemis 2015; Bertin et al. 2018; Salomies et al. 2019). The limited tooth replacement in mammals and developmental basis of continuous tooth regeneration in all vertebrates can only be evolutionarily well-contextualized from studies of the rampant tooth replacement found in groups like cichlids (Fraser et al. 2009; Ellis et al. 2015; Hulsey et al. 2016; Hulsey et al. 2020). Fortunately, technological innovations, such as the increasing availability of CT scanners, are ushering in a renaissance in our understanding of how vertebrate dentitions are maintained and replaced (Bemis and Bemis 2015; Dumont et al. 2016; D'Emic et al. 2019; Kolman et al. 2019). It is also allowing us to better parameterize the mechanical properties of teeth as well as how these functional attributes contribute to ecological divergence (Anderson and LaBarbera 2008; Anderson et al. 2016; Van

Casteren and Crofts 2019). Understanding how teeth are replaced will continue to enhance our view of how tooth phenotypes in cichlids and other vertebrates repeatedly evolve.

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References

Anderson PSL, LaBarbera M. 2008. Functional consequences of tooth design: effects of blade shape on energetics of cutting. J Exp Biol 211:3619–26.

Anderson PSL, LaCosse J, Pankow M. 2016. Point of impact: the effects of size and speed on puncture mechanics. Interface Focus 6:20150111.

Arbour JH, Montaña CG, Winemiller KO, Pease AA, Soria-Barreto M, Cochran-Biederman JL, López-Fernández H. 2020. Macroevolutionary analyses indicate that repeated adaptive shifts towards predatory diets affect functional diversity in Neotropical cichlids. Biol J Linn Soc Lond 129:844–61.

Barluenga M, Stölting KN, Salzburger W, Muschick M, Meyer A. 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. Nature 439:719–23.

Bemis KE, Bemis W. 2015. Functional and developmental morphology of tooth replacement in the Atlantic Wolffish, *Anarhichas lupus* (Teleostei: Zoarcoidei: Anarhichadidae). Copeia 103:886–901.

Bemis KE, Burke SM, St. John CA, Hilton EJ, Bemis WE. 2019. Tooth development and replacement in the Atlantic Cutlassfish, *Trichiurus lepturus*, with comparisons to other Scombroidei. J Morph 280:78–94.

Bemis KE, Tyler JC, Bemis WE, Kumar K, Rana RS, Smith T. 2017. A gymnodont fish jaw with remarkable molariform teeth from the early Eocene of Gujarat, India (Teleostei, Tetraodontiformes). J Vert Paleo 37:e1369422.

Bergmann GT, Motta PJ. 2005. Diet and morphology through ontogeny of the nonindigenous Mayan cichlid 'Cichlasoma (Nandopsis)' *urophthalmus* (Günther 1862) in southern Florida. Env Biol Fish 72:205–11.

Bertin TJC, Thivichon-Prince B, LeBlanc ARH, Caldwell MW, Viriot L. 2018. Current perspectives on tooth implantation, attachment, and replacement in Amniota. Front Physiol 9:1–20.

Bolnick DI, Fitzpatrick BM. 2007. Sympatric speciation: models and empirical evidence. Ann Rev Ecol Syst 38:459–87.

Burress ED. 2016. Ecological diversification associated with the pharyngeal jaw diversity of Neotropical cichlid fishes. J Anim Ecol 85:302–13.

Burress ED, Martinez CM, Wainwright PC. 2020. Decoupled jaws promote trophic diversity in cichlid fishes. Evolution 74:950–61.

Chaves-Campos J, Johnson SG, Hulsey CD. 2011. Spatial geographic mosaic in an aquatic predator-prey network. PLoS One 6:e22472.

Crompton AW, Hiiemae K. 1969. How mammalian molar teeth work. Discovery 5:23–4.

Didier DA, Stahl BJ, Zangerl R. 1994. Development and growth of compound tooth plates in *Callorhinchus milii* (Chondrichthyes, Holocephali). J Morph 222:73–89.

D'Emic MD, O'Connor PM, Pascucci TR, Gavras JN, Mardakhayava E, Lund E. 2019. Evolution of high tooth replacement rates in theropod dinosaurs. PLoS One 14:e0224734.

Dumont ER, Dávalos LM, Goldberg A, Santana SE, Rex K, Voigt CC. 2012. Morphological innovation, diversification and invasion of a new adaptive zone. Proc Biol Sci 279:1797–805.

Dumont M, Tafforeau P, Bertin T, Bhullar B-A, Field D, Schulp A, Strilisky B, Thivichon-Prince B, Viriot L, Louchart A. 2016. Synchrotron imaging of dentition provides insights into the biology of *Hesperornis* and *Ichthyornis*, the "last" toothed birds. BMC Evol Bio 16:1–28.

Eastman JT. 1977. The pharyngeal bones and teeth of Catostomid fishes. Am Mid Nat 97:68–88.

Edmund AG. 1960. Tooth replacement phenomena in lower vertebrates. R Ont Mus Life Sci Div Contr 52:1–190.

Ellis NA, Donde NN, Miller CT. 2016. Early development and replacement of the stickleback dentition. J Morph 277:1072–83.

Ellis NA, Glazer AM, Donde NN, Cleves PA, Agoglia RM, Miller CT. 2015. Distinct developmental genetic mechanisms underlie convergently evolved tooth gain in sticklebacks. Development 142:2442–51.

Estes R, Williams EE. 1984. Ontogenetic variation in the molariform teeth of lizards. J Vert Paleo 4:96–107.

Evans AR, Sanson GD. 2006. Spatial and functional modeling of carnivore and insectivore molariform teeth. J Morphol 267:649–62.

Fraser GJ, Hulsey CD, Bloomquist RF, Uyesugi K, Manley NR, Streelman JT. 2009. An ancient gene network is co-opted for teeth on old and new jaws. PLoS Biol 7:e100003.

Fraser GJ, Bloomquist RF, Streelman JT. 2013. Common developmental pathways link tooth shape to regeneration. Dev Biol 377:399–414.

Fryer G, Iles TD. 1972. The cichlid fishes of the Great Lakes of Africa. Edinburgh: Oliver and Boyd.

Grubich J. 2003. Morphological convergence of pharyngeal jaw structure in durophagous perciform fish. Biol J Linn Soc Lond 80:147–65.

Gunter H, Meyer A. 2014. Molecular investigation of mechanical strain-induced phenotypic plasticity in the ecologically important pharyngeal jaws of cichlid fish. J Appl Ichth 30:630–5.

Gunter HM, Schneider RF, Karner I, Sturmbauer C, Meyer A. 2017. Molecular investigation of genetic assimilation during the adaptive radiations of East African cichlid fishes. Molec Ecol 26:6634–53.

Herring SW. 1993. Functional morphology of mammalian mastication. Am Zool 33:289–99.

Huang J, Motani R, Jiang D, Ren X-X, Tintori A, Rieppel O, Zhou M, Hu Y-C, Zhang R. 2020. Repeated evolution of durophagy during ichthyosaur radiation after mass extinction indicated by hidden dentition. Sci Rep 10:7798.

Hulsey CD. 2006. Function of a key morphological innovation: fusion of the cichlid pharyngeal jaw. Proc Biol Sci 273:669–75.

Hulsey CD. 2009. Cichlid genomics and phenotypic diversity in a comparative context. Integr Comp Biol 49:618–29.

Hulsey CD, Alfaro ME, Zheng J, Meyer A, Holzman R. 2019. Pleiotropic jaw morphology links the evolution of mechanical modularity and functional feeding convergence in Lake Malawi cichlids. Proc Biol Sci 286:20182358.

Hulsey CD, Cohen KE, Johanson Z, Karagic N, Meyer A, Miller CT, Sadier A, Summers AP, Fraser GJ. 2020. Grand challenges in comparative tooth biology. Integr Comp Biol (doi: 10.1093/icb/icaa038).

Hulsey CD, Fraser GF, Meyer A. 2016. Biting into the genome to phenome map: developmental genetic modularity of cichlid fish dentitions. Integr Comp Biol 56:373–88.

Hulsey CD, Hendrickson DA, de León FJG. 2005. Trophic morphology, feeding performance, and prey use in the polymorphic fish Herichthys minckleyi. Evol Ecol Res 7:303–24.

Hulsey CD, García de León FJ, Sánchez Johnson Y, Hendrickson DA, Near TJ. 2004. Temporal diversification of Mesoamerican cichlid fishes across a major biogeographic boundary. Mol Phylogenet Evol 31:754–64.

Hulsey CD, García de León FJ, Rodiles-Hernández R. 2006a. Micro- and macroevolutionary decoupling of cichlid jaws: a test of Liem's key innovation hypothesis. Evolution 60:2096–109.

Hulsey CD, García de León FJ, Meyer A. 2015. Sexual dimorphism in a trophically polymorphic cichlid fish?. J Morph 276:1448–54.

Hulsey CD, Hollingsworth PR, Fordyce JA. 2010. Temporal diversification of Central American cichlids. BMC Evol Biol 10:279.

Hulsey CD, Holzman R, Meyer A. 2018. Dissecting a potential spandrel of adaptive radiation: body depth and pectoral fin ecomorphology coevolve in Lake Malawi cichlid fishes. Ecol Evol 8:11945–53.

Hulsey CD, Machado-Schiaffino G, Keicher L, Ellis-Soto D, Henning F, Meyer A. 2017. The integrated genomic architecture and evolution of dental divergence in East African cichlid fishes (Haplochromis chilotes x H. nyererei.). G3 7:3195–202.

Hulsey CD, Marks J, Hendrickson DA, Williamson CA, Cohen AE, Stephens MJ. 2006b. Feeding specialization in Herichthys minckleyi: a trophically polymorphic fish. J Fish Biol 68:1–12.

Hulsey CD, Mims MC, Streelman JT. 2007. Do constructional constraints influence cichlid craniofacial diversification?. Proc Biol Sci 274:1867–75.

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

Hung NM, Ryan TM, Stauffer JR, Madsen H. 2015. Does hardness of food affect the development of pharyngeal teeth of the black carp, Mylopharyngodon piceus (Pisces: Cyprinidae)?. Biol Control 80:156–9.

Huysseune A. 1995. Phenotypic plasticity in the lower pharyngeal jaw dentition of Astatoreochromis alluaudi (Teleostei: Cichlidae). Archs Oral Biol 40:1005–14.

Huysseune A, Sire JY. 1998. Evolution of patterns and processes in teeth and tooth-related tissues in non-mammalian vertebrates. Eur J Oral Sci 106:437–81.

Ilves KL, Torti D, López-Fernández H. 2018. Exon-based phylogenomics strengthens the phylogeny of Neotropical cichlids and identifies remaining conflicting clades (Cichliformes: Cichlidae: Cichlinae). Mol Phylogenet Evol 118:232–43.

Johanson Z, Underwood C, Manzanares E, Fernandez V, Clark B, Meredith Smith M. 2020 Evolution of the dentition in holocephalans (Chondrichthyes). Integr Comp Biol (doi: 10.1093/icb/icaa093).

Karagic N, Meyer A, Hulsey CD. 2020a. Phenotypic plasticity in vertebrate dentitions. Integr Comp Biol (doi: 10.1093/icb/icaa077).

Karagic N, Schneider RF, Meyer A, HulseyCD. Forthcoming 2020b. A genomic cluster with novel and conserved genes is associated withcichlid dental developmental convergence. Molec Biol Evol (doi:10.1093/molbev/msaa153).

Kolman MA, Cohen KE, Bemis KE, Summers AP, Irish FJ, Hernandez PL. 2019. Tooth and consequences: heterodonty and dental replacement in piranhas and pacus (Serrasalmidae). Evol Devel 21:247–62.

Kornfield IL, Taylor JN. 1983. A newspecies of polymorphic fish, Cichlasoma minckleyi, from Cuatro Ciénegas, Mexico (Teleostei, Cichlidae). Proc Biol Soc Wash 96:253–69.

Leuzinger L, Cavin L, López-Arbarello A, Billon-Bruyat J-P. 2020. Peculiar tooth renewal in a Jurassic ray-finned fish (Lepisosteiformes, †Scheenstia sp.). Paleontology 63:117–29.

Liem KF. 1973. Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. Syst Zool 22:425–41.

Mehta RS, Wainwright PC. 2007. Raptorial jaws in the throat help moray eels swallow large prey. Nature 449:79–82.

Meyer A. 1989. Costs and benefits of morphological specialization: feeding performance in the trophically polymorphic Neotropical cichlid fish, Cichlasoma citrinellum. Oecologia 80:431–6.

Meyer A. 1990. Ecological and evolutionary aspects of the trophic polymorphism in Cichlasoma citrinellum (Pisces: Cichlidae). Biol J Linn Soc Lond 39:279–99.

Meyer A. 1993. Trophic polymorphisms in cichlid fish: do they represent intermediate steps during sympatric speciation and explain their rapid adaptive radiation? In: Schröder JH, Bauer J, Schartl M, editors. Trends in ichthyology. Oxford, UK: Blackwell. p. 257–66.

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 Evol Biol 11:116.

Paradis E, Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35:526–8.

R Core Team (2017). R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing (https://www.R-project.org/).

Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol Evol 3:217–23.

Rüber L, Verheyen E, Meyer A. 1999. Replicated evolution of trophic specializations in an endemic lineage from Lake Tanganyika. Proc Natl Acad Sci U S A 96:10230–5.

Santana SE, Strait S, Dumont ER. 2011. The better to eat you with: functional correlates of tooth structure in bats. Funct Ecol 25:839–47.

Salomies L, Eymann J, Khan I, Di-Poï N. 2019. The alternative regenerative strategy of bearded dragon unveils the key processes underlying vertebrate tooth renewal. Elife 8:e47702.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–5.

Schneider RF, Li Y, Meyer A, Gunter H. 2014. Regulatory gene networks that shape the development of adaptive phenotypic plasticity in a cichlid fish. Mol Ecol 23:4511–26.

Schneider RF, Meyer A. 2017. How plasticity, genetic assimilation and cryptic genetic variation may contribute to adaptive radiations. Mol Ecol 26:330–50.

Smith MM, Fraser GJ, Mitsiadis T. 2009. Dental lamina as source of odontogenic stem cells: evolutionary origins and developmental control of tooth generation in gnathostomes. J Exp Zool B Mol Dev Evol 312B:260–80.

Smith TB, Skúlason S. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annu Rev Ecol Syst 27:111–33.

Soria-Barreto M, Rodiles-Hernández R, Winemiller KO. 2019. Trophic ecomorphology of cichlid fishes of Selva Lacandona, Usumacinta, Mexico. Environ Biol Fish 102:985–96.

Stiassny MLJ, Meyer A. 1999. Cichlids of the Rift Lakes. Sci Am 280:64–9.

Stock SR. 1999. X-ray microtomography of materials. Int Mat Rev 44:141–64.

Streelman JT, Webb JF, Albertson RC, Kocher TD. 2003. The cusp of evolution and development: a model of cichlid tooth shape diversity. Evol Dev 5:600–8.

Suga S, Taki Y, Ogawa M. 1992. Iron in the enameloid of perciform fish. J Dent Res 71:1316–25.

Trapani J. 2003. Morphological variability in the Cuatro Ciénegas cichlid, *Cichlasoma minckleyi*. J Fish Biol 62:276–98.

Trapani J. 2004. A morphometric analysis of polymorphism in the pharyngeal dentition of *Cichlasoma minckleyi* (Teleostei, Cichlidae). Arch Oral Biol 49:825–35.

Tucker AS, Fraser GJ. 2014. Evolution and developmental diversity of tooth regeneration. Semin Cell Dev Biol 25–26:71–80.

Underwood CJ, Johanson Z, Welten M, Metscher B, Rasch LJ, Fraser GJ, Smith MM. 2015. Development and evolution of dentition pattern and tooth order in the skates and rays (Batoidea; Chondrichthyes). PLoS One 10:e0122553.

Van Casteren A, Crofts SB. 2019. The materials of mastication: material science of the humble tooth. Integr Comp Biol 59:1681–9.

Vermeij GJ. 1987. Evolution and escalation: an ecological history of life. Princeton (NJ): Princeton University Press.

Wainwright PC. 1987. Biomechanical limits to ecological performance: mollusk crushing by the Caribbean hogfish, *Lachnolaimus maximus* (Labridae). J Zool Lond 213:283–98.

Wainwright PC. 1988. Morphology and ecology: the functional basis of feeding constraints in Caribbean labrid fishes. Ecology 69:635–45.

Wainwright PC. 2006. Functional morphology of the pharyngeal jaw apparatus. In: Shadwick, R Lauder GV, editors. Biomechanics of fishes. San Diego (CA): Academic Press. p. 77–101.

Wainwright PC, Osenberg CW, Mittelbach GG. 1991. Trophic polymorphism in the pumpkinseed sunfish (*Lepomis gibbosus*): environmental effects on ontogeny. Funct Ecol 5:40–55.

Wainwright PC, Ferry-Graham LA, Waltzek TB, Carroll AM, Hulsey CD, Grubich JR. 2001. Examination of ram and suction feeding performance in cichlid fishes. J Exp Biol 219:119–3051.

Winemiller KO, Kelso-Winemiller LC, Brenkert AL. 1995. Ecological diversification and convergence in fluvial cichlid fishes. Environ Biol Fishes 44:235–61.

Wu P, Wu X, Jiang TX, Elsey RM, Temple BL, Divers SJ, Glenn TC, Yuan K, Chen MH, Widelitz RB, et al. 2013. Specialized stem cell niche enables repetitive renewal of alligator teeth. Proc Natl Acad Sci U S A 110:E2009–18.