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Function of a key morphological innovation: fusion of the cichlid pharyngeal jaw

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The pharyngeal jaw of cichlids may represent a key innovation that facilitated their unparalleled trophic divergence. In cichlids, 'fusion' of the lower pharyngeal jaw (LPJ) results from suturing between the two lower ceratobranchials. To examine, what novel abilities a more extensively fused pharyngeal jaw may confer, the function of LPJ suturing was examined in Heroine cichlids. Greater LPJ suturing, pharyngeal jaw splitting under compression and the forces used to crush molluscs in the wild suggest increased LPJ fusion in the trophically polymorphic *Herichthys minckleyi* operates to strengthen the pharyngeal jaw. Among Heroine cichlid species, the presence of an external LPJ suture and feeding specialization on molluscs was evolutionarily quite variable, but greater LPJ fusion estimated from the amount of external suturing was highly correlated with molluscivory. Throughout cichlid diversification, increased pharyngeal jaw fusion via suturing has likely helped to reinforce the LPJ during pharyngeal processing thereby facilitating the ability of cichlids to exploit durable prey.

Keywords: Cichlidae; comparative methods; feeding; macroevolution; molluscivory

1. INTRODUCTION

The innovations in the cichlid pharyngeal jaw may have facilitated the utilization of novel prey and ultimately led to the unparalleled trophic diversification of these fishes (Liem 1973). Although most bony fishes have pharyngeal gill arches modified to process prey (Liem 1986; Wainwright 1989), the cichlid pharyngeal jaw uniquely exhibits novel upper pharyngeal jaw joints, a 'muscular sling' and suturing between the two-fifth ceratobranchial elements (figure 1). This suturing results in a functionally fused lower pharyngeal jaw (LPJ) (Liem 1973), but the degree of fusion via suturing is highly variable (Kullander 1998). As the cichlid LPJ both resists and exerts forces during prey processing, this variability in LPJ fusion should relate to forces exerted on prey (Stiassny & Jensen 1987; Kullander 1998). To examine the trophic consequences of cichlid pharyngeal jaw fusion, I investigated whether greater LPJ suturing enhances the ability to exploit durable prey in the trophically polymorphic *Herichthys minckleyi* and tested the evolutionary association in Heroine cichlids between LPJ suturing and crushing hard-shelled molluscs.

Suturing integrates the two halves of the LPJ into a single unit. Because the pharyngeal jaw is the primary location of prey processing in most bony fishes (Liem 1973), pharyngeal jaw modifications frequently determine, what prey fishes can exploit (Wainwright 1989; Galis & Drucker 1996; Grubich 2003). The importance of pharyngeal jaw modifications to cichlid trophic divergence is clearly evident in species that are polymorphic in their pharyngeal morphology. For example, in *H. minckleyi* (Kornfield & Taylor 1983), one pharyngeal morphotype specializes in crushing molluscs (molariform), while the other pharyngeal jaw type (papilliform) utilizes prey that require less force to process (Hulsey *et al.* 2005). These alternative pharyngeal morphotypes (figure 2*a*,*b*) occur sympatrically (Kornfield & Koehn 1975), interbreed (Kornfield & Taylor 1983) and differ in little other than what prey they utilize (Sage & Selander 1975). Of all the fresh water prey, molluscs that molariform *H. minckleyi* and other cichlids crush should require the most forceful pharyngeal bite to process (Vermeij & Covich 1978; Vermeij 1987). Therefore, if greater pharyngeal jaw fusion is important in adapting the jaw to exploit more durable prey, molariform *H. minckleyi* should have more heavily sutured LPJs and better resist compressive forces encountered when crushing molluscs.

Adaptations for exploiting particular prey likely arise within populations (Meyer 1990; Schluter & Nagel 1995), but macroevolutionary divergence is often the best evidence available that a particular innovation was key to a clade's diversification (Simpson 1953). Linking modifications within a species like *H. minckleyi* to changes among species would increase the mechanistic understanding of how LPJ fusion may have generally influenced cichlid trophic divergence. As molluscivory has likely evolved multiple times in the Heroine cichlid clade that includes *H. minckleyi* (Winemiller *et al.* 1995; Hulsey *et al.* 2005), this cichlid lineage is ideal for examining the macroevolutionary association between greater cichlid LPJ fusion and crushing durable mollusc shells.

I examined cichlid LPJ fusion using morphometrics, empirical tests of the strength of the LPJ, diet analyses and phylogenetic comparative methods. I first quantified external suturing in *H. minckleyi* molariforms and papilliforms. Then I tested if, the forces that split the LPJ along the suture differed between molariforms and papilliforms and if suturing may reduce damage to the pharyngeal

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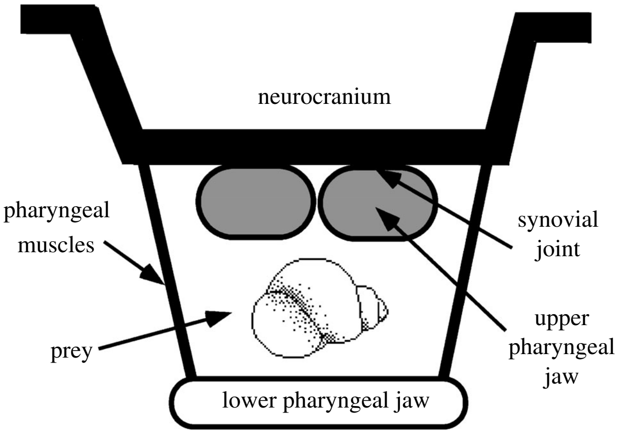

Figure 1. The labroid pharyngeal anatomy, implicated as a key innovation (Liem 1973) has three basic components: (*a*) the muscular sling formed from the pharyngeal muscles, (*b*) two synovial joints in the upper pharyngeal jaw and (*c*) a single 'fused' LPJ. In cichlids, the fusion of the LPJ is formed from the two-fifth ceratobranchials being sutured together.

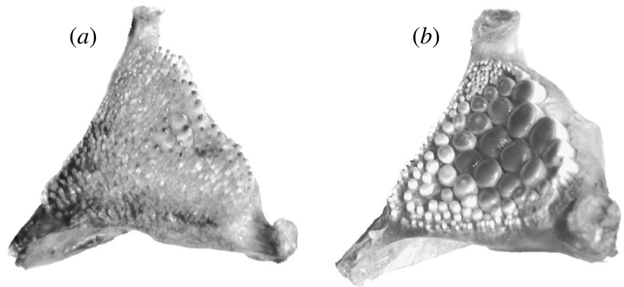

Figure 2. The dorsal view of LPJs of *H. minckleyi*. (*a*) The papilliforms are specialized to shred plants. (*b*) The molariforms are specialized to crush snails and are diagnosable by their large molariform teeth.

apparatus when this species crushes molluscs. I also quantified LPJ suturing and the extent of specialization on molluscs in 31 other Heroine cichlids and examined the phylogenetically corrected correlation between LPJ suturing and molluscivory.

2. MATERIAL AND METHODS

(a) *Suture quantification*

To measure the amount of LPJ suturing, the LPJ was removed from fishes that were collected from their native range (sites available from the author). After measuring standard length (SL), fishes were cleared using trypsin and dyed with Alcian blue cartilage stain and Alizarin red bone stain (Dinkerhus & Uhler 1977). The LPJ of the specimens examined could not be destroyed to examine internal interdigitation. Therefore, I quantified the external suturing of the LPJ (figure 3). Although external suturing is not a direct measure of the internal interdigitation of the pharyngeal jaw, three-dimensional computed tomography scans of the LPJ suggest greater external suturing reflects greater internal integration between the paired ceratobranchial bones that form the LPJ of *H. minckleyi* (Hulsey, personal observation).

To estimate the percentage of the LPJ that was sutured, the total length of the jaw, L_T, from anterior tip of the keel to the posterior centre of the jaw was measured to the nearest 0.1 mm using calipers (figure 3*b*). Then, the linear distance from the anterior-most initiation of suturing to the posterior-most end, L_S, along the centre of the jaw was measured (figure 3*c*). If, there was no suture, L_S was zero. Subsequently, the suturing of the dried LPJ was photographed using a digital microscope. The image was then imported into NIH Image (http://rsb.info.nih.gov/nih-image/about.html) and two measurements were taken. First, the winding length, L_W, of the suture was traced digitally (figure 3*c*) and its length calculated. Then, the linear length, L_S, between the anterior-most point, where suturing of the jaw began and the posterior-most end of jaw suturing was calculated using a straight line. The winding length was then divided by the linear length. This value was multiplied by the percentage of the length of the LPJ that was sutured in order to calculate the percentage the suture increased in the perimeter of contact, P_L (equation (2.1)), between the two halves of the pharyngeal jaw as compared to an unsutured jaw

$$P_L = (L_S/L_T)(L_W/L_S). \qquad (2.1)$$

If there was no suturing, P_L was equal to zero.

(b) *Quantifying suture and its function in H. minckleyi*

I compared P_L between individuals exhibiting the alternative pharyngeal dentition in *H. minckleyi* (figure 2). Nine molariform (Range: 69.9–146.0 mm SL) and nine papilliform *H. minckleyi* (Range: 66.4–133.2 mm SL) were cleared and stained, pharyngeal jaws extracted and suture P_L quantified. Then, with an ANCOVA, I tested whether P_L was significantly different between morphotypes using SL as a covariate.

I also determined if the LPJ in the *H. minckleyi* pharyngeal morphotypes resisted different amounts of compressive force. For 12 molariforms (Range: 80–147.2 mm SL) and 12 papilliforms (Range: 77.1–145.4 mm SL), wild-caught fishes were sacrificed with an overdose of MS222, their SL measured and their LPJ removed by dissection. Subsequently, the LPJ was laid on the lower force plate of an Accuforce Cadet force gauge (0–1000 Newton, Ametek, Inc. Pennsylvania, USA) with the dorsal side of the jaw facing the upper force plate. I then placed a *Mexithauma quadripaludium* snail, approximately 5 mm in shell length, aperture down, on the dorsal crushing surface of the LPJ to mimic the position of a snail during a pharyngeal crushing event. This snail is frequently crushed by molariform *H. minckleyi* (Hulsey *et al.* 2005). The snail and LPJ were then squeezed between the opposing force plates of the force gauge until, the LPJ split in half along the suture. Using SL as a covariate, an ANCOVA was used to test if the force recorded in Newtons (N) needed to split the LPJ of each morphotype along the suture was statistically distinguishable. Finally, the compressive resistance of each morphotype's LPJ was compared to the maximum force molariforms exert to crush snails in the wild ($n=33$, $\log N = 1.34 (\log \mathrm{SL}) - 0.75$, $R^2 = 0.69$) using an ANCOVA with SL as a covariate. This maximum molariform crushing force was estimated from snail opercula taken from gut contents of wild-caught fishes (Hulsey *et al.* 2005).

(c) *Molluscivory*

To determine if LPJ suturing was evolutionarily correlated with molluscivory, I obtained the percent volumetric contribution of molluscs to the gut contents of 32 Heroine species (see electronic supplementary material, appendix 1). For 22 species, the proportional contribution of molluscs to their diet was extracted from published sources. However,

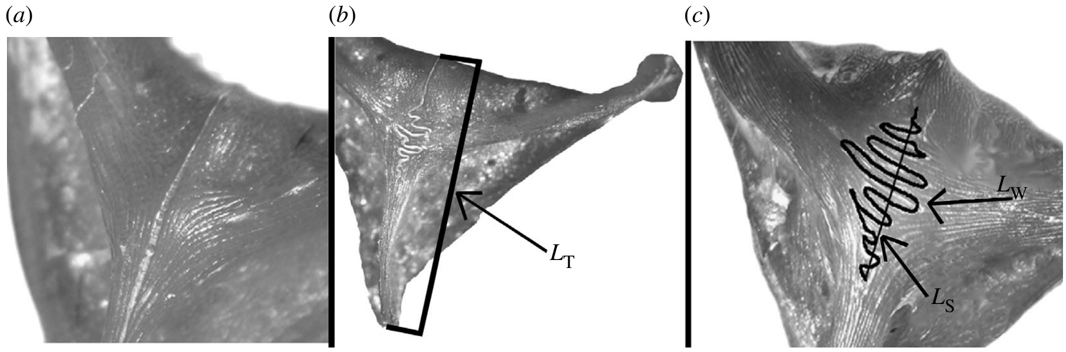

Figure 3. There is substantial variation in suturing of the ventral LPJ in cichlids. In (*a*) *A. robertsoni*, the two halves of the LPJ meet but there is no external suture, while other species (*b* and *c*) exhibits extensive suturing. (*b*) To estimate the suture length, the length of the jaw from the tip of the LPJ keel to the posterior centre end of the LPJ, L_T, was first measured using calipers and then (*c*) the length of the suture, L_S, was measured. Subsequently, the dried bone was photographed using a digital microscope and the suture quantified. The L_T, L_S and L_W were used to calculate (equation (2.1)) the percent the suture increased the perimeter of contact between the two halves of the LPJ, or P_L, as compared to an unsutured jaw.

only the frequency of occurrence of prey was reported for five of these species (Chavez-Lomeli *et al.* 1989). In two of these five species, molluscs were never recovered. But, for *Paratheraps fenestratus*, *Astatheros robertsoni* and *Thorichthys meeki* molluscs were recorded and for these the contribution of molluscs was estimated. The volumetric contribution of all prey types to the diet of each species was estimated from the frequencies reported. These frequencies were transformed using the average of a prey type's volumetric contribution when included in the diet of a single *H. minckleyi* (Hulsey *et al.* in press). The estimated contribution for all major prey types was added together and molluscivory for the three species was estimated. For 10 additional species, I quantified molluscivory from gut contents (figure 4). The gut contents were examined in fishes collected from localities reported in Hulsey *et al.* (2004) in approximately 10 individuals per species (*Astatheros macracanthus*, $n=5$; *Herichthys bartoni*, $n=10$; *Herichthys labridens* C; $n=10$; *H. labridens* ML $n=6$; *Herichthys tamasopoensis*, $n=10$; *Paraneetroplus bulleri*, $n=10$; *Paratheraps guttulatus*, $n=10$; *Thorichthys callolepis*, $n=9$; *Thorichthys ellioti*, $n=10$; *Thorichthys helleri*, $n=10$). The volumetric contributions of all diet items were identified as molluscs or other (Thorpe & Covich 1991) and measured using water displacement. For the phylogenetic correlations, arcsin transformations of molluscivory were used because the proportions were non-normally distributed.

(d) *Comparative analyses of LPJ suturing and molluscivory*

After P_L was quantified as detailed above for approximately three specimens per species in 31 Heroine species (excluding *H. minckleyi*), the correlation between molluscivory and P_L was examined (figure 4). The range of L_S/L_T and L_W/L_S are reported, but the species mean P_L was used in the comparative analyses. Because species are not evolutionarily independent (Felsenstein 1985), the correlation was examined using an independent contrast analysis. For the phylogenetic backbone of this analysis, I used the phylogeny in Hulsey *et al.* (2004) based on sequences of the cytochrome *b* gene (1137 bp). The phylogenetic topology (figure 4) with branch lengths was first imported into Tree Edit 1.0 (Rambaut & Charleston 2002). The branch lengths in the topology were smoothed using non-parametric rate smoothing (Sanderson 1997) with mid-point rooting because there was substantial heterogeneity in branch lengths due to non-clocklike molecular evolution (Hulsey *et al.* 2004). Species for which the LPJs were not examined and all but one sequence for each species examined were then pruned from the topology. The branch lengths and topology for the remaining species were exported into comparative analysis by independent contrasts (CAIC) (Purvis & Rambaut 1995).

The phylogenetic independent contrast analyses were performed in several ways. First, the correlation of P_L and arcsin transformed values of molluscivory were examined using the 'crunch' algorithm that assumes all variables are continuous. Then, because many species had zero or low contribution of molluscs to the diet, I also examined the correlation when mollusc feeding was treated as a categorical variable. Species that included less then 2% molluscs in their diet were categorized as (0) non-molluscivores and species with greater then 2% molluscs in their diet as (1) molluscivores. Then, with the 'brunch' algorithm, which allows dichotomous variables to be tested in an independent contrast framework in CAIC, it was determined if P_L was consistently greater in those clades coded as molluscivores. Finally, because the P_L of the jaws in *H. minckleyi* appeared to be slightly correlated with SL, I reanalysed the above correlations using the residuals of a reduced major axis regression between average SL of specimens and P_L. Also, the presence of greater than 2% molluscs in the diet was mapped onto the phylogeny using parsimony (Maddison & Maddison 2000) to provide an initial estimate of the number of times molluscivory may have evolved in Heroine cichlids.

3. RESULTS

(a) *Quantifying suture and its function in H. minckleyi*

In *H. minckleyi*, both (L_S/L_T) and (L_W/L_L) were greater in molariforms (see electronic supplementary material, appendix 1). The molariforms had significantly greater P_L than papilliforms (ANCOVA, $n=9$ molariforms and 9 papilliforms, $F=11.2$, $p=0.004$), although, there was also an effect of SL on the amount of suturing ($F=15.4$, $p=0.001$). When the jaws of *H. minckleyi* were crushed, the LPJ always split along the suture. The teeth of the papilliforms sometimes broke prior to jaw splitting, but the molariforms' large molars were never visibly damaged. The force (figure 5) needed to split molariforms' jaws increased substantially with SL ($\log N=3.53\ (\log \mathrm{SL})-4.75$, $R^2=0.72$). The increase in force needed to split the

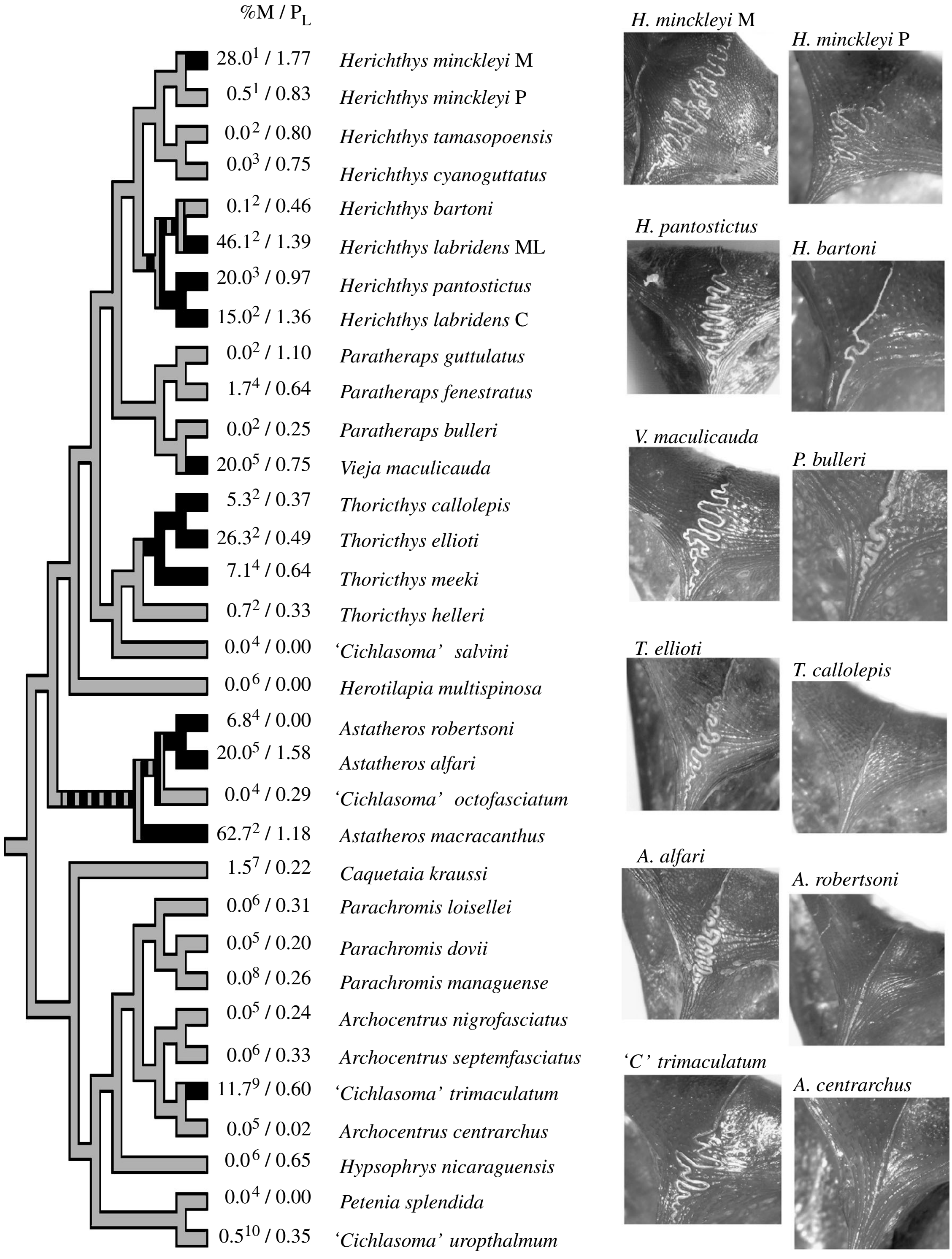

Figure 4. The phylogenetic relationships, evolution of molluscivory and photos of LPJ suturing in representative Heroine species. The percent contribution of molluscs to the diet of each species and the mean P_L are depicted next to the species name. The subscript above the percent indicates the source of the diet information: 1, Hulsey *et al.* 2005; 2, this study; 3, Darnell 1962; 4, Chavez-Lomeli *et al.* 1989; 5, Winemiller *et al.* 1995; 6, Bussing 1993; 7, Winemiller 1989; 8, Gestring & Shafland 1997; 9, Yanez-Arancibia 1978; 10, Martinez-Palacios & Ross 1988. The evolution of molluscivory (greater than 2.0% molluscs in the diet) is mapped onto the phylogeny using parsimony. The parsimony reconstructions suggest molluscivory may have evolved six times independently in Heroine cichlids. The six examples of closely related non-molluscivorous and molluscivorous cichlid LPJs display the variability in Heroine LPJ fusion.

papilliform jaw ($\log N = 0.67 (\log SL) + 0.39$, $R^2 = 0.72$) was much less than the force needed to split the molariform LPJ (ANCOVA, $n = 12$ molariform LPJ and $n = 12$ papilliform LPJ, $F = 11.3$, $p = 0.002$). The LPJ of the molariforms required greater force to split than the maximum force this morphotype used in the wild to crush snails (ANCOVA, $n = 12$ molariform LPJ and $n = 33$ estimates of maximum force from molariform gut contents, $F = 27.3$, $p < 0.001$). However, this was not true in the papilliforms (ANCOVA, $n = 12$ papilliform LPJ, $F = 29.2$, $p < 0.001$). The jaw of the papilliforms split along the suture at forces that were generally less than

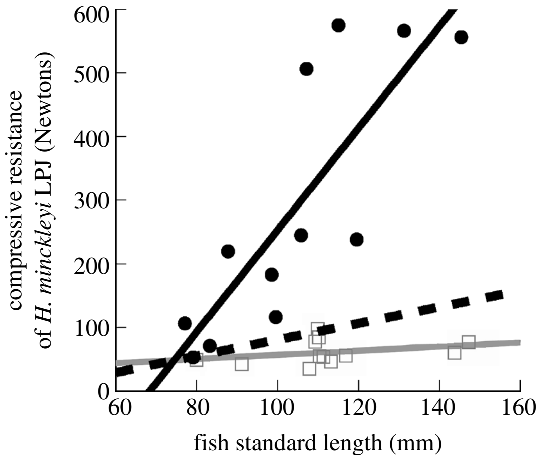

Figure 5. The LPJ of an ontogenetic series of molariform and papilliform *H. minckleyi* were crushed using a force gauge. When the jaws split at the suture, the force was recorded. The compressive resistance of the jaws was then compared to the estimated maximum force molariform *H. minckleyi* used to crush snails in the wild (broken lines; Hulsey *et al.* 2005). The failure of molariform jaws (filled circle) was much greater than the maximum force they use to crush snails in the wild ($p<0.001$). However, the ontogenetic trajectory of failure of papilliform jaws (open square) was significantly lower than both the resistance of the molariform jaws ($p=0.002$) and the forces molariforms used in the wild to crush snails ($p<0.001$).

what an equivalent sized molariform used to crush snails in the wild.

(b) *Molluscivory*

Astatheros alfari, *A. macracanthus*, *A. robertsoni*, '*Cichlasoma*' *trimaculatum*, *Paratheraps maculicauda*, *T. callolepis*, *T. ellioti*, *T. meeki*, *H. labridens* 'Cascadas', *H. labridens* 'Media Luna', *Herichthys pantostictus* and molariform *H. minckleyi* all included greater than 5% molluscs in their diet (figure 4). Many species included a small amount of molluscs (less than 2%), or no molluscs in their diet. Unordered parsimony reconstructions of molluscivory (greater than 2% in the diet) suggest this feeding habit evolved at least six times independently in Heroine cichlids (figure 4).

(c) *Suture quantification*

Cichlasoma salvini, *Herotilapia multispinosa*, *A. robertsoni* and *Petenia splendida* completely lacked suturing ($P_L=0.00$). *Caquetaia kraussi* ($P_L=0.19$), *Nandopsis dovii* ($P_L=0.20$) and *Archocentrus centrarchus* ($P_L=0.02$) displayed very limited suturing. Although *A. robertsoni* had an unsutured LPJ, its close relative *A. alfari* had the greatest proportion of its jaw sutured (55%) of any Heroine cichlid studied (see electronic supplementary material, appendix 1). For the Heroine species, 26% was the average proportion of the LPJ that was sutured within species, there was variability in the proportion of the LPJ that was sutured as well as the amount the winding length increased suturing. However, the limited range of values obtained from the few individuals examined within species suggest P_L differs consistently and substantially among species. The cichlid species having the greatest amount of suturing were *A. macracanthus* ($P_L=1.18$), *H. labridens* Media Luna ($P_L=1.39$) *H. labridens* Cascadas ($P_L=1.36$) and molariform *H. minckleyi* ($P_L=1.77$).

(d) *Comparative analyses of LPJ suturing and molluscivory*

A significant correlation between P_L and molluscivory was robust to all coding of the variables. The correlation of independent contrasts was highly significant whether quantitative measures (d.f.$=30$, slope$=0.37$, $r=0.83$, $p<0.001$) or categorical designations (d.f.$=7$, slope$=0.10$, $r=0.75$, $p=0.031$) of molluscivory were used. When the residuals of the reduced major axis regression of P_L versus specimen SL were used, similar results were obtained (continuous values of molluscivory: d.f.$=30$, slope$=0.36$, $r=0.84$, $p<0.001$; and categorical values of molluscivory: d.f.$=7$, slope$=0.10$, $r=0.75$, $p=0.033$).

4. DISCUSSION

In the polymorphic *H. minckleyi*, the amount of suturing adds to the list of putatively adaptive characteristics that differ between molariform snail crushers and papilliform plant processors (Kornfield & Taylor 1983; Liem & Kaufman 1984; Hulsey *et al.* 2005). Molariform *H. minckleyi* possess the greatest average amount of external suturing of any Heroine cichlid examined (figures 3*c* and 4) and much greater suturing throughout ontogeny than papilliforms. However, it is unclear, if the amount of suturing is genetically controlled in this species or in any other Heroine. Although, the different pharyngeal morphologies in *H. minckleyi* appear to have some genetic basis (Trapani 2003), much of the pharyngeal jaw variation may be a phenotypically plastic response to crushing the unusually robust snail prey found in their native habitat (Vermeij & Covich 1978; Liem & Kaufman 1984; Hulsey *et al.* 2005).

In *H. minckleyi*, the LPJ consistently split along the suture prior to extensive damage occurring to the remainder of the jaw. It took substantially more force to split the molariform LPJ along the suture than the force used by molariforms to crush snails in the wild. Whereas a molariform that is 150 mm in SL can produce 115 N of crushing force (Hulsey *et al.* 2005), its LPJ can resist approximately 600 N of crushing force before splitting along the suture. A similarly sized papilliform LPJ resists only about 60 N before splitting at the suture. Because greater suturing should act to reduce damage to the jaw and damage to the feeding apparatus should be selected against (Vermeij 1987; Van Valkenburgh 1988), greater LPJ fusion through increased suturing may be a critical modification of the molariform jaw. The minimal fusion of the papilliform LPJ and its inability to resist substantial crushing forces may explain why papilliforms rarely utilize snails as prey (Hulsey *et al.* 2005). Strikingly, if papilliforms generated the forces that molariforms frequently use to crush molluscs, papilliform *H. minckleyi* would likely split their LPJ into separate halves at the suture.

Specialization in molluscs probably evolved several times within Heroine cichlids (Winemiller *et al.* 1995, figure 4). Twelve of the 32 species included herein had gut contents composed of more than 5% molluscs by volume and molluscivory may have evolved at least six times, independently. Notably, molluscivory has also evolved numerous times in other cichlid clades such as *Aequidens* (Winemiller *et al.* 1995), *Astatoreochromis*, *Lamprologus* (Liem 1973) and *Serranochromis* (Winemiller *et al.* 1995). Suturing of Heroine cichlid jaws is also exceptionally variable (figure 4) and external suturing has probably evolved and been lost multiple times in Heroines, as it has in other cichlids (Kullander 1998). Pharyngeal musculature and other skeletal elements are undoubtedly important in adapting the cichlid pharyngeal jaw to mollusc crushing (Liem 1973; Hulsey *et al.* 2005; Wainwright 2005). However, there is a clear relationship between greater suturing and molluscivory both among evolutionarily independent lineages of Heroine cichlids and within *H. minckleyi*. Micro- and macroevolutionary lability in LPJ fusion likely makes it easy for cichlids to evolve to exploit durable prey.

Cichlids share several morphological innovations in their pharyngeal jaw with wrasses (Labridae), damselfish (Pomacentridae), parrotfish (Scaridae) and the surfperches (Embiotocidae). These major groups of fishes were once included in a putatively closely related group named the Labroidei (Kaufman & Liem 1982) that is now recognized as being paraphyletic (Streelman & Karl 1997). Monophyly of the Labroidei was originally hypothesized because of their structurally similar pharyngeal jaws, although, the LPJ in non-cichlid labroids exists as a single fused bone that lacks a suture (Liem 1986; Stiassny & Jensen 1987). Like cichlids with extensive LPJ suturing, many wrasses and surfperch feed upon hard-shelled crustaceans or molluscs (Liem 1986; Randall *et al.* 1997) and parrotfish pharyngeal jaws are likely modified to break down extremely tough prey such as coral (Bellwood 1995). As in cichlids with extensive LPJ suturing, labroid LPJ fusion may have facilitated the exploitation of durable prey (Wainwright *et al.* 2004; Wainwright 2005). Most bony fishes and, therefore, the ancestors to all groups with a labroid pharyngeal jaw likely had a divided LPJ with no suturing (Liem 1986; Wainwright 1989). However, LPJ fusion through suturing is not limited to cichlids. Suturing of the two ceratobranchials composing the cichlid LPJ resemble the condition found in a several non-Labroidei fish clades such as *Gerres*, *Lepomis* and *Pogonias* that lack a muscular sling but frequently consume molluscs (Stiassny & Jensen 1987; Galis & Drucker 1996; Grubich 2003). The repeated coupling of greater LPJ fusion and durophagy during teleost evolution makes the apparent pharyngeal jaw convergence between cichlids and other labroids functionally less surprising.

Both the splitting of LPJs in *H. minckleyi* and the phylogenetic comparative analyses indicate a more extensively fused jaw is advantageous for the exploitation of durable prey. However, the variation in cichlid suturing suggests there are probably disadvantages to completely fused jaws. When manipulating prey, less fusion might facilitate independent movement or greater bending of the two halves of the LPJ (Wainwright 2005). Fused jaws may also decrease the pharyngeal gape and hinder swallowing larger prey like fishes (Wainwright 1987). Piscivory is rare in the other labroid groups (Randall *et al.* 1997; Wainwright *et al.* 2004) and cichlid groups that are primarily piscivorous like *Cichla*, *Crenicichla* and *Petenia* (Chavez-Lomeli *et al.* 1988; Winemiller *et al.* 1995; Hulsey & García de León in press) all have limited LPJ fusion (Kullander 1998). Having a flexibly sutured LPJ as opposed to a single bony element may have permitted cichlids to exploit both large prey types such as fishes as well as hard-shelled prey like no other group of aquatic vertebrates.

Evaluating if a complex key innovation (*sensu* Simpson 1953) like the cichlid pharyngeal jaw is responsible for trophic diversification is difficult. Evolutionary novelties in organismal design are not always replicated (Levinton 1988; Padian 2001) and novel structures cause changes that could themselves be interpreted as innovations (Cracraft 1990). However, when variability in transitional phenotypes exists, what favoured the evolution of a putative innovation can be tested (Greene 1983). Furthermore, verifying that a phenotype is favoured under realistic ecological circumstances can provide support for the hypothesis that particular character states, like greater LPJ fusion, are crucial to specialization on particular prey (Wainwright 1987). In order to evaluate the evolutionary and ecological consequences of their unique trophic apparatus, it may be key to more critically assess the structural and functional consequences of diversity in the cichlid pharyngeal jaw.

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REFERENCES

Bellwood, D. R. 1995 Direct estimate of bioerosion by two parrotfish species, *Chlorururs gibbus* and *Chlorurus sordidus*, on the great barrier reef, Australia. *Mar. Biol.* **3**, 419–429.

Bussing, W. A. 1993 Fish communities and environmental characteristics of a tropical rain forest river in Costa Rica. *Rev. Biol. Trop.* **41**, 791–809.

Chavez-Lomeli, M. O., Mattheeuws, A. E. & Perez-Vega, M. H. 1988 Biología de los peces del río San Pedro en vista de determinar su potencial para la piscicultura. *Inst. Nac. Invest. Rec. Biol.* **1**, 1–222.

Cracraft, J. 1990 The origin of evolutionary novelties: pattern and processes at different hierarchical levels. In *Evolutionary innovations* (ed. M. H. Nitecki), pp. 21–44. Chicago, IL: University of Chicago Press.

Darnell, R. M. 1962 Fishes of the Rio Tamesi and related coastal lagoons in East-Central Mexico. *Publ. Inst. Mar. Sci. Univ. Texas* **8**, 299–365.

Dinkerhus, G. & Uhler, L. H. 1977 Enzyme clearing of Alcian bluestained whole vertebrates for demonstration of cartilage. *Stain Technol.* **52**, 229–232.

Felsenstein, J. 1985 Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15.

Galis, F. & Drucker, E. G. 1996 Pharyngeal biting mechanics in centrarchids and cichlids: insights into a key evolutionary innovation. *J. Evol. Biol.* **9**, 641–670.

Gestring, K. & Shafland, P. 1997 Selected life history attributes of the exotic jaguar guapote (*Cichlasoma managuense*) in Florida. *Florida Scientist* **60**, 137–142.

Greene, H. W. 1983 Dietary correlates of the origin and radiation of snakes. *Am. Zool.* **23**, 431–441.

Grubich, J. 2003 Morphological convergence of pharyngeal jaw structure in durophagous perciform fish. *Biol. J. Linn. Soc.* **80**, 147–165.

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

Hulsey, C. D., García de León, F. J., Sanchez-Johnson, Y., Hendrickson, D. A. & Near, T. J. 2004 Temporal diversification of Mesoamerican cichlid fishes across a major biogeographic boundary. *Mol. Phylogenet. Evol.* **31**, 754–764.

Hulsey, C. D., Hendrickson, D. A. & García de León, F. J. 2005 Trophic morphology, feeding performance and prey use in the polymorphic fish *Herichthys minckleyi*. *Evol. Ecol. Res.* **7**, 1–22.

Hulsey, C. D., Marks, J., Hendrickson, D. A., Williamson, C. A., Cohen, A. E., Stephens, M. J. In press. Feeding specialization in *Herichthys minckleyi*: a trophically polymorphic fish. *J. Fish. Biol*

Kaufman, L. & Liem, K. F. 1982 Fishes of the suborder Labroidei (Pisces: Perciformes): phylogeny, ecology and evolutionary significance. *Brevoria* **472**, 1–19.

Kornfield, I. L. & Koehn, R. K. 1975 Genetic variation and speciation in new world cichlids. *Evolution* **94**, 427–437.

Kornfield, I. L. & Taylor, J. N. 1983 A new species of polymorphic fish, *Cichlasoma minckleyi*, from Cuatro Ciénegas, Mexico (Teleostei: Cichlidae). *Proc. Biol. Soc. Wash.* **96**, 253–269.

Kullander, S. O. 1998 A phylogeny and classification of the South American Cichlidae (Teleostei: Perciformes). In *Phylogeny and classification of neotropical fishes* (ed. L. R. Malabarba, R. E. Reis, R. P. Vari, Z. M. Lucena & C. A. S. Lucena), pp. 461–498. Puerto Alegre, Brazil: Edipucrs.

Levinton, J. S. 1988 *Genetics, paleontology and macroevolution*. Cambridge, UK: Cambridge University Press.

Liem, K. F. 1973 Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. *Syst. Zool.* **22**, 425–441.

Liem, K. F. 1986 The pharyngeal jaw apparatus of the Embiotocidae (Teleostei): a functional and evolutionary perspective. *Copeia* **2**, 311–323.

Liem, K. F. & Kaufman, L. S. 1984 Intraspecific macroevolution: functional biology of the polymorphic cichlid species *Cichlasoma minckleyi*. In *Evolution of fish species flocks* (ed. A. A. Echelle & I. Kornfield), pp. 203–215. Orono, Maine: University of Maine Press.

Maddison, D. R. & Maddison, W. P. 2000 MacClade 4.0. Sinauer Associates, Inc.

Martinez-Palacios, C. A. & Ross, L. G. 1988 The feeding ecology of the Central American cichlid *Cichlasoma uropthalmus* (Gunther). *Florida Scientist* **33**, 665–670.

Meyer, A. 1990 Ecological and evolutionary aspects of the trophic polymorphism in *Cichlasoma citrinellum* (Pisces: Cichlidae). *Biol. J. Linn. Soc.* **39**, 279–299.

Padian, K. 2001 Cross-testing adaptive hypotheses: phylogenetic analysis and the origin of bird flight. *Am. Zool.* **41**, 598–607.

Purvis, A. & Rambaut, A. 1995 Comparative analysis by independent contrasts (CAIC): an apple macintosh application for analyzing comparative data. *Bioinformatics* **11**, 247–251.

Rambaut, A. & Charleston, M. 2002 TreeEdit: phylogenetic tree editor v.1.0 alpha 10. http://evolve.zoo.ox.ac.uk/software/TreeEdit/main.html.

Randall, J. E., Allen, G. R. & Steene, R. C. 1997 *Fishes of the Great Barrier Reef and Coral Sea*. Bathurst, Australia: Crawford House Publishing.

Sage, R. D. & Selander, R. K. 1975 Trophic radiation through polymorphism in cichlid fishes. *Proc. Natl Acad. Sci. USA* **72**, 4669–4673.

Sanderson, M. J. 1997 A nonparametric approach to estimating divergence times in the absence of rate constancy. *Mol. Biol. Evol.* **14**, 1218–1231.

Schluter, D. & Nagel, L. M. 1995 Parallel speciation by natural selection. *Am. Nat.* **146**, 292–301.

Simpson 1953 *The major features of evolution*. New York: Columbia University Press.

Stiassny, M. L. J. & Jensen, J. S. 1987 Labroid interrelationships revisited: morphological complexity, key innovations and the study of comparative diversity. *Bull. Mus. Comp. Zool.* **151**, 269–319.

Streelman, J. T. & Karl, S. A. 1997 Reconstructing labroid evolution with single-copy nuclear DNA. *Proc. R. Soc. B* **264**, 1011–1020. (doi:10.1098/rspb.1997.0140)

Thorpe, J. H. & Covich, A. P. 1991 *Ecology and classification of North American freshwater invertebrates*. San Diego, CA: Academic Press.

Trapani, J. 2003 Morphological variability in the Cuatro Cienegas cichlid, *Cichlasoma minckleyi*. *J. Fish Biol.* **62**, 276–298.

Van Valkenburgh, B. 1988 Incidence of tooth breakage among large, predatory mammals. *Am. Nat.* **131**, 291–302.

Vermeij, G. J. 1987 *Evolution and escalation: an ecological history of life*. Princeton, NJ: Princeton University Press.

Vermeij, G. J. & Covich, A. P. 1978 Coevolution of freshwater gastropods and their predators. *Am. Nat.* **112**, 833–843.

Wainwright, P. C. 1987 Biomechanical limits to ecological performance: mollusc crushing by the Caribbean hogfish, *Lachnolaimus maximus* (Labridae). *J. Zool. Lond.* **213**, 283–298.

Wainwright, P. C. 1989 Functional morphology of the pharyngeal jaws in perciform fishes: an experimental analysis of the Haemulidae. *J. Morphol.* **200**, 231–245.

Wainwright, P. C. 2005 Functional morphology of the pharyngeal jaw apparatus. In *Biomechanics of fishes* (ed. R. Shadwick & G. V. Lauder), pp. 77–101. Chicago, IL: Elsevier Press.

Wainwright, P. C., Bellwood, D. R., Westneat, M. W., Grubich, J. R. & Hoey, A. S. 2004 A functional morphospace for the skull of the labrid fishes: patterns of diversity in a complex biomechanical system. *Biol. J. Linn. Soc.* **82**, 1–25.

Winemiller, K. O. 1989 Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan Ilanos. *Environ. Biol. Fish.* **26**, 177–200.

Winemiller, K. O., Kelso-Winemiller, L. C. & Brenkert, A. L. 1995 Ecological diversification and convergence in fluvial cichlid fishes. *Environ. Biol. Fish.* **44**, 235–261.

Yanez-Arancibia, A. 1978 Taxonomía, ecología, y estructura de las comunidades de peces en lagunas costeras con bocas efímeras del Pacifico de Mexico. *Public. Esp. Cen. Cien. Mar. Limn.* **2**, 1–306.