Co-evolution of the premaxilla and jaw protrusion in cichlid fishes (Heroine: Cichlidae)

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The ability of Perciform fishes to protrude their jaw has likely been critical to the trophic diversification of this group, which includes approximately 20% of all vertebrates. The length of the ascending process of the premaxilla is thought to influence the maximum extent that cichlids and other Perciforms protrude their oral jaw. Using a combination of morphometrics, kinematics, and new phylogenetic hypotheses for 20 Heroine cichlid species, we tested the evolutionary relationship between the length of the premaxillary ascending process and maximum jaw protrusion. In this clade, the length of the ascending process of the premaxilla ranged from 11.6–32.7% with respect to standard length whereas maximum jaw protrusion ranged from 3.5–23.4% with respect to standard length. The evolutionary relationships among the Heroine cichlids obtained from the genetic partitions cytochrome *b*, S7, and RAG1 showed limited concordance. However, correlations between the length of the ascending process and maximum jaw protrusion were highly significant when examined as independent contrasts using all three topologies. Evolutionary change in the length of the ascending process of the premaxilla is likely critical for determining the amount of jaw protrusion in Perciform groups such as cichlid fishes.   © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **100**, 619–629.

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INTRODUCTION

The ability of Perciform fishes to protrude their oral jaws has likely contributed to the unparalleled diversity of this vertebrate clade (Alexander, 1967; Lauder, 1982; Ferry-Graham & Lauder, 2001). This group of fishes comprises 20% of all vertebrates and exhibits a stunning array of trophic specializations that are tied to the ability to protrude the oral jaws (Schaeffer & Rosen, 1961; Ferry-Graham & Lauder, 2001). For example, jaw protrusion can increase the speed a predator approaches evasive prey fish (Waltzek & Wainwright, 2003), can adapt feeding movements to the position of benthic dwelling prey types (Motta, 1984), can increase the distance over which a predator can suck difficult to obtain items into the mouth (Ferry-Graham, Wainwright & Bellwood, 2001a; Wainwright *et al.*, 2001), and can augment the hydrodynamic force exerted on attached and evasive prey (Holzman *et al.*, 2008a,b). Although several mechanisms are considered to contribute to the ability of Perciforms to protrude their jaws, the shape of the premaxilla that forms the upper oral jaw of Perciform fishes is likely critical (Witte, 1984). The length of the ascending process of the premaxilla could dictate the maximum extent to which Perciformes are able to protrude their upper jaw. To test this idea, we examine whether the length of the premaxillary ascending process (PAP) coevolves with the amount of maximum jaw protrusion (MJP) in a trophically diverse group of cichlid fishes.

The PAP is relatively long in groups of Perciformes that protrude their jaws extensively (Westneat & Wainwright, 1989; Westneat, 1991; Ferry-Graham *et al.*, 2001b; Waltzek & Wainwright, 2003). Because

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the PAP in Perciforms likely guides the upper jaw out during protrusion and back into its resting position as the jaws are closed (Alexander, 1967), its length should reflect the maximum amount that a fish can protrude its jaw. It is possible that the ascending process is able to protrude past its insertion point along the midline of the head. If this occurs, we would expect MJP in some cichlids to exceed their PAP length. Moreover, other mechanisms, such as rotation of the lower jaw, cranial elevation, and the four bar linkage system of the anterior jaws, are also important in the evolution of jaw protrusion in Perciforms (Lauder & Liem, 1981; Westneat, 1995; Waltzek & Wainwright, 2003; Westneat, 2004; Hulsey & García de León, 2005). Thus, the diversity of mechanisms determining MJP could lead to a weak correspondence between PAP length and MJP in cichlid species.

Cichlid fish groups such as the Heroine cichlids of Central America exhibit considerable trophic diversity (Eaton, 1943; Liem & Osse, 1975; Winemiller, Kelso-Winemiller & Brenkert, 1995; Hulsey, 2006) and a substantial component of that divergence is associated with jaw protrusion. Heroine cichlids such as *Petenia splendida* are known to exhibit some of the most extensive abilities to protrude their jaw of any Perciform. The ascending process of the premaxilla in this species is also exceptionally long, suggesting that a correlation between PAP length and MJP could be high (Waltzek & Wainwright, 2003). However, to date, protrusion in Heroine cichlids has been primarily measured on preserved specimens and the maximum protrusion ability of live individuals is unclear (Hulsey & García de León 2005). It is also generally unknown whether species that do not exhibit substantial jaw protrusion have relatively short ascending processes. If PAP length is critical for determining the maximum amount that most cichlids can protrude their jaws, we would expect a strong evolutionary correlation between PAP length and the MJP that a particular cichlid species exhibits.

To test the importance of the premaxillary ascending process to maximum jaw protrusion, it would be ideal to examine the co-evolution of these traits in a well-resolved phylogenetic context. However, the phylogenetic relationships among Heroine cichlids continually prove difficult to resolve (Kullander, 1998; Hulsey *et al.*, 2004; Chakrabarty, 2006; Higham *et al.*, 2007; Rican, Zardoya & Doadrio, 2008). Determining the evolutionary relationships among species in genera such as *Petenia*, *Caquetaia*, and *Parachromi*s that all have long ascending processes (Eaton, 1943; Waltzek & Wainwright, 2003) appears especially critical for determining how MJP and the length of the PAP coevolve. Many previous studies of the relationships among Heroine cichlids

have relied extensively on the phylogenetic relationships recovered from mitochondrial DNA (Hulsey *et al.*, 2004). With the advent of rapidly evolving nuclear markers, more robust analyses of the relationships among these fishes are now possible. Resolving the relationships among these species would also allow the evaluation of whether extensive jaw protrusion has appeared in multiple evolutionary lineages of Heroine cichlids.

In the present study, we combine morphological measurements, kinematic videos of jaw protrusion, and two new phylogenetic hypotheses of 20 Heroine cichlid species to test the evolutionary relationship between MJP and the length of the PAP. First, we examined PAP length in morphological specimens of each cichlid species. Then, we recorded high-speed video sequences of these species when they were consuming evasive prey to estimate the MJP of each species. We also examined the phylogenetic relationships among these cichlids using one mitochondrial and two nuclear genetic markers. Finally, we employed phylogenetic independent contrasts to test the evolutionary correlation between MJP and PAP length.

MATERIAL AND METHODS

MORPHOLOGY

A combination of specimens collected from the wild by the authors and wild-caught accessioned museum specimens were used in the quantification of morphology. Collection localities of all specimens are the same as those utilized for the analyses conducted by Hulsey, García de León & Rodiles–Hernández (2006). For the morphological analyses, up to three specimens of each species were cleared using trypsin and double-stained using an Alcian-blue cartilage stain and alizarin red bone stain (Dingerkus & Uhler, 1977) for the examination of PAP length. Because of availability, only one *Parachromis managuensis* and two specimens of *Nandopsis tetracanthus* and *Hypsophrys nicaraguensis* were examined. The standard length (SL) of all preserved specimens was first measured. The length of the PAP (Fig. 1) was measured from its most posterior process to the tip of the tooth on the most anterior part of the premaxilla. For analyses, PAP length was expressed as a percentage of SL.

KINEMATICS

All cichlids used in the kinematic analyses were purchased commercially and housed at the University of California (Davis, CA, USA). During experiments, fish were fed similarly sized guppies (*Poecilia reticulata*) that were approximately 2–3 cm in length. We
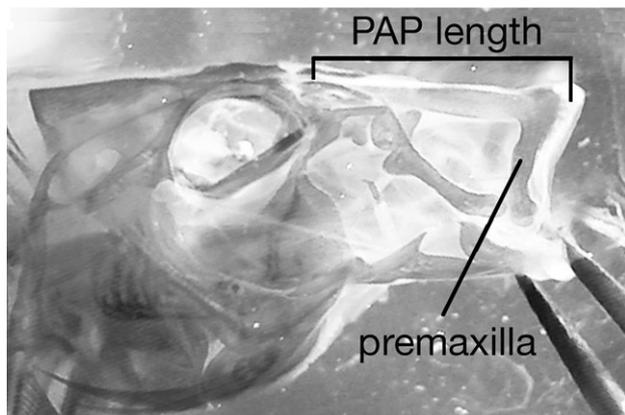
Figure 1. The length of the premaxilla ascending process (PAP) was measured in cleared and stained cichlid specimens of the 20 Heroine species examined. The PAP length was measured from its posterior tip to the tip of the tooth on the end of the premaxilla.

used this prey item because it represents a relatively evasive prey species (Wainwright *et al.*, 2001), and we wanted to elicit as close to maximum protrusion as possible from each cichlid. The size of our prey was well within the acceptable range expected based on the predator's mouth size (Costa, 2009). One individual from each species was tested, and at least four video sequences per individual were obtained. Although one individual provides no intra-specific replication and could potentially increase the error in our analyses, we consider that the most extensive variation in MJP likely lies among the species examined and not within species. Furthermore, any significant results recovered would indicate that this level of within species replication provides sufficient power to test the hypotheses examined. All maintenance and experimental procedures used in this research complied with guidelines for the use and care of animals in research at the University of California, Davis.

We recorded the jaw movements in lateral view from each fish by using a high-speed NAC Memrecam Ci digital system (Japan) operating at 500 images per second. Grids (1×1 cm) were placed behind the fish to provide a distance scale. Additionally, we took a picture of a ruler inside the tank at the location of the prey to verify the scaling. Two floodlights (600 W) on either side of the camera illuminated the experimental tank during the experiment. After the experiments, the fish were sacrificed with an overdose of anesthetic (MS-222) and the SL of the fish was measured. These measurements of SL were previously reported in Higham *et al.* (2007), except for the values for *Parachromis dovii*, '*Cichlasoma*' *grammodes*, and *Parachromis friedrichstahli*, which were measured using digital images.

For the kinematic analysis of MJP, we examined only those trials where the trajectory of the fish was parallel to the long axis of the tank and therefore orthogonal to the camera. We also only analyzed sequences in which the prey was completely consumed. All sequences were converted to stacks of digital images and imported into ImageJ, version 1.33 (National Institutes of Health) for digitizing. We digitized points in two frames (Fig. 2). The first (closed) was a randomly chosen frame before the feeding event when the jaw was completely closed. The second (max protrusion) was the image when the upper jaw was maximally extended. For the two frames examined in each sequence, we digitized the distance between the anterior tip of the upper jaw and the most posterior point on the eye. The difference in the two measurements (max protrusion minus close) for each fish was analyzed for each sequence. The average of all measurements is given, although the single greatest estimate of MJP obtained for each species is also reported and was used in the comparative analyses.

PHYLOGENETIC ANALYSIS

Exactly 1000 bp of the RAG1 protein coding locus of the 20 Heroine cichlid species (Table 1) and the outgroup *Geophagus brasiliensis* (Farias *et al.*, 2001) were sequenced using primers from Grande, Laten & Lopez (2004). These sequences (GU595398–GU595416) have been submitted to GenBank. In addition to these new sequences, the cytochrome *b* mitochondrial gene (1137 bp) and the S7 ribosomal intron (approximately 490 bp) previously generated for these species were analyzed in a phylogenetic context. The individuals sequenced for the RAG1 were collected from sites reported in previous studies (e.g. Hulsey *et al.*, 2004).

For sequencing, total genomic DNA was isolated from axial muscle using Puregene extraction. A 1-µL aliquot of this solution was used to provide a DNA template for the polymerase chain reaction (PCR) performed using standard protocols. Positively amplified DNA was then purified using an enzymatic combination of 1 µL of exonuclease I (10.0 U µL^{-1}) and 1 µL of shrimp alkaline phosphatase (2.0 U µL^{-1}) per 10 µL of PCR product. Sequences were read at the HighThroughput Sequencing Facility at the University of Washington. Complete gene sequences were assembled from individual sequencing reactions using the program SEQUENCHER, version 4.1 (Gene Codes). For analyses, sequences were aligned using CLUSTAL X (Larkin *et al.*, 2007).

For the Bayesian analyses, the cytochrome *b* gene, S7 intron, and RAG1 exon were analyzed independently. For analyses, the cytochrome *b* and RAG1
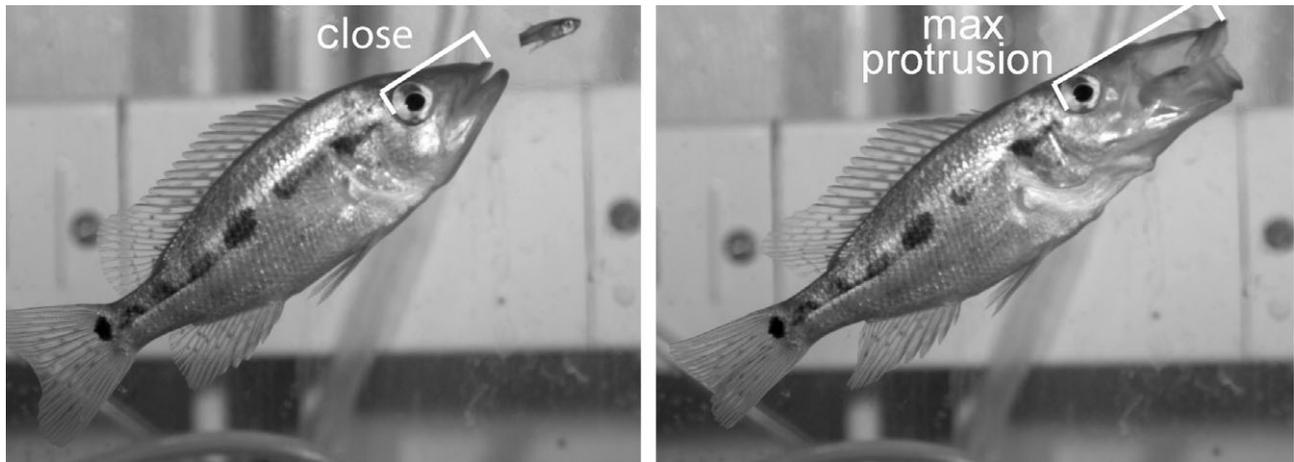
Figure 2. Maximum jaw protrusion (MJP) was measured using high-speed (500 frames per second) video recordings of each species. At least four video sequences per species were examined when fish were fed *Poecilia reticulata*. Measurements from the posterior-most point of the eye to the tip of the upper jaw were measured in calibrated digital stills taken from each video sequence. The first measurements were made when the jaws of the fish were 'closed' prior to prey capture and the second measurement was made when the jaws were maximally protruded or 'max protrusion'. The difference between these measurements was used as the estimate of the MJP for each species.

Table 1. The sequences used in the reconstruction of Heroine cichlid phylogenetic relationships

Species	Cytochrome *b*	S7	RAG1
Amititlania nigrofasciatus	AF009934	EF433034	GU595399
Amphilophus citrinellus	AB018985	DQ119256	GU595398
Caquetaia kraussii	AF009938	DQ836815	GU595400
Caquetaia myersi	AY050615	DQ836805	EF362577
Caquetaia umbrifera	AF009940	DQ836816	GU595404
'Cichlasoma' salvini	AY324029	EF433015	GU595402
'Cichlasoma' trimaculatum	AY324031	DQ836822	GU595403
'Cichlasoma' festae	AY050610	DQ119274	GU595407
'Cichlasoma' grammodes	DQ990718	EF433016	GU595416
Herichthys pearsei	DQ494388	DQ836823	GU595408
Herichthys minckleyi	AY323996	DQ836821	GU595409
Hypsophrys nicaraguensis	AF009930	DQ836819	GU595410
Nandopsis haitiensis	DQ494391	DQ836806	GU595405
Nandopsis tetracanthus	DQ494386	DQ836810	GU595406
Parachromis managuensis	AY050613	DQ836813	GU595411
Parachromis dovii	DQ990701	DQ119262	GU595414
Paracrhomis friedrichstahli	DH235781	DH235782	GU595415
Petenia splendida	AF370679	DQ836820	GU595412
Rocio octofasciatum	AY324017	DQ836808	GU595401
Vieja maculicauda	U97165	DQ836818	GU595413
Geophagus brasiliensis	AF370659	EU199082	EU706360

genes were partitioned into their three codon sites using MACCLADE, version 4.0 (Maddison & Maddison, 2000). The S7 intron was treated as a single partition and indels were treated as missing data. MODELTEST, version 3.06 (Posada & Crandall, 1998), was used to identify the best model of molecular evolution for each protein-coding codon site and the entire S7 partition. MrBayes, version 3.0 (Ronquist & Huelsenbeck, 2003), was utilized to perform the Bayesian analyses aiming to find the best approximations of the maximum likelihood tree. The analyses treated the transition-transversion matrices, number of invariant sites, and gamma shape parameters as unlinked and independent for codon site in

the two protein coding loci. Flat prior probability distributions for all parameters were assumed before analysis. For each of three gene regions, we ran three separate Bayesian analyses for 1 000 000 generations with four Markov chains in each run. We sampled trees from the Markov chain Monte Carlo search algorithm every 1000 generations. At the end of each analysis, the log-likelihood scores were plotted against generation time to identify the point at which log likelihood values reached a stable equilibrium. In all three, the equilibrium appeared to be reached at approximately 50 000 generations, and therefore, sample points prior to generation 100 000 in each run were discarded as 'burn-in' samples. The remaining samples from all runs combined were used to produce a single majority rule consensus tree in PAUP* 4.0b10 (Swofford, 2002) for each of the three separate genetic partitions. The percentages of all trees recovered for a particular clade (the clade's posterior probability) were depicted on the single best likelihood tree topology recovered during the three Bayesian runs performed on each locus.

COMPARATIVE ANALYSIS

Correlations between the PAP length and MJP were examined both without taking phylogenetic relationships into account (tip values) and also in a phylogenetic context.

We considered that if PAP length sets a constraint on MJP, empirical species values for their relationship should always fall below a 1 : 1 relationship of PAP length and MJP. The species tip values for PAP length and MJP were both plotted against one another aiming to examine their relationship and how the species values compared with the theoretical constraint of a 1 : 1 relationship between these values. To determine whether PAP length could function as a constraint, we also performed a paired one-tailed t-test on the empirical values to determine whether MJP was consistently lower than PAP length.

However, correlations among species values are not statistically independent because of the shared evolutionary history of species (Felsenstein, 1985). Therefore, we calculated the phylogenetic independent contrast (PIC) to test the hypothesis that evolutionary changes in PAP lengths are associated with evolutionary changes in MJP. Correlations between PICs were calculated using the single best topology recovered for each of the three genes. For the independent contrasts, the phylogenetic topology with branch lengths was imported into MESQUITE, version 1.06 (Maddison & Maddison, 2005). We then used the PDAP package implemented in MESQUITE (Midford *et al.* 2005) to obtain the independent contrasts. The correlation between the PIC values was

then determined with a reduced major axis regression because there is considerable variation in both variables. The relationship between size corrected PAP length and MJP were then painted onto mirror images of the S7 phylogeny using MESQUITE with the values broken into approximately 3% intervals. The quantitative measurements were then painted onto the two trees using parsimony reconstructions implemented in MESQUITE.

RESULTS

PAP length (Table 2) ranged from a low of 10.6% of SL in *Herichthys pearsei* to 32.7% of SL in *Caquetaia myersi*. The mean value of PAP length for all species was 17.5% of SL. Size corrected MJP ranged from 23.4% in *C. myersi* to 3.5% in *Herichthys minckleyi*. The mean value of MJP was 10.6% of SL. It appeared that close to maximum effort was generally elicited in the videos because the mean value of jaw protrusion was generally close to the MJP value for the species.

When only the size corrected species tip values were examined, there was a clear relationship between PAP length and MJP (Fig. 3). However, the species values of size corrected PAP length were consistently greater than the values recovered for size
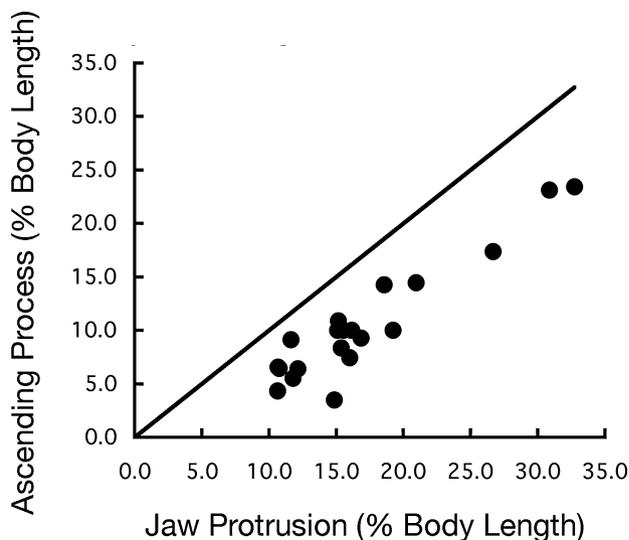

Figure 3. The relationship between maximum jaw protrusion (MJP) and premaxillary ascending process (PAP) length species tip values for the 20 Heroines species is shown. The relationship between these data points and a 1 : 1 relationship between MJP and PAP length is depicted. Because the empirical estimates of MJP never exceed the PAP length ($P < 0.001$), this suggests that the PAP length sets a morphological constraint on the ability of the cichlid species to protrude their jaws.

Table 2. The measurements of standard length of morphological specimens (MSL) and the length of the premaxillary ascending process (PAP) for the 20 species of Heroine cichlids

Species	MSL (mm)	PAP (mm)	PAP % MSL	VSL (mm)	MJP maximum/ mean (mm)	MJP % VSL
Amititlania nigrofasciatus	58.5	7.1	12.1	64	4.1/3.2	6.4
Amphilophus citrinellus	78.0	12.0	15.4	85	7.1/5.8	8.4
Caquetaia kraussii	83.6	22.3	26.7	83	14.4/13.3	17.3
Caquetaia myersi	95.3	31.2	32.7	94	22.0/17.4	23.4
Caquetaia umbrifera	64.4	13.5	21.0	65	9.4/8.5	14.5
'Cichlasoma' salvini	73.0	11.8	16.2	77	7.7/5.6	10.0
'Cichlasoma' trimaculatum	77.5	11.7	15.1	76	7.6/6.1	10.0
'Cichlasoma' festae	63.3	9.6	15.2	67	7.3/5.5	10.9
'Cichlasoma' grammodes	72.1	12.6	17.5	85	7.2/5.7	8.5
Herichthys pearsei	82.7	8.8	10.6	83	3.6/2.1	4.3
Herichthys minckleyi	90.5	13.4	14.9	77	2.7/2.2	3.5
Hypsophrys nicaraguensis	79.0	8.5	10.8	79	5.1/3.9	6.5
Nandopsis haitiensis	63.8	9.9	15.5	65	6.5/6.0	10.0
Nandopsis tetracanthus	62.5	10.0	16.0	62	4.6/4.0	7.4
Parachromis managuensis	64.6	12.0	18.6	61	8.7/7.6	14.3
Parachromis dovii	79.5	15.3	19.2	83	8.3/6.8	10.0
Paracrhomis friedrichstahli	57.6	9.7	16.8	96	8.9/7.3	9.3
Petenia splendida	83.4	25.8	30.9	67	15.5/14.5	23.1
Rocio octofasciatum	74.1	8.6	11.6	69	6.3/5.1	9.1
Vieja maculicauda	67.0	7.9	11.8	69	3.8/2.8	5.5

The standard length from the video (VSL) and the maximum and mean jaw protrusion (MJP) of each species are also shown. Both PAP length and MJP are shown as a percentage of their respective measures of standard length. Except for *Parachromis dovii*, *Cichlasoma grammodes*, and *Parachromis friedrichstahlii*, the measurements of VSL were previously reported in Higham *et al.* (2007) and were measured to a different significant digit.
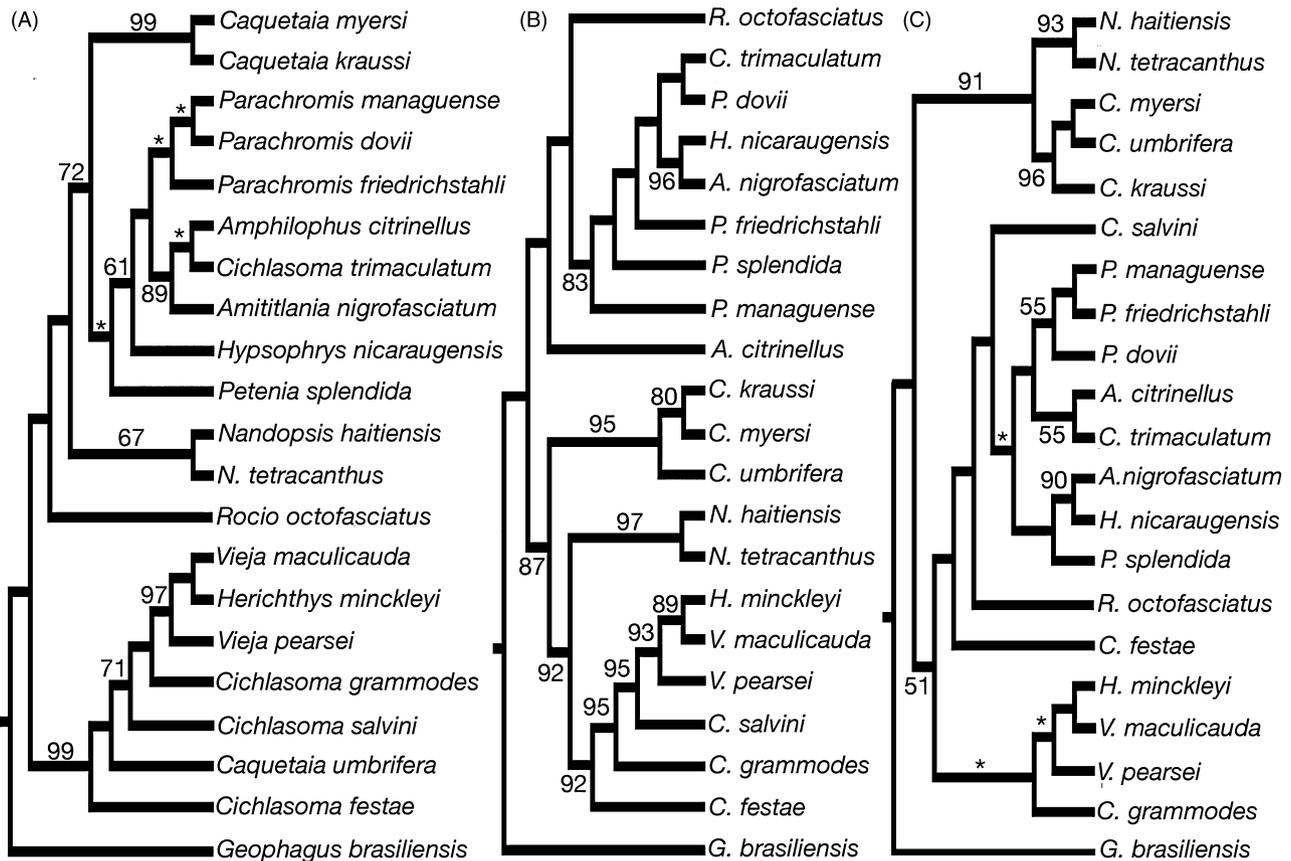
Figure 4. The phylogenetic relationships reconstructed for the 20 Heroine species examined for morphology and kinematics are depicted. The three genes: (A) cytochrome *b*, (B) S7 ribosomal intron, and (C) RAG 1 recovered somewhat discordant relationships among the 20 species examined.

corrected MJP. For all the species examined, MJP expressed as a percentage of PAP length was 59.0% on average and MJP was never larger than PAP length. The one-tailed *t*-test between PAP length and MJP was highly significant ($P < 0.001$) indicating that MJP of Heroine species was significantly lower than the constraint imposed by PAP length.

The phylogenetic relationships recovered (Fig. 4) from the three different loci were fairly discordant. However, there are some commonalities among the relationships inferred using the three genes. Both *Nandopsis haitiensis* and *N. tetracanthus* were recovered as closely related, although the exact relationship of this clade to others was highly variable. *Caquetaia* and *P. splendida*, two groups that exhibit extensive jaw protrusion, were never found to form a monophyletic clade. However, *P. splendida* consistently showed affinities to the *Parachromis* species that also display substantial jaw protrusion. Similarly, *'Cichlasoma' grammodes* was always recovered as relatively closely related to *Vieja* and *H. minckleyi*, which also consistently grouped together.

Several phylogenetic relationships were extremely variable between the three genetic partitions. *Rocio octofasciatum* showed variable affinities when the three topologies were compared, as did *'Cichlasoma' festae*. All of the *Caquetaia* species examined were recovered as monophyletic with S7 and RAG1, although not with cytochrome *b*. The three *Parachromis* species formed a clade with cytochrome *b* and RAG1, but not with S7. *Amititlania nigrofasciatus*, *Amphilophus citrinellus*, *'Cichlasoma' trimaculatum*, and *H. nicaraguensis* repeatedly fell in the same region of the tree but showed highly variable relationships.

The analyses of independent contrasts consistently gave highly significant results (Fig. 5) using all three of the different phylogenetic hypotheses recovered. The analyses of cytochrome *b* PICs ($r = 0.88$; $P < 0.0001$; Fig. 5), S7 intron PICs ($r = 0.89$; $P < 0.0001$), and RAG1 PICs ($r = 0.93$; $P < 0.0001$) all recovered highly significant correlation coefficients between PAP length and MJP. The strong correlation recovered between PAP length and MJP across the
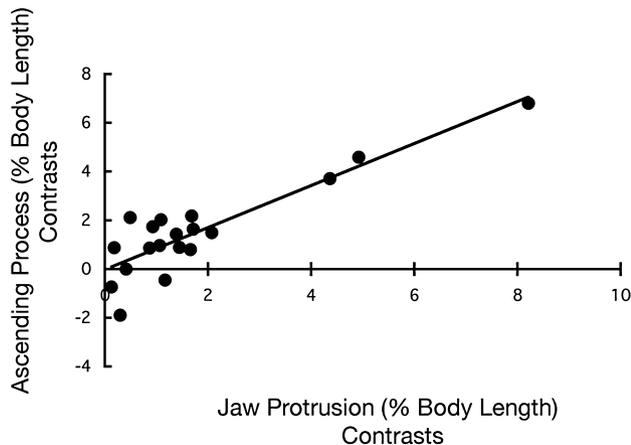
Figure 5. The phylogenetically independent contrast correlation between maximum jaw protrusion (MJP) and premaxillary ascending process (PAP) length for the cytochrome *b* gene are shown. There is a highly predictive relationship between the two variables, regardless of which of the three phylogenies produced in the present study was used to account for shared phylogenetic history. A change in MJP is clearly correlated with a change in PAP length during the evolution of Heroine cichlids.

phylogeny is obvious when this relationship is depicted on any one of the phylogenetic hypotheses (Fig. 6).

DISCUSSION

The length of the PAP of the oral jaws varies extensively in Heroine cichlids. The PAP was in the range 10.6–32.7% with respect to body length. *Petenia splendida* and the two *Caquetaia* species examined show the longest PAP of any of the Heroine cichlids examined. *Herichthys pearsei* has the shortest PAP as a percentage of body length. This range of PAP length is reflected by the range of MJP variation that Heroine cichlids exhibit. *Petenia splendida* and *C. myersi* exhibit the most extensive jaw protrusion of any of the Heroine cichlids examined. Species such as *A. nigrofasciatus* and *H. minckleyi* possess some of the smallest amounts of jaw protrusion in the Heroine cichlids. The amount of MJP as a percentage of body length has changed substantially during the evolution of Central American Heroines.

The three phylogenetic hypotheses recovered for the three different genetic loci were quite distinct from one another. The phylogeny and taxonomy of Heroine cichlids has long been chaotic (Kullander,
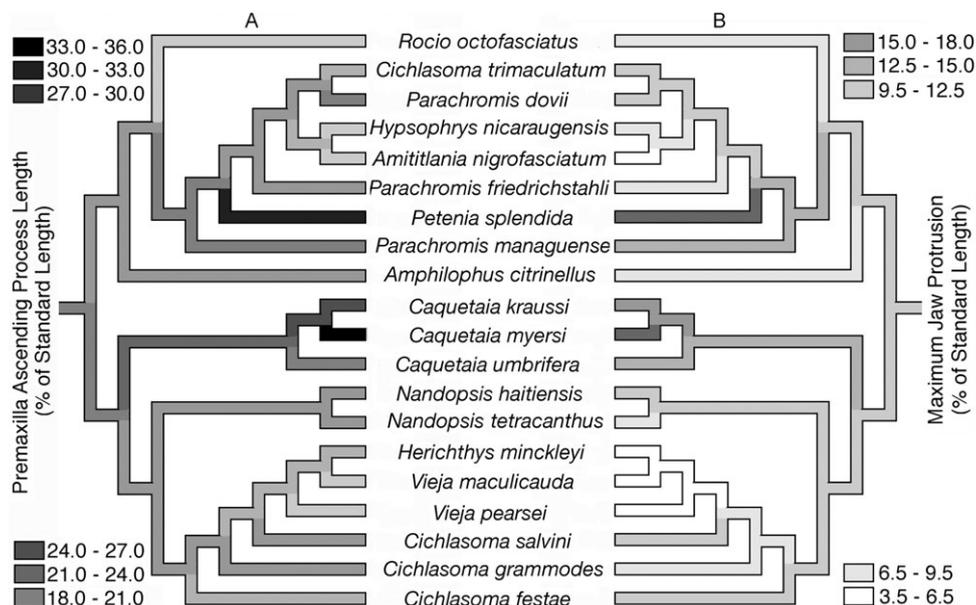

Figure 6. Co-evolution of the premaxilla and jaw protrusion in Heroine cichlids. premaxillary ascending process (PAP) length (A) and maximum jaw protrusion (MJP) (B) expressed as a percentage of standard length (SL) and mapped onto mirror images of the best S7 phylogeny. Darker shades represent a greater percentage of SL and the measurements are divided into approximately 3% increments of SL, as depicted in the rectangles above. The quantitative measurements were painted onto the trees using parsimony reconstructions implemented in MESQUITE. As a rule, PAP length is longer than MJP in any given species, although there is a clear association among the variables across the Heroine species that are shown in the middle between the phylogenetic mappings of the two variables.

1998; Farias *et al*., 2001), with many species inferred from morphology to have uncertain affinities with the group and to be ambiguously referred to generically as '*Cichlasoma*' (Kullander, 1998). Unfortunately, a stable taxonomy and understanding of the phylogenetic relationships of many species remains elusive. Many of these '*Cichlasoma*' species and species such as *R. octofasciatum* do not exhibit consistent phylogenetic relationships with respect to different genetic partitions. The general lack of concordance of the three best phylogenies recovered here for each gene should generally serve as a warning for phylogenetic studies that concatenate different genetic partitions into one data file. As the availability of genetic markers from different parts of the genome have become available, this discord between gene trees and species phylogenies has become obvious. Future systematic and comparative studies will have to incorporate methods that provide robust inferences in the face of this gene and species tree discordance (Maddison & Knowles, 2006) especially if a stable taxonomy for these fishes is to be found.

Although both the PAP length and jaw protrusion are evolutionarily quite variable, evolutionary change in the length of the PAP is clearly positively correlated with the evolution of jaw protrusion in Heroine cichlids (Fig. 6). This result was robust to comparative analyses performed using all three distinct phylogenetic topologies derived from the three genes. The consistent phylogenetic divergence between groups such as *P. splendida* and *Caquetaia* species, which both have extensive jaw protrusion and are repeatedly found to be non-monophyletic, contributes to the strength of the macroevolutionary correlation we recovered. Likewise, the evolution of relatively short MJP characterizes numerous other phylogenetic lineages in this cichlid clade (Fig. 6). Future studies of jaw protrusion in this group should examine the advantages and constraints characterizing species with limited MJP.

Whatever its exact pattern of macroevolutionary divergence in Heroine cichlids, the length of the PAP likely constrains the maximum extent the upper jaw can be protruded in cichlids. The species values of length of PAP as a percentage of SL were consistently more than the values recovered for MJP as a percentage of SL. Because the amount of jaw protrusion as a percent of body length is never as great as PAP length for a given species, it is unlikely that these cichlids can ever extend their jaws a greater distance than the length of their PAP. This finding is consistent with the length of the PAP setting a constraint on the maximum jaw protrusion an individual fish could exhibit. One of the primary goals of ecomorphology is to identify the types of morphometric characters that can be used to make consistent and clear inferences on the maximum abilities of an organism (Wainwright, 1996). Other simple ecomorphological variables, such as gape width setting a maximum size of prey that a predatory fish can ingest (Mittelbach & Persson, 1998; Costa, 2009), or the cross-sectional area of the pharyngeal biting muscles setting a maximum on the force a crushing predator can exert on a prey (Wainwright, 1987; Hulsey & García de León, 2005), have substantial explanatory power. Similarly, PAP length appears to provide a highly predictive maximum for the amount a predatory Perciform can protrude its jaws.

Numerous other mechanisms such as rotation of the lower jaw, cranial elevation, and the four bar linkage system of the anterior jaws are likely important in jaw protrusion of cichlids and many other Perciform fishes (Westneat, 1995; Waltzek & Wainwright, 2003; Hulsey & García de León, 2005). In some cases, these components or other elements such as ligaments that connect these mechanisms to the jaw might operate to fundamentally constrain the MJP of most fish. Additionally, it is possible that further studies could show that PAP length is more critical in constraining MJP in species with large PAP lengths. Nevertheless, the shape of the premaxilla is likely critical during jaw protrusion (Witte, 1984). The tight evolutionary relationship between length of the ascending process and the amount of jaw protrusion found in the present study across the Heroine phylogeny suggests the ascending process is critical in the evolution of cichlid feeding. Further comparative analyses of other groups of Perciform fishes exhibiting substantial variation in jaw protrusion should also be conducted to determine whether the correlated evolution recovered in the present study comprises a general pattern. How the feeding abilities that jaw protrusion enhances have contributed to Perciform diversification will become increasingly clear as we more explicitly examine the mechanisms responsible for both limiting and enhancing jaw protrusion.

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