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MICRO- AND MACROEVOLUTIONARY DECOUPLING OF CICHLID JAWS: A TEST OF LIEM'S KEY INNOVATION HYPOTHESIS

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Abstract.—The extent to which elements of functional systems can change independently (modularity) likely influences the diversification of lineages. Major innovations in organismal design, like the pharyngeal jaw in cichlid fishes, may be key to a group's success when they relax constraints on diversification by increasing phenotypic modularity. In cichlid fishes, pharyngeal jaw modifications that enhanced the ability to breakdown prey may have freed their oral jaws from serving their ancestral dual role as a site of both prey capture and prey processing. This functional decoupling that allowed the oral jaws to become devoted solely to prey capture has been hypothesized to have permitted the two sets of cichlid jaws to evolve independently. We tested the hypothesis that oral and pharyngeal jaw mechanics are evolutionarily decoupled both within and among Neotropical Heroine cichlids. In the trophically polymorphic species *Herichthys minckleyi*, molariforms that exhibit enlarged molarlike pharyngeal jaw teeth were found to have approximately 400% greater lower jaw mass compared to *H. minckleyi* with the alternative papilliform pharyngeal morphology. However, oral jaw gape, lower jaw velocity ratios, anterior jaw linkage mechanics, and jaw protrusion did not differ between the morphotypes. In 40 other Heroine species, there was a weak correlation between oral jaw mechanics and pharyngeal jaw mass when phylogenetic history was ignored. Yet, after expansion of the cytochrome *b* phylogeny for Heroines, change in oral jaw mechanics was found to be independent of evolutionary change in pharyngeal jaw mass based on independent contrasts. Evolutionary decoupling of oral and pharyngeal jaw mechanics has likely played a critical role in the unparalleled trophic diversification of cichlid fishes.

Key words.—Cichlidae, comparative methods, duplication, integration, pleiotropy.

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Increases in the particulate nature of systems likely facilitate diversification (Wagner and Altenberg 1996). By allowing biological units to follow independent evolutionary trajectories, recurring events like speciation (Slowinski and Guyer 1993) and gene duplication (Force et al. 2005) that result in evolutionary subdivision may be the primary forces structuring organismal diversity. But not all evolutionary events are repeated. This is problematic because diversification within species is often used to infer the mechanisms that, iterated numerous times, produce macroevolutionary patterns (Losos et al. 1997, 1998; Reznick et al. 1997). Similarly, macroevolutionary studies document variation among clades and seek to infer processes generating population-level variation (Vermeij 1987; Hansen and Martins 1996). However, neither microevolution nor macroevolution alone can explain organismal diversity (Vermeij 1974; Erwin 2000). Key innovations (Simpson 1953) such as the cichlid pharyngeal jaw (Liem 1973) that have arisen only once may augment a clade's success independent of microevolutionary forces (McShea 1998). Yet, novel traits like the cichlid pharyngeal jaw should contribute most to diversification when promoting divergence both within species and in entire clades. One way the pharyngeal jaw may have been key to cichlid success at multiple stages of their radiation is through enhancing the modularity or functional decoupling between the pharyngeal and the oral jaws during evolution (Liem 1973). To test whether the two jaws in cichlids can evolve independently, we investigated whether change in oral jaw mechanics is correlated with modifications of the pharyngeal jaw among Neotropical Heroine cichlids and within the trophically polymorphic species *Herichthys minckleyi.*

Cichlids and most bony teleost fishes have two sets of jaws (Fig. 1). They have oral jaws, used primarily to capture prey (Schaeffer and Rosen 1961), and also modified gill arches called pharyngeal jaws that are used to crush, shred, and prepare prey for digestion (Schaeffer and Rosen 1961; Liem 1973). Primitively, fish oral jaws were used extensively in prey processing, but during teleost evolution, clades have sequentially arisen that exhibit greater elaboration of the pharyngeal jaw (Liem and Greenwood 1981; Lauder 1985). As the ancestral condition of simple toothed pharyngeal processes found in basal teleosts was transformed into the sophisticated and highly articulated perciform apparatus during evolution, this has putatively resulted in increased power and effectiveness for processing prey (Liem 1973). The structural novelties in the cichlid pharyngeal jaw may have made cichlids that much more efficient at processing prey when compared to other perchlike fishes exhibiting a more ancestral pharyngeal jaw form (Liem 1973; Stiassny and Jensen 1987; Galis and Drucker 1996).

In the cichlid lower pharyngeal jaw (LPJ), the putatively novel muscular sling, innovative upper jaw joints, and unique lower jaw fusion may have improved their pharyngeal processing capabilities and ultimately facilitated the trophic diversification of these fishes (Liem 1973; Hulsey 2006; Wainwright 2006). Indeed, when compared to all other fish groups, the unparalleled trophic diversity (Fryer and Iles 1972) and species richness (Greenwood 1964) of cichlids suggest that

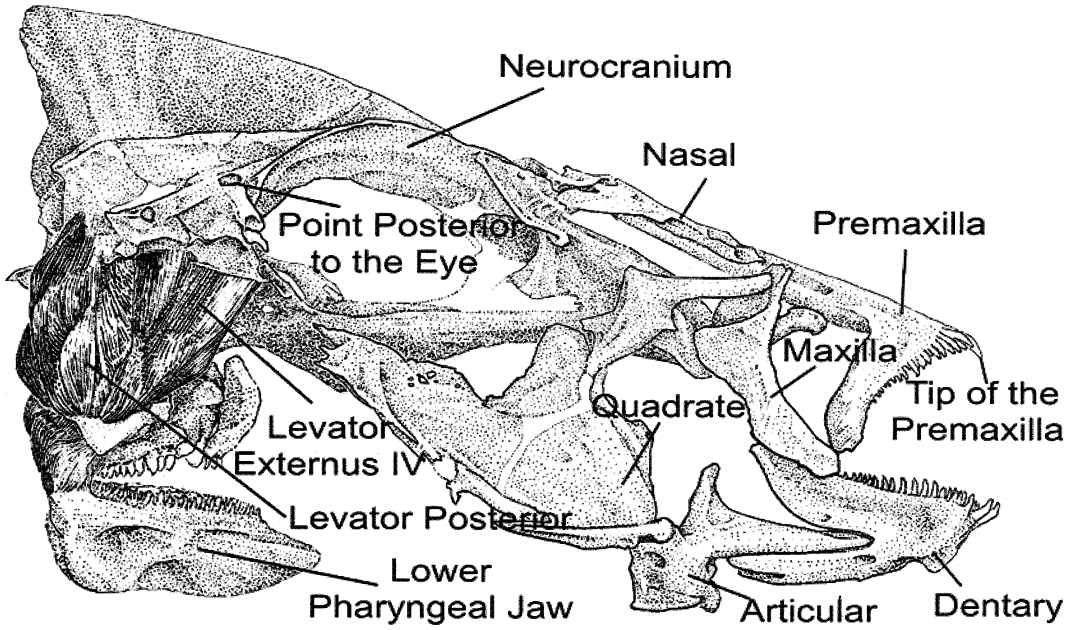

FIG. 1. The pharyngeal and oral jaws of *Herichthys minckleyi*. The levator externi IV and levator posterior (LEIV/LP) are almost inseparably associated in many fish and function as the primary pharyngeal crushing muscles that form the novel labroid muscular sling. These muscles attach onto the lower pharyngeal jaw (LPJ), the fused fifth ceratobranchials, that forms another part of the key innovation in the cichlid pharyngeal jaw. Bones critical to the mechanics of the oral jaw are also shown. The lower jaw made up by the articular and dentary rotate on the quadrate as the jaw is opened. The premaxilla is protruded during a feeding strike and is pushed open in part due to the mechanics of the four bar linkage in which lower jaw rotation is transmitted to the rotating maxilla. This rotation of the maxilla, which is coupled to movement of the nasal, rests on the neurocranium where the eye is located. One postulate of the pharyngeal jaw key innovation hypothesis (Liem 1973) is that mechanical specialization in the oral jaws and specialization in the pharyngeal jaws are decoupled evolutionarily.

some unique mechanism is necessary to explain their diversification. One postulate of Liem's key innovation hypothesis (1973) is that the efficiency the cichlid pharyngeal jaw conferred in prey processing frees cichlid oral jaws to diverge independently as instruments for highly effective prey capture. This greater modularity could be important because it reduces the pleiotropy, or the potential negative effects of adaptive change in one set of jaws on the evolution of the other set of jaws (Wagner and Altenberg 1996), during cichlid diversification. Although decoupling is frequently examined in genetic (Albertson and Kocher 2005; Albertson et al. 2005; Shapiro 2005) and developmental systems (Matsuoka et al. 2005), Liem's hypothesis explicitly predicts that how the two jaws mechanically function should be modular. However, whether the mechanics of the cichlid pharyngeal jaw are decoupled from specialization in the oral jaws and can diversify independently has never been tested comparatively in any cichlid group.

Heroine cichlids are an ideal clade for examining mechanical independence, or modularity, in the two jaws comprising the cichlid trophic apparatus. Heroines likely represent the most trophically diverse radiation of Neotropical cichlids (Winemiller et al 1995). The oral jaws in this monophyletic clade (Farias et al. 2001; Hulsey et al. 2004) are thought to exhibit substantial anatomical diversity for exploiting disparate prey types (Eaton 1943; Waltzek and Wainwright 2003). Pharyngeal jaw variation is also substantial both among species and within polymorphic species (Meyer 1990) such as *Herichthys minckleyi* wherein sympatric individuals vary extensively in pharyngeal musculature, skeletal elements, prey processing abilities, and diet (Liem and Kaufman 1984; Hulsey et al. 2005a). However, the phylogeny and taxonomy of Heroine cichlids has long been chaotic (Kullander 1983; 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). The addition of other taxa could increase the robustness of our inferences about the clade's evolutionary history (Hillis 1996). Moreover, if phylogenetic relationships were estimated, macroevolutionary patterns of pharyngeal and oral jaw mechanical decoupling could be thoroughly examined among Heroine cichlids.

In polymorphic Heroine cichlids like *H. minckleyi* we could test Liem's decoupling hypothesis on a microevolutionary level within single species. Within closely related groups, modular phenotypic units can be delineated using quantitative genetics (Albertson et al. 2005), developmental studies of gene expression (Fraser et al. 2004), or examinations of the correlations among phenotypic traits (Klingenberg et al. 2003; Monteiro et al. 2005). For example, comparing oral jaw divergence between alternative pharyngeal morphotypes in the polymorphic *H. minckleyi* should allow us to assess whether specialization in the pharyngeal jaw occurs independently of oral jaw divergence in a single species. In *H. minckleyi*, molariforms (Fig. 2) exhibit enlarged molariform teeth, huge crushing muscles, and robust jaws while papilliforms are diagnosable by their small pointed teeth, generally more gracile jaws, and smaller pharyngeal muscles (Sage and

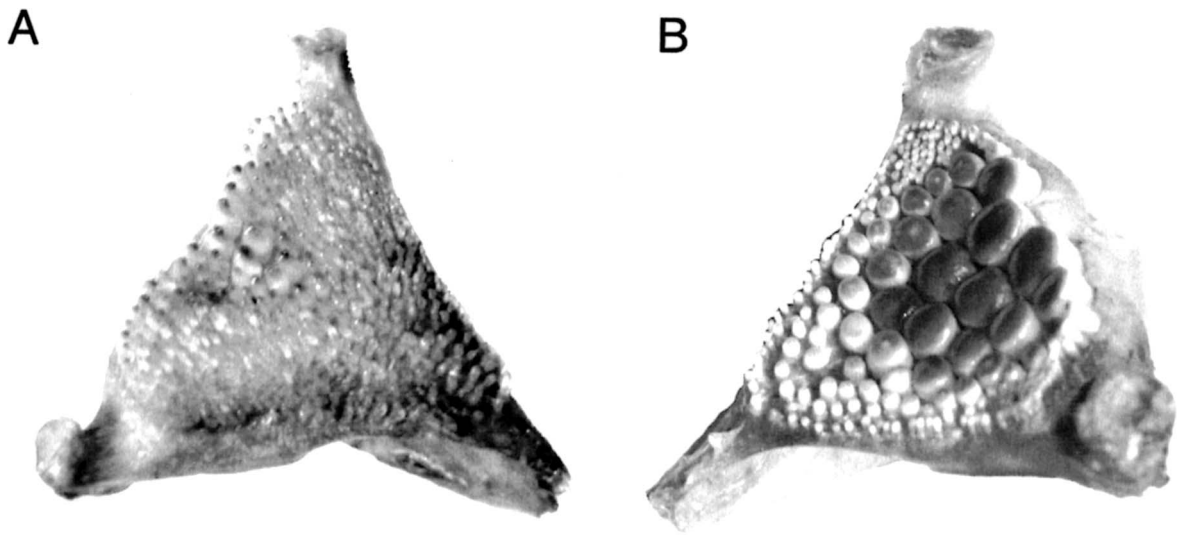

FIG. 2. The dorsal view of the lower pharyngeal jaw (LPJ; shown laterally in Fig. 1) of papilliform (A) and molariform (B) *H. minckleyi*. The two LPJ types in *H. minckleyi* can be diagnosed by the presence or absence of large rounded molariform teeth. The molariforms tend to have much more robust LPJs and enlarged LEIV/LP muscles. Papilliforms have more gracile pharyngeal jaws and smaller LEIV/LP muscles.

Selander 1975; Liem and Kaufman 1984). Molariforms are apparently specialized to crush snails while papilliforms are specialized to shred plant material (Hulsey et al. 2005a). These two pharyngeal morphotypes are considered the same species (Kornfield and Taylor 1983) because they interbreed (Kornfield et al. 1982), are sympatric (Minckley 1969), and are indistinguishable at all genetic loci examined (Sage and Selander 1975; Kornfield and Koehn 1975; Kornfield et al. 1982). Several studies have also examined variation in prey capture and head morphology between the alternative morphotypes in *H. minckleyi* (Wainwright et al. 2001; Swanson et al. 2003; Trapani 2003; Hulsey et al. 2005a), but none have determined whether skeletal mechanical elements differ in their oral jaws or how these mechanics covary with pharyngeal jaw phenotype.

To investigate evolutionary decoupling in the jaws of *H. minckleyi* and other Heroine cichlids, it would be most informative to examine characters having mechanical and putatively functional consequences for feeding. For example, maximum oral jaw protrusion can influence the ability to capture evasive prey (Motta 1984; Hulsey and García de León 2005), and oral gape should determine the maximum prey size a predator can ingest (Wainwright 1996). Also, most skeletal components of the cichlid oral jaw function as lever systems. Therefore, quantifying how jaw elements like the lower jaw and anterior jaw four-bar linkage transmit motion and force should reflect functionally important divergence (Westneat 1990). These leverlike aspects of the oral jaw exhibit associations with trophic habit in many fish (Wainwright and Richard 1995; Westneat 1995) including cichlids (Barel 1983; Hulsey and García de León 2005). Likewise, characteristics of the pharyngeal jaw such as crushing muscle mass that determines the physiological ability to produce force during prey processing (Liem 1973, Wainwright 1987) and mass of the lower pharyngeal jaw (LPJ) that withstands the forces generated by these muscles (Wainwright et al. 2004) should likewise reflect pharyngeal jaw divergence. Based on Liem's hypothesis (1973), we would expect evolutionary change in oral jaw mechanics to be independent of change in the pharyngeal jaw among Heroine cichlids.

Modularity in jaw diversification could be exhibited in several ways during cichlid macroevolution (Vermeij 1974; McShea 1998). Cichlid jaws may be so decoupled that the two jaw types have no correlation among extant species. If this pattern were recovered, not only might the two jaws be functionally decoupled, but jaw phenotypes might be completely decoupled from any inertial effects of evolutionary history on divergence. Alternatively, oral and pharyngeal jaw mechanics within species might have evolved independently, but the range of phenotypes a particular species could have evolved would have been historically constrained (Hansen and Martins 1996). Correlations of phylogenetically independent contrasts can be used to factor out this historical influence on the phenotypes a species exhibits (Felsenstein 1985). This is especially true if limited evolutionary history exists between nodes at which the phylogenetically independent contrasts, or amount of inferred phenotypic change along branches of a phylogeny, are reconstructed (Martins 1996). A significant correlation between contrasts in oral jaw mechanics and the amount of inferred change in pharyngeal jaw mechanics would reject Liem's hypothesis (1973) that the two types of jaws do evolve independently. A significant phylogenetically independent correlation is the type of pattern we would expect for functionally integrated characters such as the two sets of mammal limbs or the paired fins of fish. When one of these structures was functionally modified during evolution, we would expect the other to have also been modified. Alternatively, if evolutionary contrasts in oral and pharyngeal jaw mechanics were uncorrelated and therefore evolutionarily independent, decoupling of the mechanics of the two jaws may have been a critical force in cichlid diversification.

We determined whether pharyngeal and oral jaw mechanics have diverged independently on either a microevolutionary level within *H. minckleyi* or macroevolutionarily among Heroine cichlids. First, we determined whether the molariform and papilliform *H. minckleyi* were significantly different in oral jaw gape, protrusion, lower jaw velocity ratio (VR), and maxillary kinematic transmission (KT). Second, we tested the hypothesis that variation in the mass of *H. minckleyi*'s LPJ reflects divergence in force production. We also reconstructed a phylogenetic hypothesis for Heroine cichlids based on the cytochrome *b* gene. Subsequently, we used both species values and independent contrasts to determine whether pharyngeal jaw mass is evolutionarily decoupled from divergence in oral jaw mechanics in 40 species of Heroine cichlids.

Materials and Methods

Jaw Mechanics

A combination of specimens collected from the wild in Mexico by the authors and wild-caught accessioned museum specimens were used in the quantification of morphology. Collection localities of all specimens are in the Appendix. For this analysis, 41 species collected from their native range, preserved in formalin, and then transferred to 70% ethanol were examined. The standard length (SL) of all preserved specimens was measured. Approximately three specimens of each species other than *H. minckleyi* were cleared using trypsin and double-stained using an Alcian-blue cartilage stain and alizarin red bone stain (Dingerkus and Uhler 1977) for the examination of morphological characteristics of the jaws. In cleared and stained specimens, the bones are clearly discernable for morphological measurements and the natural articulations of the skull are retained. Because of availability, only two specimens of *Astatheros alfari*, *Herotilapia multispinosa*, *Nandopsis tetracanthus*, and *Hypsophrys nicaraguensis*, and one each of *Parachromis loisellei*, *Herichthys deppii*, and *Parachromis managuensis* were examined.

For the oral jaws, we first measured both the amount each species was able to maximally protrude its jaws as well as the maximum oral jaw gape. Jaw protrusion was measured as the distance from the anterior tip of the premaxilla to the most posterior point of the eye when the jaws were maximally extended. Then, we subtracted the distance from the tip of the premaxilla to the eye when the jaw was completely closed to determine maximum protrusion. For 31 of these species, the measurements of maximum protrusion were taken from Hulsey and García de León (2005). The maximum gape was measured as the horizontal distance within the oral jaw between the two coronoid processes of the articular bones when the oral jaws of the fishes were extended.

We also measured several components of the oral jaw that can be modeled as simple lever systems (Fig. 3). We first measured components of the lower jaw opening lever system (Wainwright and Richard 1995). The out-lever for the opening system was measured as the linear distance between the articular-quadrate joint and the anterior tip of the dentary bone on the lower jaw (Fig. 3, Z). The in-lever for lower jaw opening was quantified as the distance from the articular-quadrate joint to the midpoint of the interopercular-mandibular ligament on the articular bone that serves to pull open the jaw. The ratio of out-lever to in-lever for this leverlike aspect of lower jaw opening should determine the fraction of motion input to the lower jaw that is transmitted to the anterior tip of the jaw (Wainwright and Richard 1995). For analyses of the mechanical properties of the lower jaw opening, the inverse of the mechanical advantage was calculated as the ratio of out-lever to in-lever, which is expressed as the velocity ratio (VR) of the lever system.

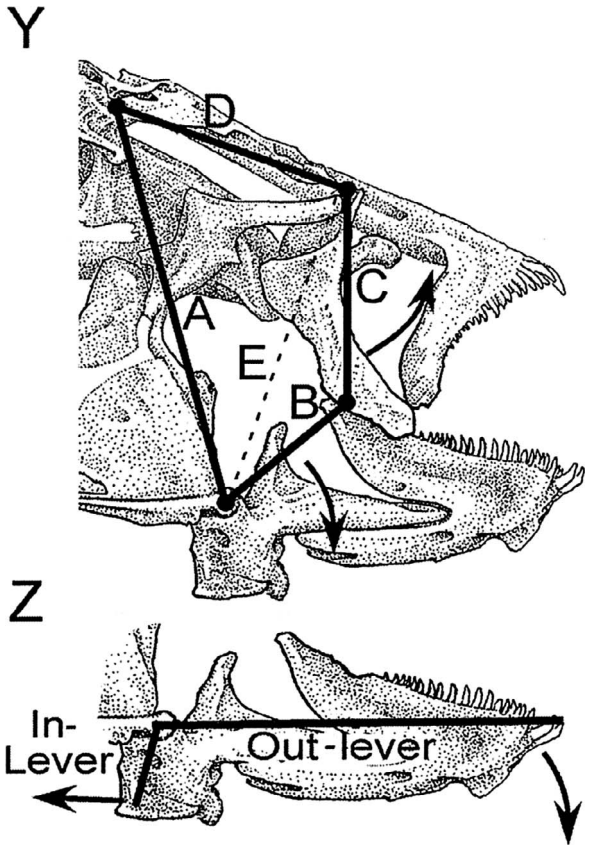

FIG. 3. The lever mechanisms of the oral jaws. The four-bar linkage (Y) was quantified to determine maxillary kinematic transmission (KT). The length from quadrate-articular joint to the point where the nasal attaches to the neurocranium was used as the fixed link (A). The length from the quadrate-articular joint to the place where the maxilla rotates on the dentary was determined as the lower jaw input link (B). Where the maxilla rotates on the dentary to the point where the nasal attaches to the maxilla was considered the maxilla link (C). The length of the nasal was used as the coupler link (D). The diagonal distance between the nasal-maxillary attachment and the quadrate-articular joint was estimated using a starting angular relationship of 15 degrees between the lower jaw link and fixed link. To estimate maxillary KT, 30 degrees of rotation was input into the lower jaw link and the amount of kinematic rotation of the maxilla estimated from the law of cosines. The KT was determined by dividing output of the linkage by the 30 degrees of input. The lower jaw opening lever system (Z) was also quantified. The in-lever was measured from the quadrate-articular joint to the interopercular ligament (not shown) which serves to pull the jaw open. The out-lever was measured from the quadrate-articular joint to the tip of the lower jaw. The velocity ratio (VR) or expected output for a given input to the lever was measured using out-lever length divided by the in-lever length. Assuming isometry through ontogeny both maxillary KT and lower jaw VR should be size independent.

TABLE 1. Sequenced species, source of species, and GenBank numbers.

Species	Collection locale	GenBank no.
Thorichthys pasionis	Lago de Illusiones, Tabasco, MX	DQ494385
Herichthys pearsei	Rio San Pedro, Chiapas, MX	DQ494388
Herichthys deppii	Rio Nautla, Veracruz, MX	DQ494384
Nandopsis tetracanthus	commercial	DQ494386
Nandopsis haitiensis	commercial	DQ494391
Theraps irregularis	commercial	DQ494383
''Cichlasoma'' intermedium	Rio San Pedro, Chiapas, MX	DQ494387
Mesonauta festivus	Guyana	DQ494392
''Cichlasoma'' lentiginosus	Rio Tzendales, Chiapas, MX	DQ494390
Vieja argentea	Rio Tzendales, Chiapas, MX	DQ494389

We also measured aspects of the oral jaws that can be modeled as a four-bar linkage system (Westneat 1990). For this anterior jaw four-bar linkage (Fig. 3, Y), the lower jaw link (A) was measured from the base of the coronoid process, at the joint where the articular rotates on the quadrate, to the ligamentous attachment of the maxilla on dentary. Then we determined the distance between this attachment point of the ventral shank of the maxilla and the point where the nasal rotates on the maxilla. This was used as the relevant length of the maxilla link (B). The nasal was measured as the link connecting the maxilla to the neurocranium (C). The distance from where the nasal attaches to the neurocranium down to the coronoid process was measured as the fixed link (D). The anatomy of these bones and the morphometrics of these linkages have been described in more detail elsewhere (Westneat 1990, 1995; Wainwright et al. 2004).

If it is planar and the lengths of all links are determined, a four-bar linkage has only one degree of freedom during movement (Muller 1987). Therefore, at any point during jaw rotation, all angles in the linkage would be defined if the angular relationship between the lower jaw and the fixed link is determined. An angular relationship of 15 degrees was used to estimate the diagonal distance (E) between the point where the nasal attaches to the suspensorium and where the lower jaw link meets the maxilla link (Fig. 3, Y). Defining the diagonal separating the linkage into two triangles allows all of the angular relationships between the links including the starting angle to be determined exactly from the law of cosines.

Finally, we estimated the input angle as the angular rotation of the input link. In the anterior jaw linkage, the input angle is the angular depression of the lower jaw. There are other kinematic outputs of the anterior jaw, but we only examine rotation of the maxilla as output (Westneat 1990). After defining the size of the physical links, the starting angle, and the input angle, we summarized the mechanical properties of each linkage by the maxillary KT for each four-bar linkage (Westneat 1990). We calculated the maxillary KT as the ratio of expected output rotation of the maxilla divided by the given 30 degrees of rotation of the lower jaw (Westneat 1994). Higher KT anterior jaw linkages transmit a greater amount of motion from the lower jaw to the maxilla than do linkages with low KT. The maxillary KTs for empirically measured linkages were calculated using macros written in Excel (Microsoft Corp., Redmond, WA). For 31 of these species, the measurements of maxillary KT were taken from Hulsey and García de León (2005).

Prior to clearing and staining *H. minckleyi* individuals, the fourth levator externi and levator posterior (LEIV/LP) were dissected from the pharyngeal apparatus (Fig. 1; Hulsey et al. 2005a). These two muscles are the primary muscles responsible for transmitting forces to the pharyngeal jaw during prey processing in cichlids. For *H. minckleyi* as well as the other 40 species, the fused fifth ceratobranchials (LPJ) were removed from the cleared and stained specimens. The pharyngeal musculature attached to the jaw was severed and the attachment of the jaw's keel to the other ceratobranchials was also cut. Then, the LPJ was cleaned of all attached musculature and allowed to dry. The dried bone was then weighed to the nearest 0.1 mg. In *H. minckleyi*, the correlation between the $\log_{10}$-transformations of $(\text{LEIV/LP mass})^{1/3}$ and $(\text{LPJ mass})^{1/3}$ was determined.

In both morphotypes of *H. minckleyi*, all of the above oral jaw mechanical variables were also measured. A limited number of these fish were caught from the pools Churince and Mojarral West in the federally protected Cuatro Ciénegas basin where *H. minckleyi* is native with permits from the Mexican government. Because individuals did not have to be cleared and stained but only slightly dissected to make the measurements, we measured 20 individuals per pharyngeal morphotype for the three oral jaw lever characters. For the gape and protrusion measurements, individuals were cleared and stained ($n = 16$ molariform, $n = 13$ papilliform). For the invasive LPJ mass and LP/LEIV measurements, we measured fewer individuals ($n = 9$ molariform, range = 70.8–146 mm SL; $n = 13$ papilliform, range = 65.4–133.2 mm SL). Because there were different numbers of individuals measured and the same individuals were not used in all analyses, each individual character was compared separately between molariform and papilliform *H. minckleyi*.

DNA Isolation and Sequencing

Gene sequences for the cytochrome *b* in 10 Heroine species (Table 1) were combined with sequences for numerous species previously analyzed in other phylogenetic studies. Seven species were collected from the wild and three were purchased commercially. For sequencing, total genomic DNA was isolated from axial muscle using Puregene (Minneapolis, MN) extraction at the University of California, Davis. A 1-μl aliquot of this solution was used to provide a DNA template for the polymerase chain reaction (PCR). The entire cytochrome *b* gene was PCR amplified using primers in Martin and Bermingham (1998) and Hulsey et al. (2004). Am-

plifications were carried out in a Perkin-Elmer (Boston, MA) DNA thermocycler. The PCR reaction volume was 50 μl [32 μl of H_2O, 5 μl 10× $MgCl_2$ PCR buffer, 2.5 μl $MgCl_2$, 4 μl dNTPs (10mM), 2.5 μl of each primer (10 μM), 0.5 μl of Taq, and 1 μl DNA (~15–20 ng)]. Thermal cycling conditions consisted of an initial denaturation step of 94°C (30 sec), 55°C (30 sec), and 72°C (1.5 min). A final incubation of 72°C for 5 min was added to ensure complete extension of amplified products. Subsequently, the 1.3 kb PCR products were electrophoretically separated from unincorporated primers and dNTPs using electrophoresis in low melting point agarose gel run in Tris-acetate buffer (pH 7.8). Gels were stained in ethidium bromide (1 mg/μl) for five minutes and destained in deionized water for 15 min. Positively amplified DNA was then purified using an enzymatic combination of 1 μl of exonuclease I (10.0 U/μl) and 1 μl shrimp alkaline phosphatase (2.0 U/μl) per 10 μl of PCR product. Treated PCR products were used as templates for Big Dye sequencing reactions (Applied Biosystems [Foster City, CA] terminator cycle sequencing reactions). Sequences were read with an ABI377 (Applied Biosystems) automated sequencer at the Automated DNA Sequencing Facility at the University of California, Davis. Complete gene sequences were assembled from individual sequencing reactions using the program Sequencher version 4.1 (Gene Codes, Ann Arbor, MI). For analyses, sequences were aligned using Clustal X (Thompson et al. 1997) and codon positions were defined using MacClade 4.0 (Maddison and Maddison 2000).

Phylogenetic Analysis

In our analysis, we included a total of 66 recognized species of Heroine cichlids (Kullander 1998), a group that is largely endemic to Central America. To polarize the Heroine group that includes *H. minckleyi*, we included nine species of cichlids from South America that are generally recognized as outgroups to this clade (Kullander 1998; Farias et al. 2001). Only one individual of all species sequenced in other studies for cytochrome *b* were included in the analysis. The GenBank numbers for previously sequenced species included in this phylogenetic analysis were, AF009924–AF 009931, AF009932, AF009934, AF009937, AF009938, AF009940–AF009945, AF009949, AF009951, AF009993 (Martin and Bermingham 1998); AF370671, AF370673–AF370679, AB018985, AF141318, AF141319 (Farias et al. 2001); U88860, U88863, U88864, U97158, U97159, U97165 (Roe et al. 1997); AY050610, AY050613, AY050615, AY050620, AY050621, AY050624–AY050626 (O. Rican, J. Zrzavy, M. Obornik, and J. Novak, unpubl. data); AY323981, AY323983, AY323988, AY323991, AY323994, AY323997, AY323998, AY324000, AY324002, AY324004, AY324007, AY324010, AY324012, AY324014, AY324017, AY324019, AY324021, AY324025, AY324029, AY324030, AF009942 (Hulsey et al. 2004). The GenBank numbers for the species sequenced in this study were DQ494383–DQ494392 (Table 1).

For the Bayesian analyses, the cytochrome *b* gene was partitioned into its three codon sites. ModelTest 3.06 (Posada and Crandall 1998) was used to identify the best model of molecular evolution for each codon site. The Bayesian analyses were executed to find approximations of the maximum likelihood tree using MrBayes 3.0 (Ronquist and Huelsenbeck 2003). The analyses treated the transition-transversion matrices, number of invariant sites, and gamma shape parameters as unlinked or independent for each codon site. Flat prior probability distribution for all parameters were assumed before analysis. 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 (MCMC) search algorithm every 100 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 five, 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 majority rule consensus tree in PAUP* 4.0b10 (Swofford 2002). The percentage of trees that recovered a particular clade (the clade's posterior probability) were depicted on the single best likelihood tree topology found during the Bayesian analyses.

Macroevolutionary Jaw Decoupling

Because VR and KT values for a species are size independent under the assumption that the components determining the mechanics change isometrically through ontogeny (Hulsey and Wainwright 2002), and because the values recovered approximated a normal distribution among the species examined, these values were not transformed for analyses. However, because most morphology changes considerably with size, fish SL was used to factor out size from the other variables. Since mass generally scales with the third power of length (Wainwright et al. 2004), the cube root of the LPJ mass of each species was first taken. Then, protrusion, gape, and $(\text{LPJ mass})^{1/3}$ were $\log_{10}$-transformed and regressed against the $\log_{10}$-transformation of the each specimen's SL. To minimize the effect of repeated comparisons on our power to detect a correlation, we reduced the covariation in oral jaw morphology of the 40 species other than *H. minckleyi* into independent axes. The residuals of protrusion and gape were combined with the lower jaw and maxillary VR values of each species in a principal component analysis (PCA). The correlations between $(\text{LPJ mass})^{1/3}$ and the species loadings from the first two PC axes were then examined.

Herichthys minckleyi, because of its potentially unique attributes, were excluded from the independent contrast analyses. For the independent contrasts, we adjusted for phylogeny using the single topology and branch lengths recovered from our three Bayesian runs that had the best likelihood score. First, the phylogenetic topology with branch lengths was imported into the program Tree Edit 1.0 (Rambaut and Charleston 2002). Then, for the species in the phylogeny that were not examined here, the sequences were pruned from the topology. The branch lengths and topology for the species left in the tree were then exported into the program CAIC (Purvis and Rambaut 1995). Using the crunch algorithm, which assumes all variables are continuous, the correlation between independent contrasts of the oral jaw first principle

TABLE 2. Phenotypic differences between molariform and papilliform *Herichthys minckleyi*. The equations for the correlations of levator externus IV/levator posterior (LEIV/LP mass)$^{1/3}$, lower pharyngeal jaw (LPJ mass)$^{1/3}$, gape, and protrusion with standard length (SL) for each pharyngeal morphotype are shown. The degrees of freedom (df) and *P*-values for the ANCOVA using SL as a covariate when comparing the morphotypes is given. For lower jaw kinematic transmission (KT) and maxillary KT, morphotype values are given as are the df and *P*-values from *t*-tests.

Characters	Papilliform	Molariform	df	*P*
Pharyngeal jaws				
LP/LEIV mass	3.5(log*x*) − 5.1	3.3(log*x*) − 4.2	20	0.002
LPJ mass	3.2(log*x*) − 4.6	4.3(log*x*) − 6.3	20	0.003
Oral jaws				
gape	1.6(log*x*) − 2.1	1.1(log*x*) − 1.1	28	0.202
protrusion	1.2(log*x*) − 1.6	0.9(log*x*) − 0.9	28	0.442
lower jaw KT	4.4 ± 0.5	4.3 ± 0.6	38	0.322
maxillary KT	0.70 ± 0.05	0.69 ± 0.07	38	0.927

scores and SL regressed residuals of (LPJ mass)$^{1/3}$ was determined. To ensure the independent contrast analyses were robust we checked the assumptions of the analyses (Purvis and Rambaut 1995). Independent contrasts were also performed on the PC1 loadings when outliers were removed.

RESULTS

Herichthys minckleyi Jaw Mechanics

Molariform and papilliform *H. minckleyi* differed extensively in pharyngeal morphology but were very similar in oral jaw mechanics (Table 2). The (LPJ mass)$^{1/3}$ was significantly different between the two morphotypes when SL was used as a covariate (ANCOVA, $F_{1,8,12} = 55.7$; $P < 0.001$). The molariform LPJ had about 300–400% greater mass than the papilliform LPJ when SL was accounted for in the comparison. In *H. minckleyi*, the LPJ mass appears to be an excellent predictor of pharyngeal crushing muscle mass

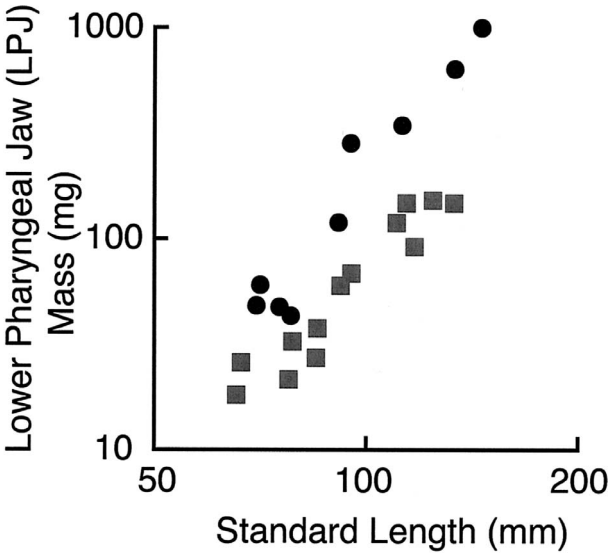

FIG. 4. The relationships between $\log_{10}$ standard length (mm) and $\log_{10}$ lower pharyngeal jaw (LPJ) mass for molariform (circles) and papilliform (squares) *H. minckleyi*. No trend lines are drawn because all statistical analyses used the cube root of LPJ mass. This transformation was done because mass generally scales to the third power of length. The molariforms have an LPJ mass 300–400% the size of papilliforms with the same Standard length.

(Fig. 4) independent of fish size (df = 20, slope = 1.0, $r = 0.88$, $P < 0.001$). The lack of difference in all four of the oral jaw variables examined contrast starkly with the differences in *H. minckleyi*'s pharyngeal jaws. The gape and protrusion of the jaws increased with SL for both papilliforms ($\log_{10}$gape = 1.6($\log_{10}$SL) − 2.1, $\log_{10}$protrusion = 1.2($\log_{10}$SL) − 1.6) and molariforms ($\log_{10}$gape = 1.1($\log_{10}$SL) − 1.1, $\log_{10}$protrusion = 0.9($\log_{10}x$) − 0.9). However, neither protrusion (ANCOVA $F_{1,14,14} = 0.6$, slope = 1.4, $P = 0.44$) nor gape (ANCOVA $F_{1,14,14} = 1.7$, slope = 1.1, $P = 0.2$) were significantly different between the two morphotypes. The average lower jaw VR of papilliforms (4.4 ± 0.5) and molariforms (4.3 ± 0.6) did not differ ($t_{39} = 1.0$; $P = 0.32$). Similarly, maxillary KT of papilliforms (0.70 ± 0.05) and molariforms (0.69 ± 0.07) were virtually identical ($t_{39} = 0.9$; $P = 0.93$).

Heroine Phylogeny

Few of the phylogenetic relationships recovered (Fig. 5) deviated extensively from those found in Hulsey et al. (2004). The relationships among the previously sequenced *Herichthys*, *Thorichthys*, and *Astatheros* + ''*Cichlasoma*'' *octofasciatum* were identical. However, the topology recovered here with the best likelihood placed the *Caquetaia* clade with the clade containing *Astatheros* and *Herichthys* (node A). In previous analyses, *Caquetaia* grouped with the similarly piscivorous *Petenia* and *Parachromis* containing clade, although in neither this nor in other analyses (Martin and Bermingham 1998; Hulsey et al. 2004) did its placement have strong bootstrap or posterior probability support. *Hypsophrys nicaraguensis* + *Neetroplus nematopus* (node 13) and *Archocentrus spilurus* also changed topological affinities within the large clade containing them (Node B) but with little support. ''*Cichlasoma*'' *salvini* was weakly supported as sister to *Thorichthys* instead of grouping with *Herotilapia multispinosa*. The weakly supported relationships among the anomalous ''*Cichlasoma*'' *umbiferum*, *Tomocichla tuba*, ''*Cichlasoma*'' *atromaculatum*, and ''*Cichlasoma*'' *festae* changed topologically from previous analyses (Hulsey et al. 2004) but with little posterior probability support.

Of the two species putatively assigned to *Herichthys*, only *H. deppii* appeared to be phylogenetically most closely affiliated with the other *Herichthys*. It is sister to *H. carpintis* and *H. tamasopoensis*. *Herichthys pearsei* was sister to an-

other group which included ''*Cichlasoma*'' *lentiginosum*, and *Theraps irregulare* + ''*Cichlasoma*'' *intermedium*. These four species formed a monophyletic clade (node C). This new clade, plus the monophyletic *Herichthys* north of the Punta del Morro, and the remaining *Vieja* + *Paratheraps* + *Paraneetroplus* species group, were all clearly related (node D) exhibiting high posterior probabilities (100%). However, the relationships between any two clades with the respect to the other clade did not have substantial support.

The relationships among some of the other species were informative but not surprising. *Thorichthys pasionis* was found to be sister to the other largely yellow *T. helleri*. *Vieja argentea* was placed in the clade containing *V. regani* and *Paraneetroplus bulleri* with high posterior probabilities (100%). There was also substantial support for *Mesonauta festivus* being closely related to *M. insignis* (100%). The resolution found for these closely related species contrasts sharply with the support for relationships deeper in the topology.

As previously found (Martin and Bermingham 1998; Farias et al. 2001; Hulsey et al. 2004), there was generally low support for nodes at the base of the Central American Heroine group. However, the Bayesian posterior probabilities showed significant support (100%) for including the Caribbean *Nandopsis tetracanthus* and *N. haitiensis* within the largely Central American Heroine group (node E). There was also some support (90%) for placing this clade containing the two Caribbean cichlids as sister group to the remainder of the Heroines (Node F).

Macroevolutionary Correlations of Jaw Mechanics

There was substantial variation in all of the mechanical variables of the jaws among the Heroine species examined (Table 3). *Petenia splendida* had both the largest size-corrected gape and maximum protrusion capabilities (14.2% of SL). *Herichthys tamasopoensis* had the least protrusion (1.4% of SL). Average lower jaw opening VR was 4.4 ± 1.0 and average maxillary KT was 0.77 ± 0.09. *Petenia splendida* had the highest lower jaw VR (7.8) and highest maxillary KT (1.06). *Archocentrus septemfasciatus* had the lowest lower jaw VR (3.3), and *Herichthys tamasopoensis* had the lowest maxillary KT (0.58). The mass of the LPJ, uncorrected for size, ranged from 119.7 milligrams in *H. labridens* Cascadas to 4.2 milligrams in *Parachromis dovii*.

The four oral jaw variables are not mechanically independent components of the oral jaw apparatus (Table 4). All four oral jaw variables loaded positively on PC1, which accounted for 67.2% of the variation in these size-corrected variables. Lower jaw opening VR and the gape residuals loaded negatively on PC2, while anterior jaw KT and the protrusion residuals loaded positively on this PC axis. These first two PC axes explained 88.7% of the variance in these four size-adjusted characters. The loadings for oral jaw PC1 were correlated with the PJ mass (Fig. 6A) in the nonphylogenetically corrected analysis (slope = −6.7, df = 38, $r = 0.34$, $P = 0.031$). The second oral jaw PC loadings were not correlated with LPJ mass ($df = 38$, $r = 0.17$, $P = 0.295$).

In comparison to the results found with uncorrected species values (Fig. 6B), when the relationship between oral jaw PC1 loadings and LPJ were analyzed with independent contrasts, there was no significant correlation (df = 38, $r = 0.14$, $P = 0.371$). There were several contrast outliers in the PC1 versus LPJ contrast analysis. Therefore, we removed three species involved in the contrasts and reanalyzed the correlation. We removed *P. splendida*, which had the highest PC1 loadings, *H. tamasopoensis*, which had the lowest PC1 loadings, and *H. labridens* ML, which had the second greatest LPJ mass. With the outliers removed, we found the same result. There was virtually no variation explained by correlating (LPJ mass)$^{1/3}$ and the oral jaw PC1 loadings (df = 35, $r = 0.00$, $P = 0.77$).

Discussion

Because we found no difference in the four mechanical characters of the oral jaw between alternative pharyngeal jaw morphotypes in *H. minckleyi*, this supports the hypothesis that the oral and pharyngeal jaws are capable of independent change within a species. Furthermore, LPJ mass among both *H. minckleyi* pharyngeal morphotypes was highly correlated with LEIV/LP muscle mass, suggesting LPJ mass likely provides a morphological indicator of divergence in pharyngeal force production abilities. Our phylogenetic results further resolved relationships in the Heroines and provided the evolutionary framework to examine evolutionary change in Heroine oral and pharyngeal jaw mechanics. As expected if functional decoupling of the jaws is a general characteristic of cichlid diversification, there was no correlation of independent contrasts between Heroine oral and pharyngeal jaw mechanics.

Jaw Decoupling in H. minckleyi

There appears to be no relationship between pharyngeal jaw morphotype and oral jaw mechanics in *H. minckleyi* (Table 3). This is not the first study to report no effect of pharyngeal divergence on oral jaw morphology in *H. minckleyi* (Sage and Selander 1975; Trapani 2003). However, it is the first study that has explicitly examined characters that can be directly inferred to mechanically influence what prey *H. minckleyi* obtains with its oral jaws. The independence of pharyngeal jaw divergence in *H. minckleyi* from mechanical differentiation in the oral jaw suggests that changes in the two types of jaws in this one species are decoupled.

The mass of the LPJ was a good predictor of LP/LEIV mass in *H. minckleyi*. This mirrors the results found in other labroid fishes (Wainwright et al. 2004), and it better justifies using the LPJ mass as a measure of functional variation within all Heroines. Because greater muscle mass and more robust jaws generally reflect ability to break down durable prey, the primary axis of functional variation distinguishing the molariforms and papilliforms is likely the ability to produce substantial forces (Hulsey et al. 2005). The oral jaw likely has little influence on trophic diversification in *H. minckleyi*. Differences in feeding abilities associated with the pharyngeal jaw alone likely underlie the morphological divergence in *H. minckleyi* (Sage and Selander 1975).

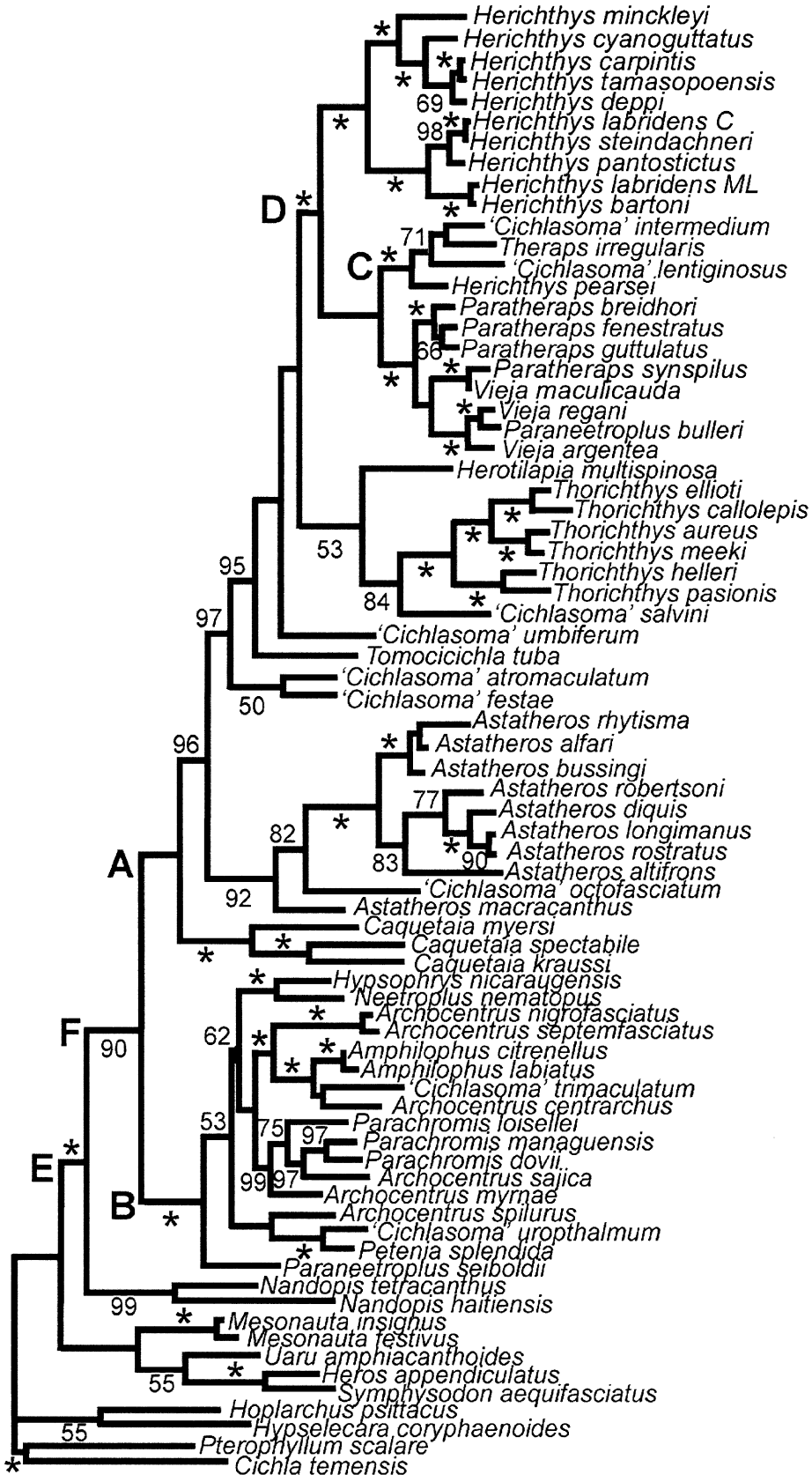
Herichthys minckleyi
Herichthys cyanoguttatus
Herichthys carpintis
Herichthys tamasopoensis
Herichthys deppi
Herichthys labridens C
Herichthys steindachneri
Herichthys pantostictus
Herichthys labridens ML
Herichthys bartoni
'Cichlasoma' intermedium
Theraps irregularis
'Cichlasoma' lentiginosus
Herichthys pearsei
Paratheraps breidhori
Paratheraps fenestratus
Paratheraps guttulatus
Paratheraps synspilus
Vieja maculicauda
Vieja regani
Paraneetroplus bulleri
Vieja argentea
Herotilapia multispinosa
Thorichthys ellioti
Thorichthys callolepis
Thorichthys aureus
Thorichthys meeki
Thorichthys helleri
Thorichthys pasionis
'Cichlasoma' salvini
'Cichlasoma' umbiferum
Tomocichla tuba
'Cichlasoma' atromaculatum
'Cichlasoma' festae
Astatheros rhytisma
Astatheros alfari
Astatheros bussingi
Astatheros robertsoni
Astatheros diquis
Astatheros longimanus
Astatheros rostratus
Astatheros altifrons
'Cichlasoma' octofasciatum
Astatheros macracanthus
Caquetaia myersi
Caquetaia spectabile
Caquetaia kraussi
Hypsophrys nicaraugensis
Neetroplus nematopus
Archocentrus nigrofasciatus
Archocentrus septemfasciatus
Amphilophus citrinellus
Amphilophus labiatus
'Cichlasoma' trimaculatum
Archocentrus centrarchus
Parachromis loisellei
Parachromis managuensis
Parachromis dovii
Archocentrus sajica
Archocentrus myrnae
Archocentrus spilurus
'Cichlasoma' uropthalmum
Petenia splendida
Paraneetroplus seiboldii
Nandopis tetracanthus
Nandopis haitiensis
Mesonauta insignus
Mesonauta festivus
Uaru amphiacanthoides
Heros appendiculatus
Symphysodon aequifasciatus
Hoplarchus psittacus
Hypselecara coryphaenoides
Pterophyllum scalare
Cichla temensis
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TABLE 3. Species name, average standard length (SL) of specimens examined, mass of the lower pharyngeal jaw, gape, protrusion, lower jaw opening velocity ratio (VR), and maxillary kinematic transmission (KT) for 40 Heroine species. Maximum protrusion and maxillary KT values were taken from 31 species (indicated by asterisks) examined in Hulsey and García de León (2005).

Species	SL (mm)	Lower pharyngeal jaw (mg)	Gape (mm)	Protrusion (mm)	Lower jaw (KT)	Maxillary (KT)
*Archocentrus centrarchus**	80.5	30.7	9.1	4.2	4.0	0.83
*Archocentrus nigrofasciatus**	59.4	10.8	5.3	2.5	3.5	0.78
*Archocentrus septemfasciatus**	66.2	15.7	9.3	2.3	3.3	0.77
*Astatheros alfari**	86.7	44.8	11.9	5.5	3.9	0.71
*Astatheros macracanthus**	83.9	42.9	11.3	3.8	3.7	0.62
*Astatheros robertsoni**	86.7	24.9	10.3	5.4	3.8	0.78
*Caquetaia kraussii**	75.0	11.1	9.7	8.5	5.2	0.96
Caquetaia myersi	74.4	6.6	10.1	11.3	6.9	0.79
''Cichlasoma'' intermedium	59.9	13.0	6.4	3.7	4.2	0.76
*''Cichlasoma'' octofasciatum**	75.8	22.3	9.8	3.3	3.4	0.71
*''Cichlasoma'' salvini**	71.8	17.9	9.2	5.9	5.4	0.90
*''Cichlasoma'' trimaculatum**	74.7	41.8	9.3	5.1	4.5	0.83
*''Cichlasoma'' uropthalmum**	84.4	30.5	10.4	5.1	5.1	0.68
*Herichthys bartoni**	81.9	27.6	12.8	2.4	4.4	0.68
*Herichthys cyanoguttatus**	88.1	62.3	11.8	4.3	4.4	0.73
Herichthys deppii	77.7	30.2	8.3	5.7	5.0	0.79
*Herichthys labridens C**	77.6	119.1	9.4	3.9	3.8	0.79
*Herichthys labridens ML**	81.6	109.7	9.5	3.7	3.4	0.71
*Herichthys pantostictus**	76.8	27.0	10.1	3.4	4.2	0.76
Herichthys pearsei	70.5	12.3	7.8	3.2	4.6	0.63
*Herichthys tamasopoensis**	89.8	38.9	10.6	1.3	3.8	0.58
*Herotilapia multispinosa**	80.4	13.3	8.3	2.8	3.7	0.65
*Hypsophrys nicaraguensis**	103.8	48.7	9.7	3.9	3.5	0.75
Nandopsis haitiensis	70.1	46.8	8.6	5.7	4.4	0.78
Nandopsis tetracanthus	53.2	12.9	7.7	4.3	5.2	0.87
*Parachromis dovii**	59.8	4.2	7.8	5.9	5.6	0.78
*Parachromis loisellei**	77.5	7.8	9.6	6.5	5.8	0.84
*Parachromis managuensis**	116.0	80.0	17.3	8.0	5.5	0.79
*Paraneetroplus bulleri**	98.2	30.7	5.6	3.5	3.7	0.74
*Paratheraps fenestratus**	107.3	64.2	11.6	4.4	3.7	0.73
*Paratheraps guttulatus**	94.1	70.4	9.1	4.2	3.5	0.76
Paratheraps synspilus	83.1	42.9	7.6	3.5	3.7	0.73
*Petenia splendida**	99.7	16.8	18.4	14.2	7.8	1.06
Theraps irregularis	84.9	15.7	7.3	5.4	4.5	0.88
*Thorichthys callolepis**	84.3	20.9	8.3	5.1	3.6	0.83
*Thorichthys ellioti**	95.6	39.3	10.2	5.5	4.0	0.76
*Thorichthys helleri**	82.2	30.2	8.2	4.4	3.9	0.79
*Thorichthys meeki**	82.0	61.4	9.6	4.9	5.5	0.73
Thorichthys pasionis	67.6	19.5	9.5	5.3	4.6	0.78
*Vieja maculicauda**	73.1	28.2	7.7	3.5	3.6	0.73

Phylogeny of Heroines

The inclusion of all of the species sequenced here increases the resolution of phylogenetic relationships of Heroines. The monophyly of the clade containing *Herichthys pearsei*, ''*Cichlasoma*'' *lentiginosum*, and *Theraps irregulare* + ''*Cichlasoma*'' *intermedium* provides resolution of an evolutionary distinct clade in Heroines. The close affinities of *Vieja argentea* to *V. regani*, *Thorichthys pasionis* to *T. helleri*, and *Mesonauta festivus* to *M. insignis* were relationships that may have been expected but nonetheless provided increased resolution for the comparative analyses. The relationships and sequence divergence of the two Caribbean cichlids, *Nandopsis tetracanthus* and *N. haitensis*, and their placement as the sister group to the clade of largely Central American Heroines, suggests that the timing (Martin and Bermingham 1998) and biogeography (Hulsey et al. 2004) of the radiation of Heroines north of the Isthmus of Panama demands more critical evaluation.

Macroevolutionary Jaw Decoupling

Oral and pharyngeal jaw mechanics in the Heroine clade are historically dependent. There was a significant nonphylogenetically corrected correlation between the oral and pharyngeal jaw mechanics in the Heroine cichlids. This type of

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FIG. 5. Phylogeny of Heroines. The best likelihood topology from three Bayesian Markov chain Monte Carlo runs for the cytochrome *b* gene separately is shown. Ten previously unsequenced species and only one sequence per species sequenced in previous studies were included in the analysis. Nodes having greater than 50% Bayesian posterior probability support are depicted. Nodes having 100% posterior probability support are depicted with an asterisk. The nodes indicated with capital letters are discussed in the Results.

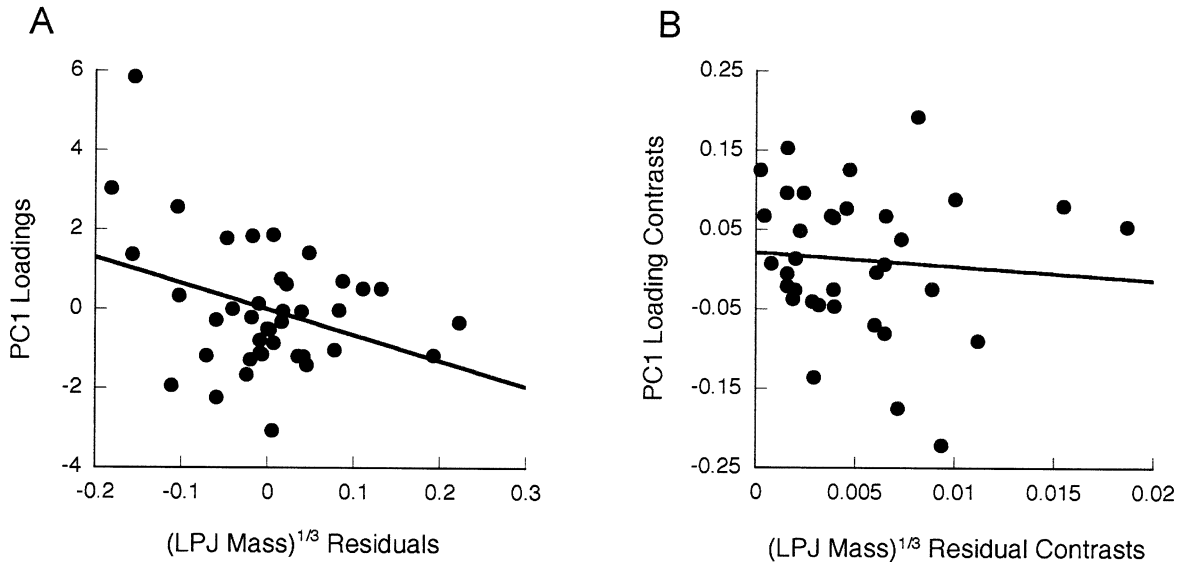

FIG. 6. Correlations of (LPJ mass)$^{1/3}$ and oral jaw PC1 loadings for 40 Heroine species. In (A), the ''tip'' values are used. There is a slight but significant correlation between the oral and pharyngeal jaw values. However, when the relationship is adjusted by phylogeny there is no correlation between (LPJ mass)$^{1/3}$ contrasts and oral jaw PC1 loading contrasts. Liem's hypothesis (1973) of evolutionary decoupling of the oral and pharyngeal jaws in Heroine cichlids cannot be rejected.

historical contingency may explain why fish in similar trophic guilds appear to be fairly similar in both their oral and pharyngeal jaws. If a fish has oral or pharyngeal jaws that are compatible with exploiting a particular prey type, their other set of jaws is more likely to evolve to enhance the ability to exploit that type of prey. But, correlations among species values do not reject the idea that adaptation in the jaws may occur independently. Also, the mechanical combinations of the oral and pharyngeal jaws in Heroine cichlids may still be more variable than combinations found in other fish clades. Future comparisons of the extant relationships between oral and pharyngeal jaw diversification of cichlids and the correlation found in other fishes are necessary to more explicitly test whether the combinations in jaw mechanics found in entire clade of cichlids is exceptionally labile. Because they lack the functionally superior cichlid pharyngeal jaw that can be rapidly modified to exploit novel prey, Liem's hypothesis would predict that evolutionary divergence in oral and pharyngeal jaw mechanics would be much more contingent on ancestral conditions in other fish clades.

Liem's (1973) proposal of modularity between the oral and pharyngeal jaws does appear to have been important in cichlid diversification. When phylogeny is accounted for explicitly, there is no correlation between the oral and pharyngeal jaw mechanics we examined in Heroine cichlids. Because our test accepts the null hypothesis of no correlation, the power to detect a significant relationship among the mechanical characteristics of the two jaws is critical. Importantly, simulations have demonstrated that analyses of phylogenetic independent contrasts should have approximately 40 species to have the power to adequately test for a phylogenetic correlation (Martins 1996). Since we have exactly 40 species in the analysis and this includes approximately half of the entire radiation of Heroines (Kullander 1998), our results are likely robust.

However, the effectiveness of phylogenetic contrasts in accounting for evolutionary history also depends on the quality of the phylogenetic hypothesis. Notably, there is limited resolution at the base of the Heroine phylogeny that could be due to saturation in cytochrome *b* (Hulsey et al. 2004). Mitochondrial genes may also introgress extensively (Machado and Hey 2003) if divergent species hybridize, as cichlids are known to do in the wild (Streelman et al. 2004). The possibility of the mitochondrial topology providing faulty inferences would be most misleading if nodes in the phylogeny exhibiting high contrast values and extensive leverage in the correlation were incorrectly recovered. For instance, the topological misplacement of enigmatic species such as *Petenia splendida* or the disparate species *Herichthys labridens* and *H. tamasopoensis* that are closely related to the polymorphic *H. minckleyi* could strongly influence our results. The two clades to which these species belong are the lineages with the most substantial amounts of change in the oral and pharyngeal jaws in Heroine cichlids. Substantial modification of the relationships of species within these major clades could significantly change the results we have recovered here. Future studies of correlated phenotypic evolution in cichlids and other groups should strive to assess both the effect of removing phenotypic outliers such as the species identified above, but also attempt to phylogenetically incorporate independent partitions from the nuclear genome

TABLE 4. Principal component analysis of the four oral jaw characters examined in the 40 Heroine species. KT, kinematic transmission; VR, velocity ratio.

	PC I	PC II
Gape	0.59	−0.77
Protrusion	0.91	0.21
Anterior jaws KT	0.80	0.45
Lower jaw VR	0.90	−0.11
Cumulative variance (%)	67.2	88.7

to provide the most robust conclusions possible about how phenotypic change maps onto phylogenetic history.

The independent divergence of jaw morphology documented here is also only interesting if the characters examined do have an influence on functional and ecological divergence in cichlids. Importantly, all four of the oral jaw characters have been documented to have an association with ecological specialization in cichlids (Barel 1983; Hulsey and García de León 2005) or in other fishes (Wainwright and Richard 1995; Westneat 1995; Hulsey and Wainwright 2002; Wainwright et al. 2004). The mass of the LPJ was the only mechanical variable of the pharyngeal jaw examined, but because of its correlation with pharyngeal muscle mass in *H. minckleyi* and other fishes (Wainwright et al. 2004), this character likely represents variation in pharyngeal force production capabilities. Force production during prey processing is likely to be a significant axis of cichlid trophic diversification because it determines whether cichlids can crush hard-shelled prey such as snails or break down tough plant material (Hulsey 2006). However, force may not be the only axis of pharyngeal diversification. Although there are likely other mechanical, functional, and ecological axes that have been important in cichlid jaw diversification that were not captured by our choice of variables, the variables examined do provide evidence of evolutionary decoupling. Because there is no correlation between the oral and pharyngeal jaw contrasts examined, Liem's (1973) hypothesis that the two jaws of cichlids are functionally decoupled during evolution cannot be rejected.

Decoupling as found here likely plays a key role in diversification in complex systems ranging from gene networks to ecosystems. However, we do not have extensive understanding of the interrelated questions of how modularity arises (Wagner and Altenberg 1996) and how decoupling at various levels of biological integration interacts to influence diversification (Hulsey et al. 2005b). At some levels of biological organization the origin of novelties that can evolve independently is almost trivial. For instance, gene duplication within genomes is likely commonplace (Shapiro 2005) and studies detailing the mechanics of divergence and the origin of modularity in gene function are growing rapidly (Force et al. 2005). However, functional decoupling at the level of the whole organism may require intermediate steps that are rarely important in the modularity of easily duplicated components like those found in genetic systems. The presence of only two sets of paired limbs in tetrapods and the gradual evolution of functional separation between the oral and pharyngeal jaws in teleost fishes (Lauder 1985) strongly suggest that duplication and functional decoupling among these types of phenotypic elements is not rapid and likely highly constrained (Vermeij 1974; McShea 1998). In part, this may be because mechanical components of macroscopic phenotypes are highly integrated into the functioning of the entire body and are generally modified from existing structures. Altering the limited number of elements available without compromising function may simply be more difficult. However, novel ideas such as the many-to-one mapping documented in the anterior jaw linkage (Alfaro et al. 2005) may provide the type of theoretical intermediate steps that complex phenotypes such as the pharyngeal jaw must pass through to enhance decoupling. In these types of linkage systems, anatomical configurations that determine one mechanical property can be optimized without compromising other distinct mechanical properties of the system providing a potential mechanical analogy to subfunctionalization (Force et al. 2005) that may lead to decoupling in genetic systems.

Modularity at levels of biological organization such as the jaws of cichlids, in which expression is necessarily the result of genetic and developmental changes, are also likely heavily constrained by how independent their genetic and developmental basis is. Interestingly, mutagenesis screens within *Danio rerio*, the zebrafish, have suggested that the genetic architecture underlying the oral and pharyngeal jaws of teleosts (Piotrowski et al. 1996) may have been extensively decoupled before the novelties in the cichlid pharyngeal jaw originated. However, even if the genetic bases of teleost jaws have long been decoupled, it remains unclear when the oral and pharyngeal jaws of teleosts became developmentally and functionally decoupled. Future studies that examine developmental and functional decoupling in the jaws of other fish groups would further inform how critical the anatomical novelties in the cichlid pharyngeal jaw were to their diversification. Furthermore, integrative studies that examine the genetic basis and genomic decoupling of explicitly mechanical traits such as has been examined in the lower jaw elements of old world cichlids (Albertson et al. 2005) could easily be used to explicitly test these interactions and the degree of decoupling in components of cichlid jaws at various levels of biological organization (Hulsey et al. 2005b).

The jaws of cichlids and other teleost fish will continue to offer excellent systems for examining the evolutionary causes as well as consequences of genetic, developmental, and functional innovation. The origination of the cichlid pharyngeal jaw likely did free this group's oral jaws from constraints imposed by serving a dual functional role as both a site of prey capture and as a mechanism responsible for prey processing (Liem 1973; Vermeij 1974). Similar to any novelty that increases the ability of elements to evolve independently, the cichlid pharyngeal jaw likely increased the extent that the oral and pharyngeal jaws could separately diverge, thus facilitating the unparalleled microevolutionary and macroevolutionary trophic diversification of cichlids.

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Appendix

Collection locations of Heroine cichlids studied. TNHC, Texas Natural History Collection; FMNH, Field Museum of Natural History; UFM, University of Florida Museum of Natural History; AMNH, American Museum of Natural History Mexico, collections by authors.

Archocentrus centrarchus: Laguna Jerico, Nicarauga FMNH, Cano Negro, Costa Rica, TNHC; *Archocentrus nigrofasciatus*: TNHC Laguna Tortuguero, Costa Rica, TNHC; *Archocentrus septemfasciatus*: Rio Tortugureo and Rio Tirimbina, Costa Rica, TNHC; *Astatheros alfari*: Bocas del Torro, Panama, UFM; *Astatheros macracanthus*: Rio de los Perros, Mexico; *Astatheros robertsoni*: Rio Tonala, Mexico; *Caquetaia kraussii*: Colombia, UFM; *Caquetaia myersi*: Napo River, Ecuador, FMNH; ''*Cichlasoma intermedium*'': Rio Icvolay, Guatemala, AMNH; ''*Cichlasoma*'' *octofasciatum*: Rio Tonala, Mexico; ''*Cichlasoma*'' *salvini*: Rio Sarabia, Lago de Illusiones, Mexico; ''*Cichlasoma*'' *trimaculatum*: Rio de los Perros, Mexico; ''*Cichlasoma*'' *uropthalmum*: Stann Creek Belize, FMNH; *Herichthys bartoni*: Laguna Media Luna, Mexico; *Herichthys cyanoguttatus*: Rio San Rafael, Mexico; *Herichthys deppii*: Rio Nautla, Mexico; *Herichthys labridens* Cascadas: Rio Tamasopo, Mexico; *Herichthys labridens* ML: Laguna Media Luna, Mexico; *Herichthys pantostictus*: Rio Guayalejo, Mexico *Herichthys pearsei*: Rio Azul, Mexico, AMNH; *Herichthys tamasopoensis*: Rio Tamasopo, Mexico; *Herotilapia multispinosa*: Laguna Jenicero, Nicaragua, FMNH; *Hypsophrys nicaraguensis*: Lago Nicaragua and Lago Managua, Nicaragua FMNH; *Nandopsis haitiensis*: Dominican Republic, UMF; *Nandopsis tetracanthus*: Canal at San Agustin, Cuba, AMNH; *Parachromis dovii*: Cano Agua Fria Viejo, Costa Rica, TNHC; *Parachromis loisellei*: Bocas del Torro, Panama, UFM; *Parachromis managuensis*: Laguna Jenicero, Nicaragua FMNH; *Paraneetroplus bulleri*: Rio Sarabia, Mexico; *Paratheraps fenestratus*: Lago Catemaco, Mexico; *Paratheraps guttulatus*: Rio Sarabia, Mexico; *Paratheraps synspilus*: Orange Walk, Belize, FMNH; *Petenia splendida*: Stann Creek and Sibun River, Belize, FMNH; *Theraps irregularis*: Rio Chixoy, Guatemala, AMNH; *Thorichthys callolepis*: Rio Almoloya, Mexico; *Thorichthys ellioti*: Lago Catemaco, Mexico; *Thorichthys helleri*: Rio Tonala, Mexico; *Thorichthys meeki*: Lago de Illusiones, Mexico; *Thorichthys pasionis*: Lago de Illusiones, Mexico; *Vieja maculicauda*: Canal Zone, Panama, FMNH.