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EVOLUTION IN FRESH WATERS DURING THE GREAT AMERICAN INTERCHANGE

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Chapter 1: Introduction

Abstract

This thesis examines the process by which continental fish faunas arise, using the Great American Biotic Interchange as a case study. The Interchange allowed South American fishes to colonize North America. I examine the history of this colonization in the Characiformes (Actinopterygii: Ostariophysi), finding repeated colonization events and a surprising non-monophyly of the group. I explore the morphological variation in the standing diversity of North American Characiformes, finding it to be a non-biased subset of the variation found in South America. I also examine intraspecific morphological variation along environmental gradients, finding that this variation can be equal to variation among species. Finally, I use ecological niche modeling to predict the future spread of characiform fishes in North America, finding that their projected future ranges may be regulated by climate, but mediated by diet.

Introduction

A key aim of evolutionary biology is understanding the history and evolutionary processes that underlie the distribution of organisms across the surface of the planet. Why some lineages are widespread while others are highly restricted, how lineage history affects the likelihood of persistence in the face of new ecological circumstances, and how geology and molecular biology can be integrated to construct internally consistent models of evolutionary history are the province of macroevolution and historical biogeography. This dissertation draws upon the peculiarities of an especially important biogeographic event, the Great American Biotic Interchange, to add to our collective understanding of earth history and the consequences of biotic mixing.

The Great American Biotic Interchange (hereafter, “Interchange,” with the general case and all other specific such events in lower-case) refers to the mixture of lineages of North and South American provenance that ensued upon the formation of the Isthmus of Panama during the Neogene Period. The precise date of the establishment of land connections between the long-separated continents is newly controversial in light of geological and molecular evidence consistent with an earlier date (Montes *et al*. 2015; Bacon *et al.* 2015a, b; Marko *et al.* 2015, O’Dea *et al.* 2016). The importance of resolving the date at which the Interchange became possible is difficult to overstate, as it affects the temporal context in which we interpret all other data pertaining to the first occurrence, rapidity of colonization, and the standing diversity of all lineages involved.

The American Interchange is uniquely useful for understanding how patterns of diversity and geographic range are established, and had a uniquely profound effect on global biodiversity. It is one of a class of comparable events, such as the mixing of Gondwanan and Laurasian taxa when India collided with the Eurasian plate (Briggs 2003, Chatterjee & Scotese 2010, Karanth 2006), or the mixing of island and continental faunas when Australasian and Eurasian elements combined to form Luzon (Vallejo 2014). However, it has several attributes that make it both more tractable and consequential than many interchanges. First, it is a comparatively recent event, which means that the geological, paleontological, and molecular signals have had less opportunity to be obscured by subsequent history. Second, it involved two biotas that had been isolated from one another quite thoroughly for a protracted interval, which leaves little ambiguity about the geographic origin of most lineages. Third, for terrestrial and freshwater organisms, the pathway to a new continent was very narrow, allowing inferences about the ecological requirements for a new colonization. Finally, and importantly, it affected taxa in virtually all habitats, since it both connected landmasses to each other and completely cut off any mid-latitude connection between the Atlantic and Pacific oceans. The separation of oceans also drastically affected ocean currents, altering global climate and likely facilitating the Ice Ages (Murdock *et al*. 1997; Haug and Tiedemann 1998).

Biogeographic history and provisional taxonomy of the characins

The Characiformes (Teleostei: Ostariophysi) are a diverse group (~2,000 species) of entirely freshwater teleosts. They have conventionally been regarded as a monophyletic group within the Ostariophysi, diagnosable by the presence, in most taxa, of an adipose fin, ctenoid scales, a prootic foramen, a transverse process on the third neural arch (Fink & Fink 1981; Fink & Fink 1996), and corroborated by some analyses of molecular data (Saitoh *et al.* 2003; Calcagnotto *et al.* 2005; Peng *et al.* 2009; Near *et al*. 2012; Betancur *et al.* 2013; Arcila *et al.* 2017). Recently, other molecular data have been interpreted as refuting the monophyly of the Characiformes. Some analyses recover a sister relationship between the Characoidei, a group that includes the great majority of living characins, and the catfishes (order Siluriformes), to the exclusion of the entirely African characiform clade Citharinoidei (*e.g.*, Chakrabarty *et al.* 2017; see Fig. 1.1). Many studies concerned with the internal relationships among characiform taxa have used catfishes to root their topologies and hence are silent on the monophyly of the group. Herein, I will use "Characiformes *sensu lato*" to refer to the putative taxon composed of catfishes, characoids, and citharinoids, and "Characiformes *sensu stricto*" to refer to the putative taxon composed of only characoids and citharinoids. Betancur-R *et al.* (2017) recognize 21 families in the Characiformes *sensu stricto*, and one additional family was recently described by de Pinna *et al.* (2017); each is briefly summarized on the following page.

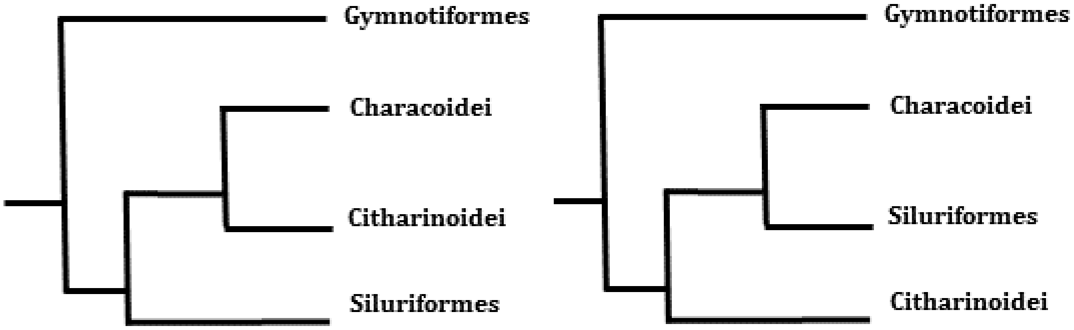

Figure 1.1: Alternative hypotheses of relationships among Characiformes and relatives. Left: monophyletic Characiformes (Saitoh *et al.* 2003; Calcagnotto *et al.* 2005; Peng *et al.* 2009; Near *et al*. 2012; Betancur *et al.* 2013; Arcila *et al.* 2017). Right: Paraphyletic Characiformes encompassing Siluriformes (Chakrabarty *et al.* 2017).

The current distribution of the Characiformes (*sensu stricto*), with more than 1,000 species in South America, and more than 200 in Africa, suggest a Gondwanan origin. This is largely congruent with their fossil record, which begins with †*Santanichthys diasii* from the Albian of Brazil (Filleul & Maisey 2004). A series of isolated dentaries from the Cretaceous of North America have been assigned to Characoidei *incertae sedis* (Newbrey *et al.* 2009); however, other workers have expressed doubt about this assignment. Likewise, †*Sorbinicharax*, another putative Cretaceous characin from Eurasia, a similarly unexpected setting for a Gondwanan group, has been reinterpreted as Teleostei *incertae sedis* (Chen *et al.* 2013; Mayrinck *et al.* 2017). Moreover, even if the Canadian fossil is in fact a characin,

Family	**common name**	**Distribution**
Citharinidae	lutefishes	Africa
Distichodontidae	distichodus	Africa
Acestrorhynchidae	pike-characins	S. Amer.
Alestidae	African tetras	Africa
Anostomidae	anostomids	S. Amer.
Bryconidae	dorados/trout-characins	S. Amer., N. Amer. to Mexico
Chalceidae	chalceus	S. Amer.
Characidae	New World tetras	S. Amer., N. Amer. to Texas
Chilodontidae	headstanders	S. Amer.
Crenuchidae	darter characins	S. Amer., N. Amer. to W. Panama
Ctenoluciidae	pike-characins	S. Amer., N. Amer. to W. Panama
Curimatidae	toothless characins	S. Amer., N. Amer. to Costa Rica
Cynodontidae	dogtooth characins	S. Amer.
Erythrinidae	trahiras	S. Amer., N. Amer. to Costa Rica
Gasteropelecidae	hatchetfishes	S. Amer., N. Amer. to W. Panama
Hemiodontidae	halftooths	S. Amer.
Hepsetidae	African pike-characins	Africa
Iguanodectidae	iguanodectids	S. Amer.
Lebiasinidae	pencilfishes	S. Amer., N. Amer. to Costa Rica
Parodontidae	scrapetooths	S. Amer. to E. Panama
Prochilodontidae	flannelmouths	S. Amer.
Serrasalmidae	piranhas	S. Amer.
Tarumaniidae	NA	S. Amer.
Triporthidae	triportheids	S. Amer.

Table 1.1: Familes of fishes in Characiformes *sensu stricto*. Citharinidae + Distichodontidae constitute the clade Citharinoidei; the remaining families constitute the Characoidei.

the complete absence of any characiform fossils from Cenozoic deposits where they might be expected to occur, such as the Green River, suggests a Gondwanan origin for at least the standing diversity (Grande 1984, Dahdul 2010). Importantly, there is no evidence that they have occurred in marine or brackish settings at any time in the Cenozoic (Betancur-R *et al*. 2015). The eastern half of Panama is geologically South American, and thus the presence of Neotropical lineages in this region cannot reliably be inferred to represent colonization events. Therefore, throughout this thesis, I will use “North America” to refer to the entire contiguous landmass north and west of central Panama, and “South America” to refer to the entire landmass south and east of central Panama.

Aims

This thesis has three major aims, focused on the history, present diversity, and future of the colonization of North America by characiform fishes. In Chapter 1, I use important new collections from western Colombia to augment taxon sampling, in order to resolve critical nodes in the phylogeny of the Characiformes *sensu lato*. Constrained with appropriate fossil occurrence data, I aim to identify the number of colonization events, as well as estimate their timing. In Chapters 2 and 3, I use an extensive morphometric data set to examine by proxy the ecology of the standing diversity of North American characins. In particular, I ask whether characins have undergone ecological diversification upon their arrival on a new continent, and whether the variation we observe is taxonomically informative. In Chapter

3, I look to the future, using a niche modeling approach to discover the correlates of characin distributions, and project those into the near future using climate change models. By combining these three approaches, I hope to draw insight into the assembly of faunas at the continental scale.

Chapter 2: Broadly sampled phylogeny of Characiformes reveals repeated colonization of North America and paraphyly of Characiformes *sensu stricto*

Abstract

Timing of the colonization of North America by South American freshwater fishes has profound implications for our understanding of the tempo of diversification and the assembly of tropical American fish faunas. This study uses expanded taxon sampling among the Characiformes, along with fossil calibrations to estimate divergence times across the Isthmus without reference to the canonical date of the Isthmus. Most such divergences are shallow (<1 ma), but this may be an artifact of the use of a locus with a low rate of evolution. The traditional order Characiformes is recovered as paraphyletic with respect to Siluriformes.

Introduction

South America experienced an unusually persistent and complete isolation from other continents, beginning in the Paleocene Epoch (Reguero & Marenssi 2014) and ending at the rise of the Isthmus of Panama, an interval of at least 40 million years. The age of final emergence of the Isthmus has long been taken to be a Pliocene event (Coates & Obando 1996); however, stratigraphic work has prompted a reappraisal of the date when overland crossing between North and South America would have become possible (Montes *et al.* 2012, Montes *et al.* 2015). A spirited debate has ensued about when the respective faunas of the American continents might have come into contact with one another (Bacon *et al.* 2015a, b; Marko *et al.* 2015; O'Dea *et al.* 2016).

In principle, genetic data from organisms in the region of the Isthmus represent independent corroborations or refutations of proposed closure dates. However, divergence time estimates have often used the canonical age of the Isthmus itself as a calibration point (Cody *et al*. 2010; Smith & Klicka 2010; Weir *et al.* 2009). This is a defensible choice if the age of the Isthmus is considered a reliable benchmark; however, if new geological evidence calls that date into question, using it as a calibration introduces circularity to the argument. If molecular divergence times are to be brought to bear on questions of geological history, they must be calibrated by other means.

Characoid fishes are a convenient group to attempt to estimate geology by proxy. Eight separate families of characoid fishes colonized *terra nova* to varying degrees during the Interchange. Even if each family pushed into new territory only once, this represents a minimum of eight potential divergence estimates. Moreover, whereas the earliest putative fossil characin is known from a brackish or marine environment, all living representatives are strictly freshwater, as are all known Cenozoic fossils (Bussing 1998; Dahdul 2010; Géry 1977). This is a crucial fact because it implies that characins could not have easily crossed an incomplete Isthmus, in contrast to clades with both marine and freshwater representatives, such as mullets, gobies, and sea catfishes (McMahan *et al.* 2013; Stange *et al.* 2017). The published fossil record of characins is also reasonably helpful, offering node calibrations from a limited number of time points, but from many parts of the phylogeny.

Previous studies of the interrelationships of characoid fishes have sampled heavily among taxa from both Central America and *cis*-Andean South America—*i.e.,* the region of South

America east of the Andes (*e.g.*, Calcagnotto *et al.* 2005; Ornelas-García *et al.* 2008; Sivasundar *et al.* 2001). In part, the focus on *cis*-Andean taxa reflects strong scientific infrastructure in Brazil, and much greater diversity of fishes in the Amazon and Orinoco regions than elsewhere in the Americas. The relative dearth of *trans*-Andean (west of the Andes) taxa is partly a result of the historical difficulty in collecting and exporting specimens from Colombia (Fernández 2017). However, recent relaxation of collection regulations, coupled with increasing peace and stability in Colombia, have made it much more accessible to both local and international researchers (Regalado 2013).

Taxa from Colombia, especially western Colombia, are essential for gauging divergence times between North and South American lineages. The closure of the Isthmus, under either model of emergence, would post-date the main Andean orogeny (Ramos 2009). It follows that North American characoids would almost certainly be derived from *trans*-Andean stocks in northern South America, and that any phylogenetic hypothesis that attempted to estimate divergences without representatives from these regions would lack power and precision. This study combines sequence data from newly collected Colombian taxa in the Atrato and Cuenca river basins with published genetic data culled from GenBank to construct a densely and broadly sampled hypothesis of relationships among Characiformes *sensu lato*, with special attention to the lineages that contributed to the Central American fauna.

Methods

Phylogenetic analysis

Tissues were extracted from 130 specimens using the DNEasy kit (Qiagen). Purified DNA was amplified for three mitochondrial and three nuclear loci, chosen for their widespread use in previous studies of ostariophysan relationships, in order to maximize taxon sampling. PCR primers for *COI*, *cytb*, *16S*, *RAG1*, and *RAG2* were based on Oliveira *et al.* 2011; *myh6* was based on Li *et al.* 2007. For all other taxa, sequence data were downloaded from NCBI GenBank (Benson *et al.* 2005) and culled using a custom R script to reduce representation to two exemplars per nominal species. The newly generated data combined with GenBank accessions yielded an alignment with 826 ingroup taxa (6 Siluriformes and 821 Characiformes *sensu stricto*) and several outgroup taxa (Black Ghost Knifefish *Apteronotus albifrons*, Bluntnose Minnow *Pimephales notatus*, Milkfish *Chanos chanos*). The 830 sequences were aligned using the Geneious alignment tool (Kearse *et al.* 2012). The alignment was analyzed using RaxML implemented on the CIPRES server (Miller *et al.*

Taxon	**Age (my)**	**Locality**	**Reference**
†*Arabocharax baidensis*	26-23[1]	Baid, Saudi Arabia	Micklich & Roscher 1990
Hoplias sp.	13.8-12[2]	La Venta, Colombia	Lundberg 1997
Hydrolycus sp.	13.8-12	La Venta, Colombia	Lundberg 1997
Colossoma macropomum	13.8-12	La Venta, Colombia	Lundberg *et al.* 1986
Acestrorhynchus sp.	13.8-12	La Venta, Colombia	Lundberg 1997
Markiana sp.	13.8-12	La Venta, Colombia	Lundberg 1997
Pygocentrus sp.	13.8-12	La Venta, Colombia	Lundberg 1997
Serrasalmus sp.	13.8-12	La Venta, Colombia	Lundberg 1997
Erythrinus sp.	13.8-12	La Venta, Colombia	Lundberg 1997
†*Mahengecharax carrolli*	46-45.7[3]	Mahenge, Tanzania	Murray 2003
†*Brycon avus*	30-25[4]	Tremembé, Brazil	Malabarba 1998
†*Cyphocharax mosesi*	30-25	Tremembé, Brazil	Malabarba 1998

Table 2.1: Fossil taxa used to calibrate the time-tree. References for formations ages: 1: Schmidt *et al.* 1982; 2: Takemura 1985; Harrison *et al.* 2001; Kellner & Campos 1999.

2010), using a GTRCAT model with 100 bootstraps. For this manuscript, only the *myh6* and *COI* loci were analyzed, and each was analyzed separately.

Fossil calibrations

Fossil occurrences were aggregated from paleontological literature. Included calibration points included Cenozoic fossils referable to either extant species or genera, placed in the topology based on taxonomic identity, or, where available, explicit character analyses in which the fossil specimens were included (Table 2.1). The time-tree was computed using the RelTime algorithm implemented in MEGA7 (Kumar *et al.* 2016).

Results

Topology

Initial analyses of both *myh6* and *COI* were impeded by the erroneous inclusion of non-characiform sequences from GenBank. The *COI* tree was misled by the inclusion of three cichlid sequences. The *myh6* tree was misled by the inclusion of a putative specimen of *Colossoma* sp. (accession number FJ918842.1), which under a BLAST query most closely matched the lightfishes (Stomiiformes: Phosichthyidae). Because an ostariophysan was designated as the outgroup, this sequence was constrained to be in the ingroup, distorting branch length estimates. Similarly, I found three cichlid sequences in the *COI* alignment after estimating the tree (accession numbers KP027397.1, KP027397.1, KP027399.1), although these were the result of an error in the query, rather than misidentified specimens. Re-analysis of the *myh6* locus with the lightfish sequence removed recovered a paraphyletic Characiformes, with a sister relationship between the Characoidei and the Siluriformes; however, statistical support for the clade (Characoidei + Siluriformes) was

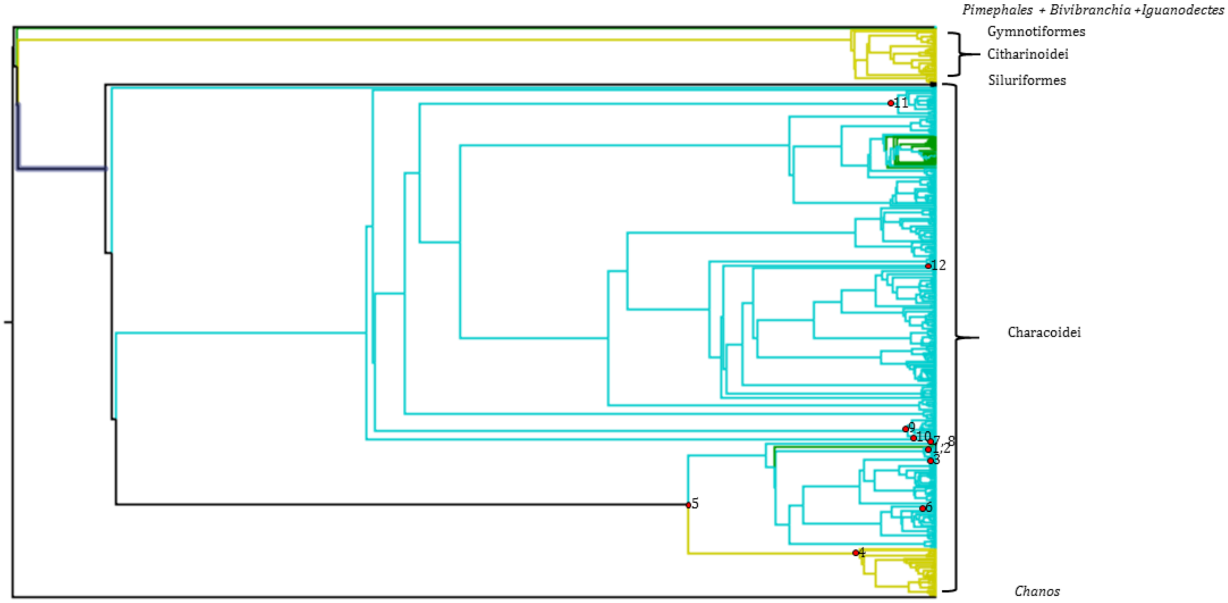

Figure 2.1: Non-time-calibrated maximum likelihood tree based on *myh6* locus. Branches in blue represent South American lineages; branches in yellow represent African lineages, and branches in green represent North American lineages. Gray branches represent non-characiform lineages or lineages which likely pre-date the breakup of South America and Africa. Numbered red dots represent fossil calibrations used in this study: 1) *Serrasalmus* sp., 2) *Pygocentrus* sp., 3) *Colossoma macropomum*, 4) †*Arabocharax baidensis*, 5) †*Mehengecharax carrolli*, 6) †*Cyphocharax mosesi*, 7) *Erythrinus* sp., 8) *Hoplias* sp., 9) *Acestrorhynchus* sp., 10) *Hydrolycus* sp., 11) †*Brycon avus*, 12) *Markiana* sp.

weak (44% bootstrap support). The clade ([Characoidei + Siluriformes] + Citharinoidei) had moderate bootstrap support (73%) but failed to circumscribe two problematic taxa (*Iguanodectes*, *Bivibranchia*, which formed a clade with the minnow *Pimephales*). Most suborders and families as given by Betancur-R. *et al*. (2017) were recovered with strong support. See Fig. 2.1 for a summary tree.

Time-tree

The root of Characiformes *sensu lato* is estimated at 232 million years, the Carnian stage of the Upper Triassic. There is no evidence that ostariophysans existed at the time, although the first teleosts appear in the fossil record in the Triassic (Arratia 2000). All cross-Isthmian sister relationships are estimated to be less than 1 million years old; for several, including the species-rich group *Astyanax*, inferred divergence time is indistinguishable from zero.

Discussion

The topology of the tree recovered herein agrees broadly with the ultra-conserved element dataset of Chakrabarty *et al*. (2017) in finding the Characiformes *sensu stricto* paraphyletic with respect to the Siluriformes (Fig. 1.1). Most major groups (suborders, families) are recovered with high statistical support, although bootstrap support is weak toward the tips. The *myh6* locus which drives the topology of this tree does not exhibit sufficient variation in close relatives to confidently resolve their relationships. The limitations of this nuclear locus extend to the dating. Sub-generic relationships, including relationships crucial to understanding expansion across the isthmus, were estimated at or near 0, reflecting the low variation in the locus except at relatively deep divergences.

Several approaches could improve estimates of divergences across the Isthmus. First, node calibrations would likely be much more reliable if more of the fossil exemplars were included in an explicit character analysis alongside extant species, in order to constrain their position in the phylogeny. Second, inclusion of additional loci from faster-evolving parts of the genome would allow better resolution toward the tips. For example, this study included some of the same taxa as a previous study, on the genus *Brycon*, which relied on mitochondrial markers and found much deeper divergences among the various species (de

Carvalho *et al.* 2011). Finally, an explicitly Bayesian analysis (*e.g.,* BEAST2, Bouckaert *et al.* 2014) conducted across a tree informed by multiple loci with different rates of evolution, would likely perform better in light of many coeval fossil calibrations. This would have the added benefit of increasing the ratio of characters to taxa, likely improving the resolution and statistical support of the topology. Taxon and character jackknifing also have potential to improve resolution. Whereas the first remedy would entail extensive additional data collection to generate a morphological character matrix, data to attempt the other remedies have already been collected and partially analyzed.

Number and direction of colonization events

The phylogenetic analysis recovers two South American members of the widespread genus *Astyanax* nested within the North American radiation. Naively interpreted, this topology—which has low statistical support—would suggest that two lineages returned to South America after the initial colonization of North America, as has been proposed for cichlids (Chakrabarty & Albert 2011). However, it is more likely that the apparent reversals are another result of the low variation at this locus for relatively recent divergences, hindering resolution in this part of the tree. If the pattern persists upon the addition of more quickly evolving loci, it would also be consistent with a demographic history that resulted in incomplete lineage sorting, as has been inferred in Neotropical livebearers (Alda *et al.* 2013).

The genus *Brycon* appears to have colonized North America twice independently. *Compsura gorgonae*, a Panama endemic whose only congener is *C. heterura* from Atlantic drainages in

Brazil, were recovered in a separate branches of a clade containing species of *Serrapinna*, *Aphyocharax*, and *Odontostilbe*. The monophyly of *Compsura* has always been dubious, and as *C. gorgonae* is the type species, *C. heterura* will eventually have to be reassigned. All other nominal genera are inferred as colonizing North America only once.

The results of this study are consistent with a younger date for the emergence of the Isthmus of Panama, but due to low variation in the only locus whose study was completed, it is impossible to draw firm conclusions. Further analysis of loci with greater variation should improve resolution at the shallow nodes joining North American taxa to their sister groups in western South America.

Chapter 3: No evidence for filtering of eco-morphology in characiform lineages during the Great American Interchange

Abstract: This study uses a geometric morphometric approach to understand the ecology and evolution of characiform fishes upon their colonization of North America. Multiple lineages colonized North America, but many did not. By examining morphospace occupation in both locations, I ask whether the North American fauna shows evidence of adaptive radiation or filtering. Explicit comparisons of disparity reveal little difference in morphospace occupation, suggesting, 1) no very strong filters, and, 2) no evidence of radiation upon reaching new river basins.

Introduction

The Great American Interchange was highly asymmetric with respect to freshwater animals, with many more lineages colonizing North America from South America than vice versa. The freshwater fish fauna of southern North America, as far north as southern Mexico, consists largely of lineages with South American origins (Berra 2007). For these South American lineages, a new continent represented an ecological opportunity, which could have created the conditions for an adaptive radiation (Losos 2010). Conversely, they might have found themselves constrained, whether by their own evolutionary history, by incumbent competitors, or by other factors. I ask whether the patterns of morphological variation observed in a large clade of freshwater fishes is more consistent with an adaptive radiation, or with some constraint.

Characiform fishes (Teleostei: Ostariophysi) have Gondwanan origins, but have colonized North America multiple times during the last few million years (Reeves & Bermingham 2006; Arroyave *et al.* 2013). Thus, they represent a test case for the dynamics of morphological evolution in response to new opportunity. I used a large landmark-based geometric morphometric data set to examine these dynamics. Specifically, I evaluated morphospace occupation of the characiform fish fauna across a broad geographical area, from western Colombia to the southernmost United States. Under a hypothesis of adaptive radiation, the expected result would be increased disparity in newly colonized regions, relative to a source region, as lineages diversify to fill new niches. Under a hypothesis of environmental filtering, differential surmounting of biogeographic barriers, or competitive exclusion, the expected result would be diminished or unchanged disparity, relative to the source region.

Methods

Materials

I examined specimens from the collections of the Field Museum (Chicago), the Tulane Biodiversity Research Institute (New Orleans), the Louisiana State Museum of Natural History (Baton Rouge), the University of Southern Mississippi (Hattiesburg), the National Museum of Natural History (Washington), and the University of Costa Rica (San José). All specimens were photographed in lateral view with an overhead digital camera. Each photograph included a known size reference, typically a ruler but occasionally a background with a grid of known dimensions. I used pins to indicate landmarks that would otherwise be difficult to locate in a photograph. Specimens too severely bent to be laid flat were excluded. I examined 267 lots, representing more than 1,800 specimens.

Digitization

Photographs were digitized using ImageJ (Rasband 1997-2012). I recorded the size-adjusted x,y coordinates of 16 homologous landmarks visible on the external surface of each individual fish, following Fink & Zelditch (1995). See Fig. 3.1 for landmark locations. Photographs where landmark locations were ambiguous were excluded. The resulting data set consists of 1,674 specimens with morphometric landmark data, all with accompanying locality data.

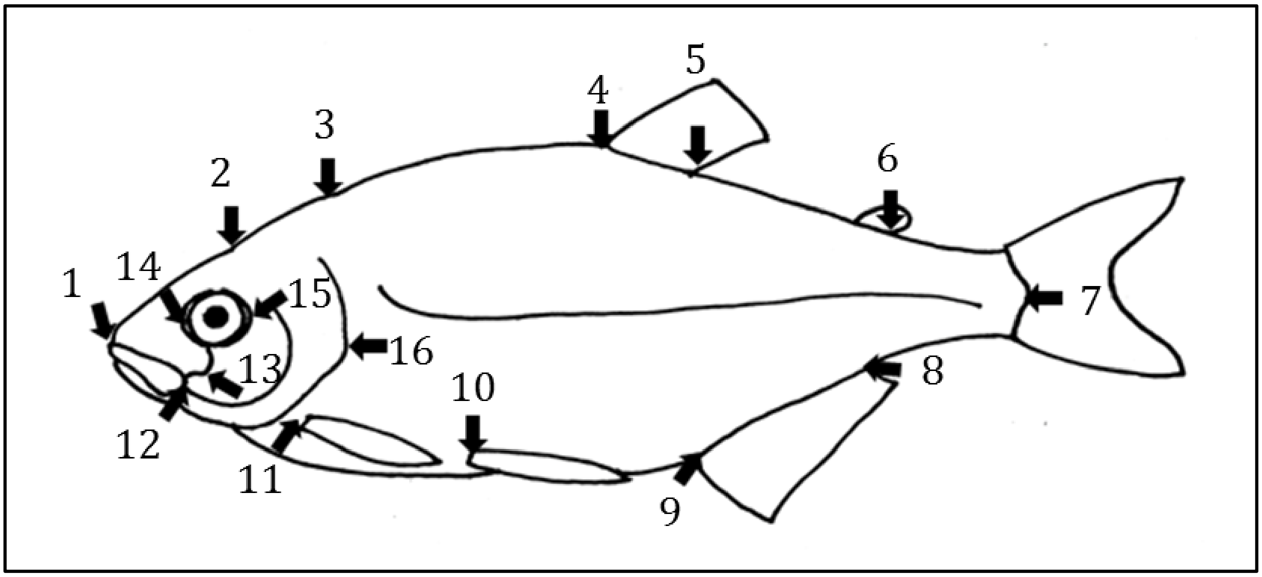

Figure 3.1: Geometric morphometric landmarks, after Fink & Zelditch (1995): 1: anterior junction of premaxillae; 2: ethmoid-frontal suture; 3: posterior tip of supraoccipital; 4: anterior dorsal fin origin; 5: posterior dorsal fin origin; 6: posterior adipose fin origin; 7: posterior edge of hypural ossification (last tail bone); 8: posterior anal fin origin; 9: anterior anal fin origin; 10: pelvic fin origin; 11: pectoral fin origin; 12: quadrate-mandibular joint; 13: junction of maxilla and 3rd infraorbital; 13: anterior margin of bony orbit; 14: posterior margin of bony orbit; 15: posterior of bony opercle.

Analysis

I divided specimens into two locality bins. The source region was taken to be the portion of Colombia west of the Andes, as well as the easternmost portion of Panama, which is geologically South American (Pennington 1981). The second bin includes western Panama and all of North America west and north. I calculated the mean shape for each nominal species (Procrustes superimposition) and exported the species mean for subsequent analyses. In most instances I followed the taxonomy given by the institutions where specimens were housed, although in a few instances I adjusted species assignments following Bussing (1998) and Miller *et al*. (2005). I conducted a principal components analysis on the species means (*n* = 17 for South America; *n* = 11 for North America). I compared disparity (bootstrapped disparity, 3600 bootstrap replicates) in the North and South American samples using the DisparityBox module of the IMP software package (Sheets *et al.* 2004).

Results

The morphospace described by the first two principal components of the combined Colombian and North American data set is strongly influenced by aspect ratio; elongate fishes such as pencilfishes and pike-characins populate the left portion of the plot, while deep-bodied and hump-backed fishes populate the right (Fig. 3.2). A deformation grid of PC1 (Fig. 3.3) shows that much of the variation arises from elongation or compression of the trunk, evident from the compression of the space between landmarks on the head and those on the fins. A deformation grid of PC2 shows greater variance in the form of the head

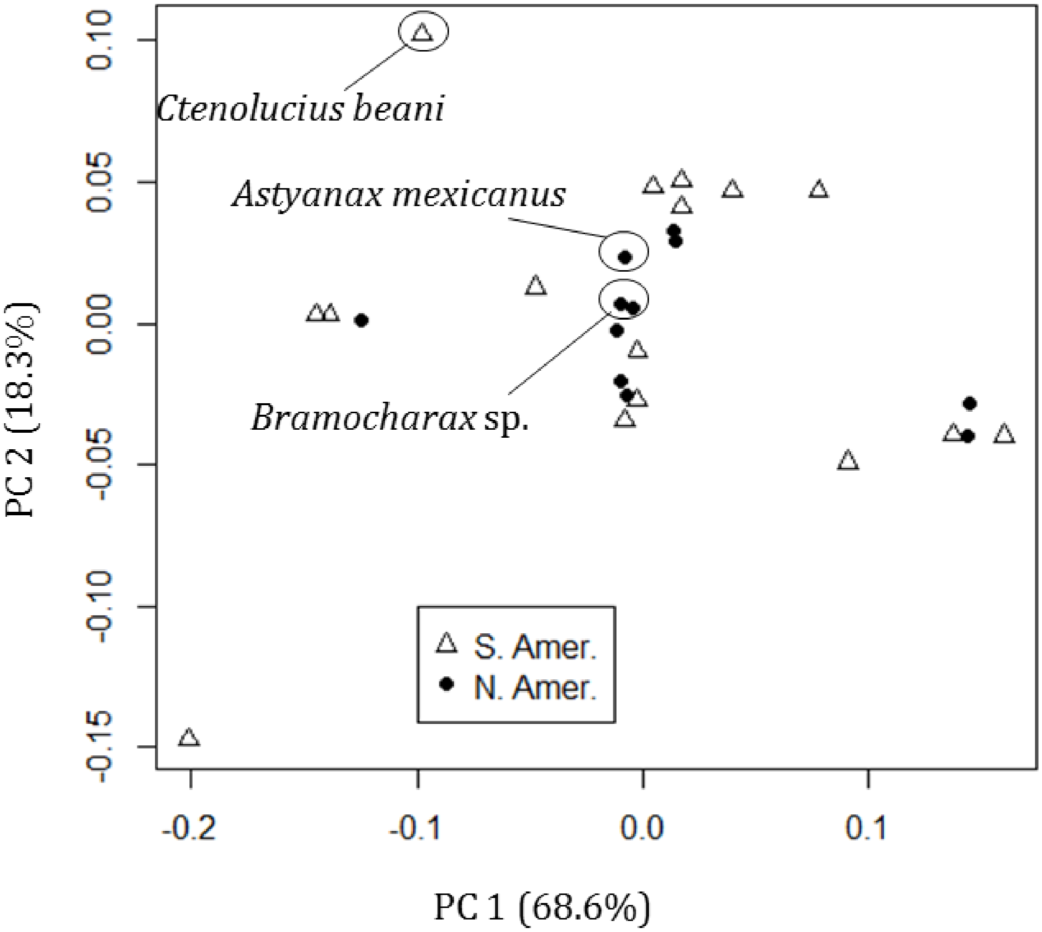

Figure 3.2: Principal components 1 (68.6% of variance) and 2 (18.3% of variance) of body shape landmark data for all characiform taxa in North America and western Colombia. Empty triangles are South American taxa; circles are North American taxa. The elongate pike-characin *Ctenolucius beani* is highlighted for its relatively aberrant morphology relative to other characins. The *"Bramocharax*" (=*Astyanax*) specimens, along with *A. mexicanus*, occupy a portion of morphospace near the group average, a region unoccupied by South American taxa in the sample.

and caudal peduncle. The top left point of the PC plot shows the pike-characin (*Ctenolucius beani)*, which has both the most elongate body and the head of the fishes in this sample.

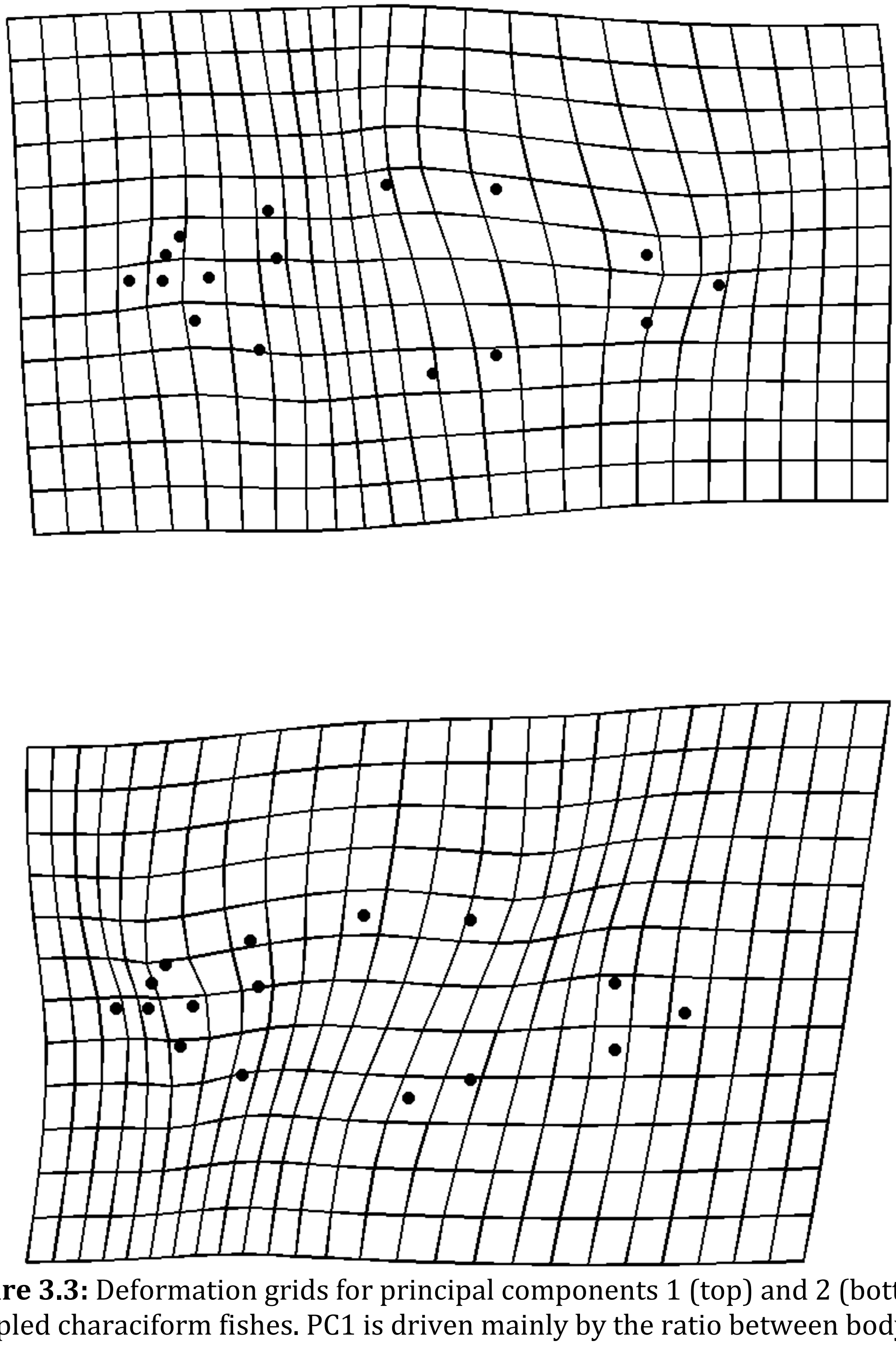

Figure 3.3: Deformation grids for principal components 1 (top) and 2 (bottom) for all sampled characiform fishes. PC1 is driven mainly by the ratio between body length and body depth; whereas PC 2 is driven by changes in head shape and variation in the length of the caudal region.

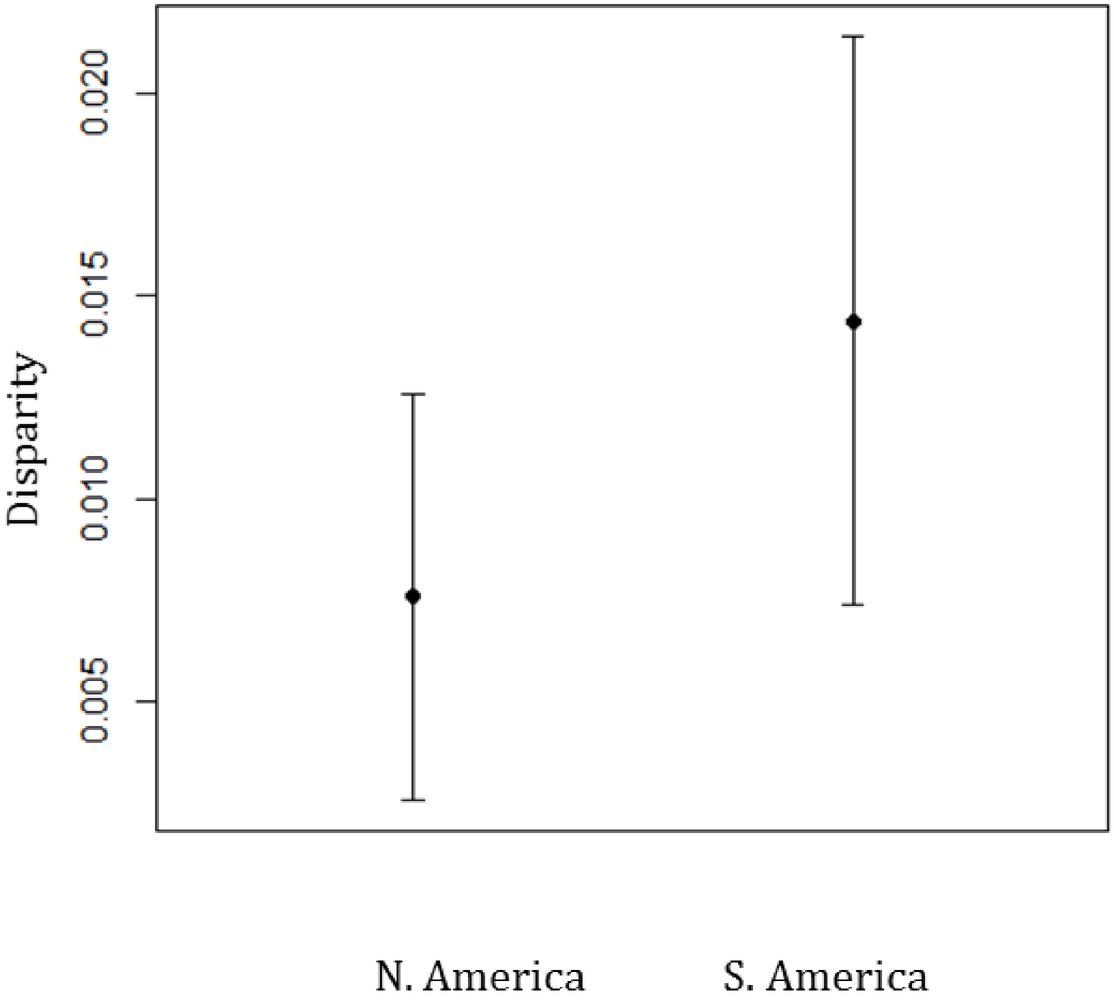

Figure 3.4: Disparity estimate for North American and South American characiform fishes based on 3600 boostrap replicates. Whereas the point estimate for South America is higher, the difference is not statistically robust.

The measured disparity of the North American fauna was 0.00760 (95% confidence interval 0.00168 to 0.0125), whereas the disparity of the South American fauna was 0.0144 (95% confidence interval 0.00703 to 0.0212). Hence, the estimated difference in disparity was 0.00680; however the confidence interval for this estimate includes zero (0.01588670 to 0.00180671) and therefore the difference in disparity is not statistically distinguishable (Fig. 3.4).

Discussion

The results reported here suggest at most a minor winnowing of morphological diversity upon colonization of North America, with no statistically distinguishable decrease in disparity. Thus, if there is an environmental filter determining the ability of a lineage to disperse northward, its effect is too minor to detect. Put another way, the North American characiform fauna appears to be a random sample of the South American fauna. This lack of selectivity is analogous to the "field of bullets" metaphor (Raup 1991) describing extinction dynamics that are not systematically biased toward any particular trait. The propensity of a given lineage to colonize North America is not dependent upon its position in the morphospace, perhaps suggesting that given more time, all lineages should be expected to establish themselves in North America.

If the North American continent had proven to be an exceptional ecological opportunity, we might observe an increase in morphospace occupation upon colonization. Such a pattern is not evident here. In geological terms, these colonization events are comparatively recent, perhaps as recent as several hundred thousand years, and no more than 8 million years. It is possible that not enough time has elapsed for the invading taxa to evolve into novel niches. A time-corrected, phylogenetically explicit analysis could probe this question. Based on the available fossil evidence, we might expect a relatively high degree of morphological conservatism. For example, the La Venta fauna from the Miocene of Colombia, dated between 13.8-12 million years ago, contains multiple taxa referable to extant genera, and some specimens indistinguishable from living species (Lundberg *et al.* 1986, Lundberg 1997). It is also possible that the presence of competitors native to North America limit their expansion, although relatively few species of Nearctic origin occur below the trans-Mexican Volcanic Belt, by which point the characiform fauna is already significantly diminished relative to points south (Berra 2007). An analysis accounting for topography might reveal a measure of habitat variability that is not evident in this broad-scale study. If, for example, there is significantly greater variation in relief in South America than in North America, the morphological diversity present in North America could actually exceed expectations.

Similarly, a phylogenetically explicit analysis might reveal interesting patterns. If the disparity exhibited by the North American characins is greater than would be expected given the residence time, one might infer the existence of incipient radiations that simply haven't had sufficient time to unfold.

Specimens of the genus *Bramocharax* sit near the center of the characin morphospace, albeit in a region not occupied by any South American taxa (Fig. 3.1). Other North American taxa are much closer to their South American relatives in this morphospace. In a sense, *Bramocharax* represents a thoroughly average characin, even though it represents a novel phenotype relative to the source populations. This pattern may be consistent with a radiation in progress, although phylogenetic correction would be required to explicitly interrogate this question (Glor 2010). The rich variation in ecomorphology shown by characiform fishes make them an excellent system for additional study with respect to questions of adaptive radiation and environmental filtering.

Chapter 4: Both elevation and species identity strongly predict body shape in *Astyanax* tetras

Abstract: Landmark-based geometric morphometrics are part of the toolkit that taxonomists use to distinguish among related taxa. Here I test whether three species of tetras (Characidae: *Astyanax*) can be reliably distinguished based on landmark data in the face of significant ecologically-based variation. I show that although there is a strong signal based on species identity, there is also a strong signal from environment, such that without *a priori* species assignments, discrimination could prove difficult.

Introduction

The delimitation of species is one of the most elementary goals of evolutionary and organismal biology. Species are, at least implicitly, the units of analysis for many studies of ecology, evolution, and behavior. Any study of this sort relies on a hypothesis that the species is a real and meaningful entity, and, depending on the aims of the study, another hypothesis about where the boundaries of that entity lie. Many lines of evidence may be available to a taxonomist to justify the twin hypotheses of species ontology and species limits. These may include discrete morphological characters, meristic data, embryological characters, developmental genetics, and molecular sequence data. Quantitative data in the form of landmark coordinates can also be a useful tool in defining taxa. Here I show that landmark data describing body shape can effectively discriminate among species, but that morphological variation corresponding to ecological setting is nearly as strong.

The use of geometric morphometrics to discover clusters in phenotype space and define the boundaries among several species is widespread. Workers have used a geometric morphometric approach in many taxonomic groups, and across a broad range of morphologies. Examples include the wings and heads of true bugs (Matias *et al.* 2001; Gurgel-Gonçalves *et al.* 2011), the genitalia of moths (Mutanen and Pretorius 2007), the teeth of sharks (Whitenack and Gottfried 2010), and the attachment and copulatory structures of platyhelminth flatworms (Soo and Lim 2015). Landmark data have also been marshaled in support of new species of fishes, including minnows (Douglas *et al.* 2001), ponyfishes (Chakrabarty *et al.* 2010), anastomoid characins (Sidlauskas *et al.* 2011), and emperor fishes (Ponton *et al.* 2013).

The New World tetra genus *Astyanax* is broadly distributed across the Americas from eastern Brazil to central Texas. Much of the taxonomy is unsettled, but the genus as currently constituted is probably not monophyletic (Schmitter-Soto 2016). Nevertheless, the species in North America and western South America appear to form a clade with a handful of other New World tetras currently assigned to the genera *Bramocharax* and *Chalceus* (Ornelas-García *et al.* 2008, Sosa unpub. data). Here, I take advantage of collections of three different species of *Astyanax* from different elevations in three widely separated rivers to compare interspecies shape variation to intraspecific variation as a function of elevation.

By quantifying shape in each of the species × elevation combinations, I ask whether the variation in shape due to species differences exceeds the variation in shape due to environmental differences between populations of the same species. If species are invariant across their range, we would be unable to determine which elevation a given specimen comes from based on shape alone (Fig. 4.1, A). If each species responds to elevation in predictable ways, but do not produce similar phenotypes to each other, even in similar environments, then we would be able to determine both the species and the location from which an individual was collected based on body shape (Fig. 4.1, B). If, however, environmental change at a given elevation requires a specific phenotype, then we would expect convergence, where elevation could be reliably determined, but species identity at one or both elevations would be difficult to discern based on body shape alone (Fig. 4.1,C).

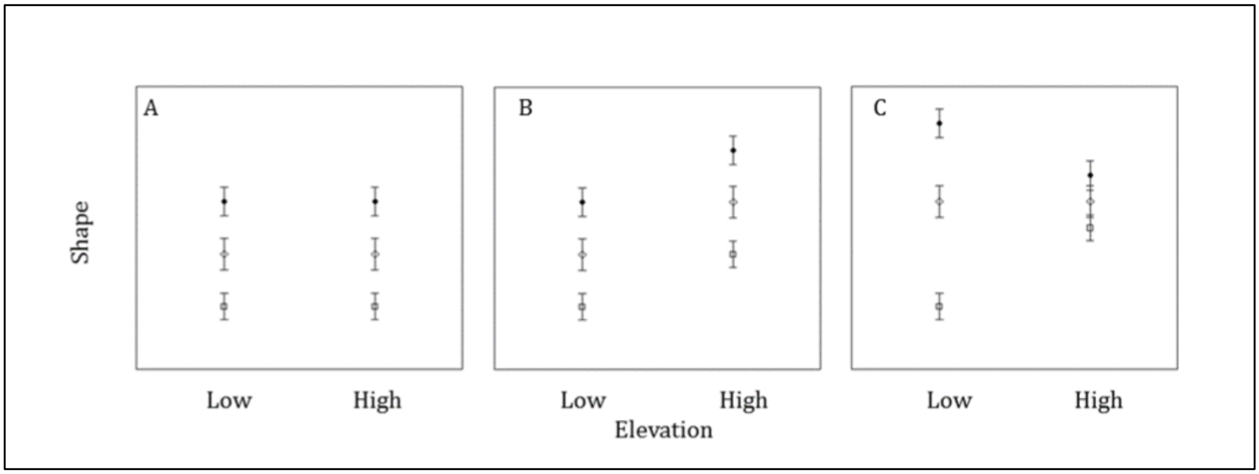

Figure 4.1: Scenarios of shape change as a function of elevation for three theoretical species. In A, each species is invariant, having the same average shape across different elevations. In B, each species responds to elevation in parallel fashion, maintaining interspecies differences but exhibiting clear environmental effects. In C, elevation imposes a sufficient constraint that all species evolve toward a narrow range of phenotypes.

Methods

Morphological data were collected as in Chapter 3 (See Fig. 3.1 for landmark configuration). From the larger morphological data set, I focused on three widely separated rivers where members of the three separate nominal species had been sampled at two different elevations. *Astyanax atratoensis* in the Atrato River in Colombia was sampled at low elevations (25-45 meters above sea level, *n*=24) and high elevations (300-400 m, *n*=16). *A. fasciatus* in the Tuira River in Panama was sampled at low (20-30 m, *n*=34) and moderate elevations (50-100 m, *n*=23). *A. aeneus* in the Coco River along the border between Honduras and Nicaragua was sampled at low (40-50 m, *n*=8) and high elevations (900-1000 m, *n*=15).

After an exploratory principal components analysis, I performed pairwise measurements of the distance between group means of all population subsets, as well as all pooled species means and the means of high- and low-population pooled samples. Each comparison was performed using an F-test (3600 bootstrap replicates) implemented in the TwoGroup module of the IMP package (Sheets *et al.* 2004). I used a canonical variates analysis on a concatenated data set of all individuals to test the ability of shape to predict group membership, based on either elevation or species identity. I performed this analysis in the CVAGen module of the IMP package (Sheets *et al.* 2004). Finally, I quantified disparity of each population to discern whether certain species or elevations were associated with greater variance.

Results

Principal components 1 and 2 are plotted for all species in Figure 4.2. For the Colombian species (*Astyanax atratoensis*), it is possible to observe significant separation in morphospace between the high- and low-elevation populations. This is also true to a lesser extent for the Honduran species (*A. aeneus*). The populations of the Panamanian species (*A.*

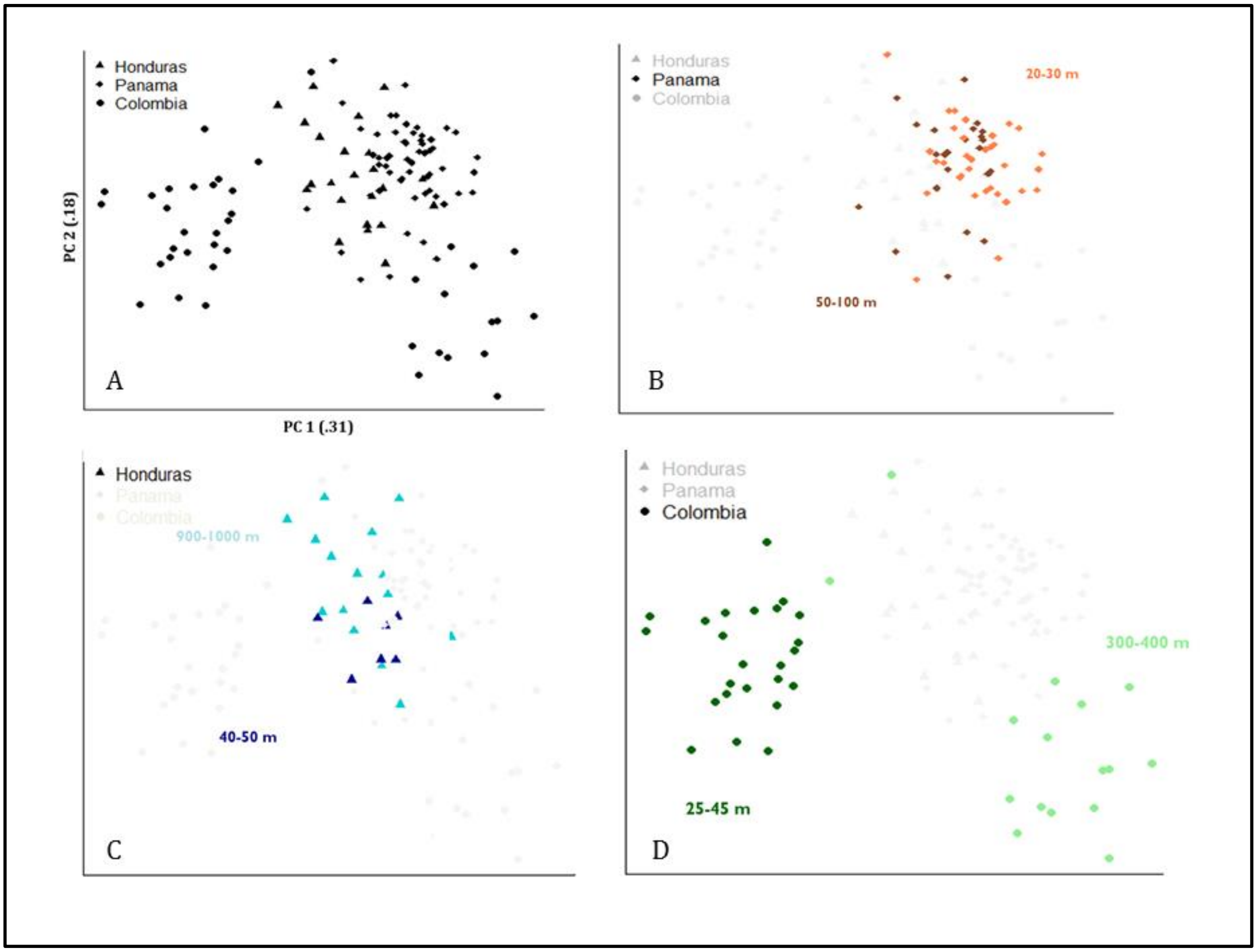

Figure 4.2: Principal components 1 and 2 for landmark coordinates of three species of *Astyanax*. A) pooled data; B) *Astyanax fasciatus*, Panama; C) *Astyanax aeneus*, Honduras; D) *Astyanax atratoensis*, Colombia. Different colors in each pane represent different elevational ranges.

fasciatus) are not readily distinguishable, and are from a relatively narrow elevational range (<80 m); they were thus pooled for subsequent analyses.

F-tests for all pairwise combinations of high- and low- elevation populations between and within species were highly significant (*p*<0.001). Each population is thus distinguishable from every other population (Table 4.1). The largest pairwise difference was between high- and low- elevation populations of the *A. atratoensis*; this difference was greater than any

Group 1	Group 2	distance	F-score
Atrato high	Atrato low	0.0758	29.36
Atrato high	Tuira	0.0455	16.55
Atrato high	Coco high	0.0542	11.42
Atrato high	Coco low	0.0520	7.06
Atrato low	Tuira	0.0634	50.94
Atrato low	Coco high	0.0565	21.08
Atrato low	Coco low	0.0660	20.17
Tuira	Coco high	0.0350	11.06
Tuira	Coco low	0.0454	11.49
Coco high	Coco low	0.0466	9.41
Atrato all	Coco all	0.0379	7.83
Atrato all	Tuira all	0.0431	21.06
Coco all	Tuira all	0.0319	11.95
>300m	<100m	0.0305	7.99

Table 4.1: Pairwise comparisons (F-test) for all populations under study. Every comparison was strongly supported ($p<0.001$). Atrato: *Astyanax atratoensis* (Colombia); Tuira: *A. fasciatus* (Panama); Coco: *A. aeneus* (Honduras).

interspecies difference by this metric. Deformation grids depicting shape change along the first principal component between each group are shown in Figure 4.3.

Canonical variates analysis readily distinguished populations by species (Fig. 4.4). Multivariate analysis of variance found strong support for shape differences corresponding to species assignments (Axis 1 $\chi^2 = 292.4$, $p < 0.001$; Axis 2 $\chi^2 = 130.6$, $p < 0.001$). An assignment test performed in CVAGen found only 6 individuals (5%) that would be assigned to the wrong species based on body shape alone. Deformation grids depicting shape change along the two canonical variates axes are shown in Figure 4.5.

Canonical variates analysis also distinguished populations by elevation (Fig. 4.6). Multivariate analysis of variance found strong support for shape differences corresponding to elevation ($\chi^2 = 152.6$, $p < 0.001$). An assignment test, however, found 54 individuals

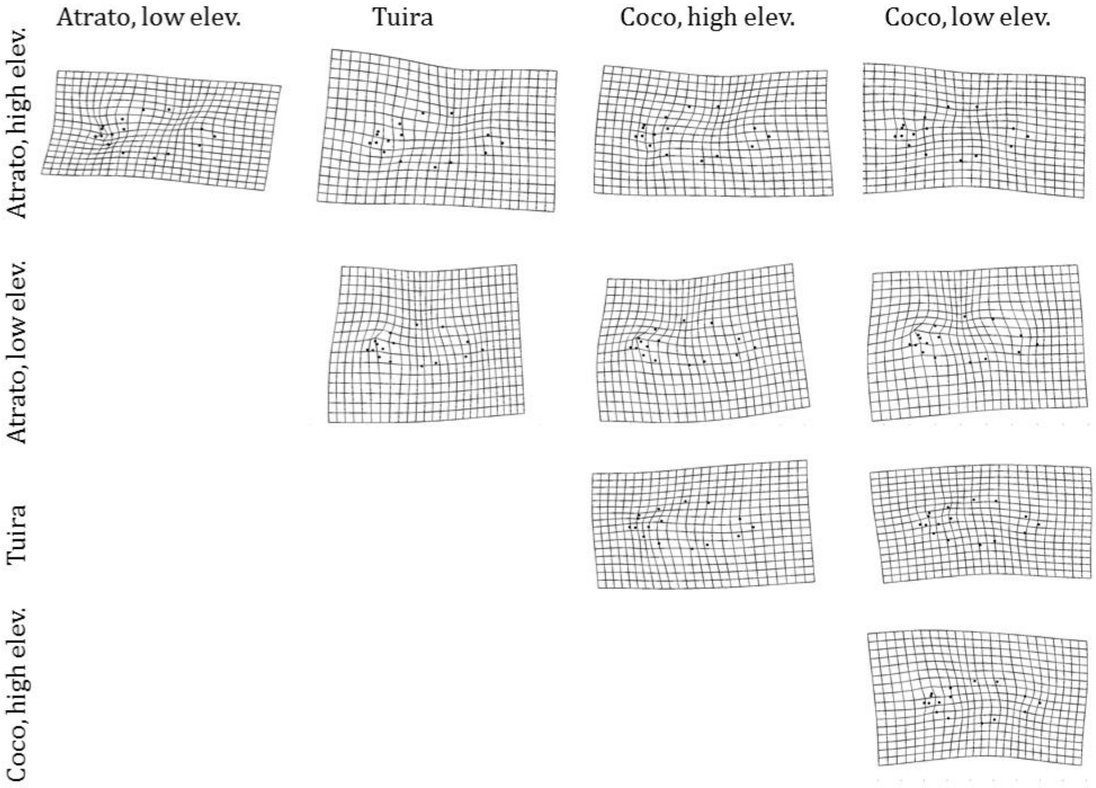

Figure 4.3: Deformation grids (first principal component) comparing each population in this study. Each grid depicts change along the first principal component axis to transform the population listed along the left edge to the population listed along the top.

(45%) that would be misassigned based on body shape alone. A deformation grid depicting shape change is shown in Figure 4.7.

Discussion

These results suggest that it is possible to use geometric morphometrics to distinguish species of *Astyanax*, but that the differences within species as a function of elevation are often similar in magnitude to interspecies differences. The low rate of mistaken assignments based on canonical variates analysis suggests that, given an *a priori*

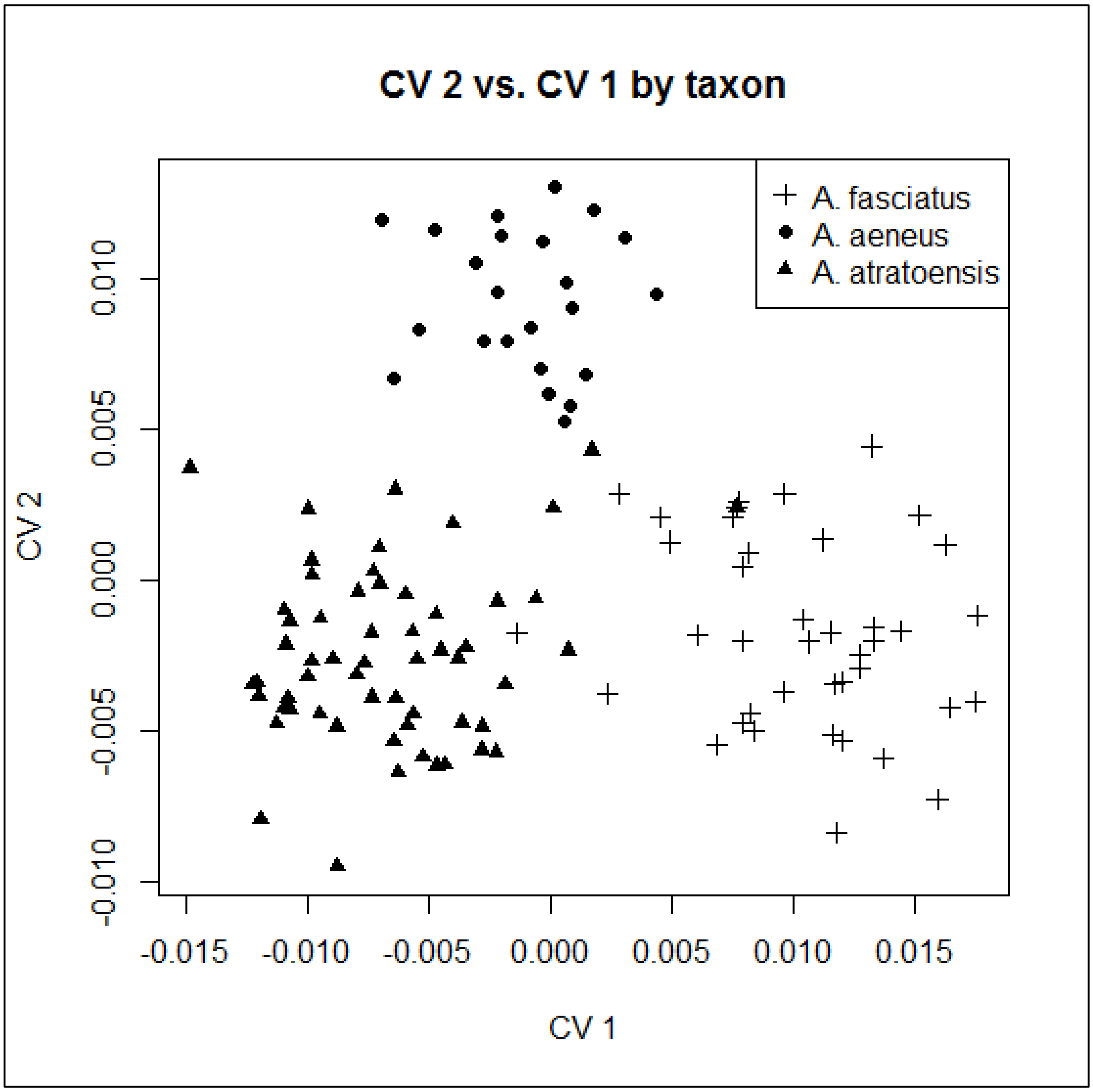

Figure 4.4: Canonical variates plot discriminating three species of *Astyanax*.

hypothesis of identity, body shape is typically a reliable way to verify taxonomy. However, this requires independent data (provenance, meristics, genetics) to generate a taxonomic hypothesis to test.

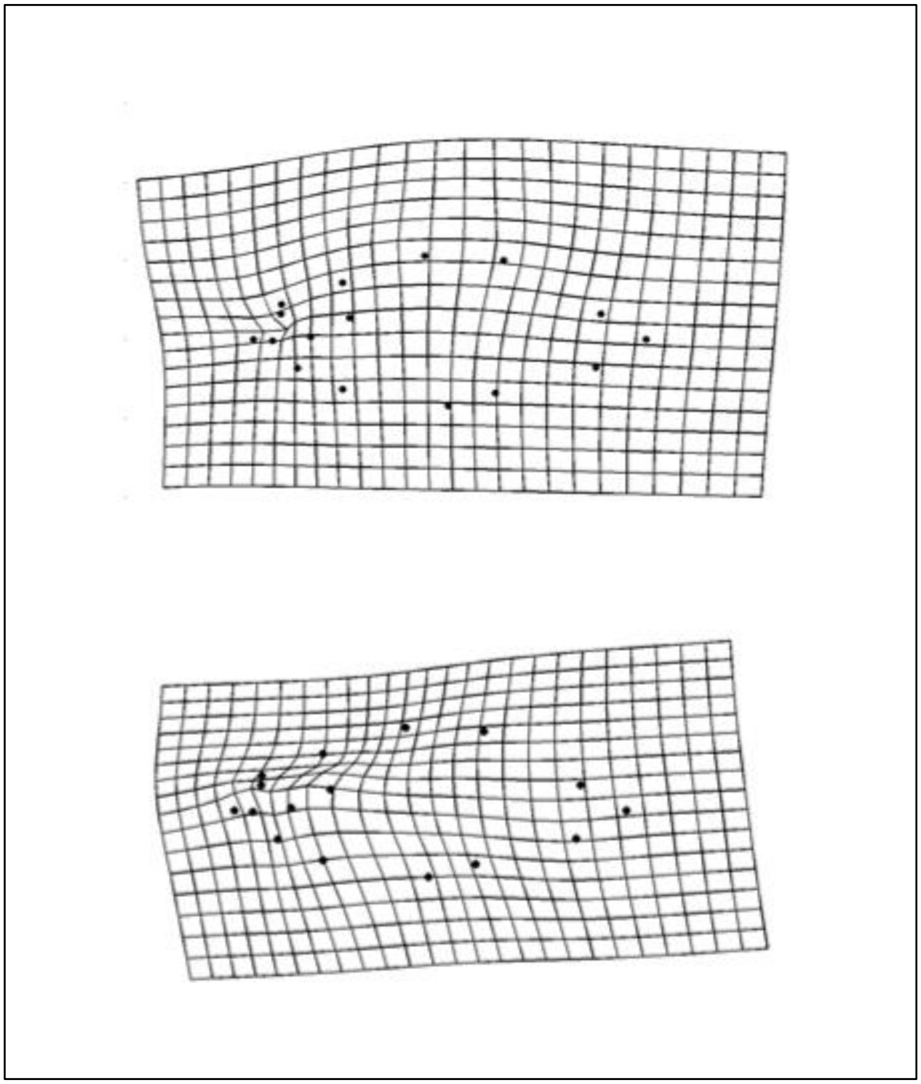

Figure 4.5: Deformation grids depicting variation on canonical variates axes 1 (top) and 2 (bottom), distinguishing the three species of *Astyanax*.

By contrast, elevational differences, while readily detectable and often substantial, do not seem to be as reliable as correlates of body shape when species are pooled. The fact that within-species differences can be quite large suggests that fishes are responding in subtly different ways to changes in elevation.

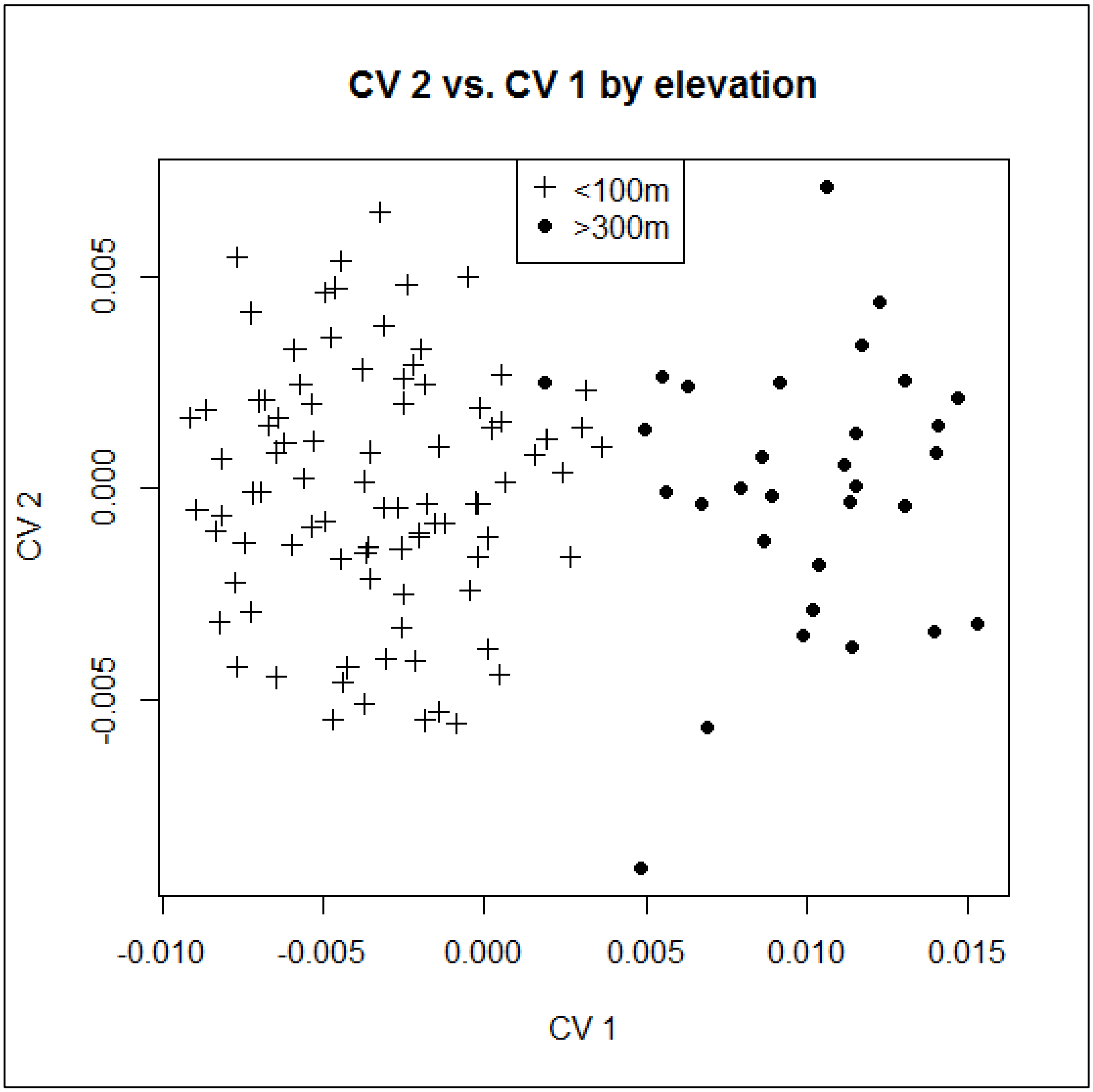

Figure 4.6: Canonical variates plot discriminating by elevation for pooled species data set.

In light of these results, I argue that landmark-based morphometric data should not be used in isolation to generate taxonomic hypotheses. However, when used in conjunction with genetic data, meristic data, or discrete character data, landmark data can be useful, even setting aside its utility in testing any number of non-taxonomic hypotheses. In

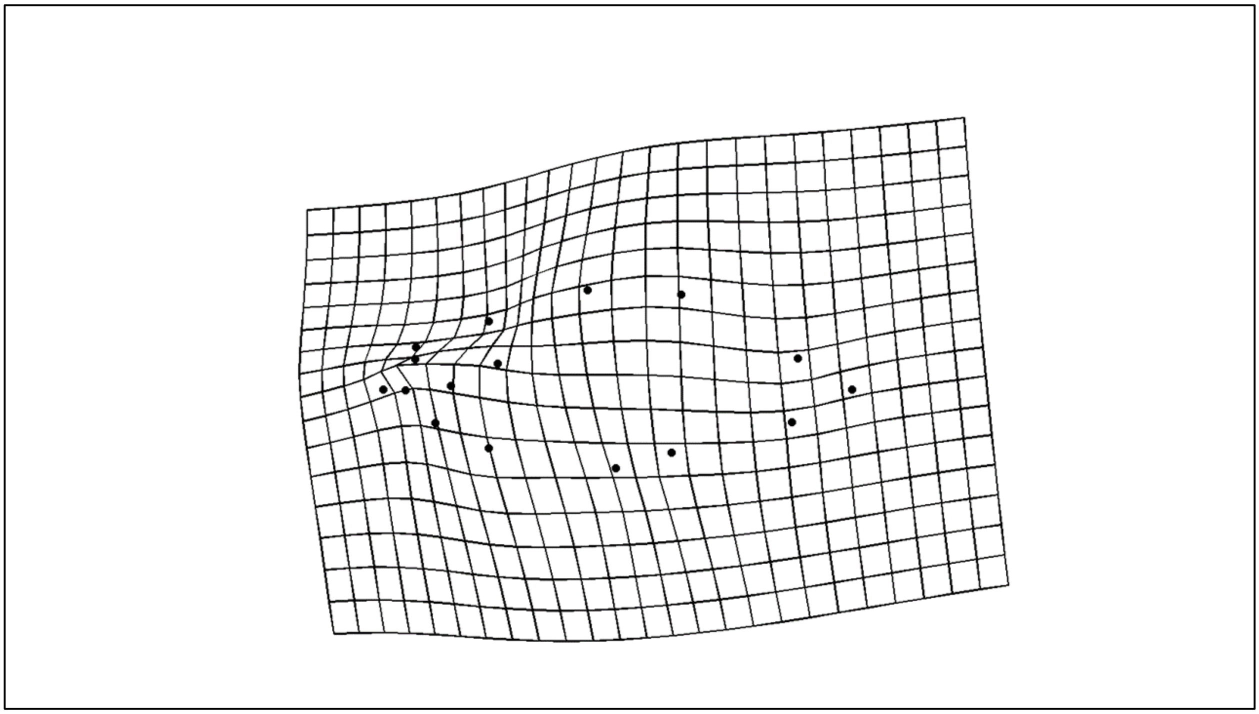

Figure 4.7: Deformation grid (canonical variates axis 1) depicting shape change corresponding to elevation

particular, given a standing hypothesis for the identity of an individual or population, morphometric data can be used as a provisional taxonomic test, though it cannot distinguish between apomorphic or plesiomorphic conditions.

Whereas it is clear that elevation has a strong effect on body shape, the mechanism is not obvious. The main axis of variation is aspect ratio, with deeper bodied fish generally found at higher elevations, and more fusiform fish found at lower elevations. It may be a stream order effect, in which particular body shapes are favored at particular elevations because of the hydrological features of the river. In particular, if higher elevation streams have a dense canopy, high allochthonous organic input, and abundant emergent vegetation, a deeper body might permit greater maneuverability (Ellerby & Gerry 2011, Claverie & Wainwright

2013). As a corollary, lower elevation streams with large channels of open water—the Rio Coco is at some points more than a kilometer across—may favor a fusiform body shape that permits sustained high swimming speeds or station holding in strong currents. Exposure to aerial predation may also have an effect. If fish in the lower reaches of a river have less emergent vegetation to use as cover, swimming speed could be much more important in those environments (Allouche & Gaudin 2001).

Importantly, the three rivers evaluated here are unlikely to permit regular gene flow among populations, due to their separation by either space or topography. Still, it is not obvious what underlies the elevational variation in *Astyanax* in a proximate sense. Body shape may be a labile trait while nevertheless being fairly strictly determined genetically; or it may be almost entirely plastic (Fischer-Rousseau *et al.* 2009; Ornelas-García *et al.* 2014). The most direct way to test for plasticity would be to raise individuals in tanks under a variety of flow conditions and assess shape variation upon maturity. Plasticity has been assessed in this way in some sport fishes (Pakkasmaa and Piironen 2000; Imre 2002). *Astyanax* tetras are amenable to lab-rearing, so this system could prove fruitful in the exploration of phenotypic plasticity. It is clear that fish environment influences body shape in diverse and complex ways, and additional investigation can help to elucidate the process by which it does so.

Chapter 5: Diet may mediate potential range expansions of Neotropical fishes under climate change

Abstract Climate disruptions caused by human activity already show evidence of affecting phenology, distribution, and behavior of a wide variety of organisms. With refinements to predictive models of near- and medium-term climate, it has become possible to project species' distribution shifts based on habitat parameters of current ranges. Using a niche modeling approach, we predict the range shifts of three lineages of freshwater characid fishes of tropical origin. All three are projected to expand their distribution; however, diet specialization is associated with a more limited future range prediction. Environmental change creates significant potential for disruption of existing temperate fish faunas.

Introduction

Anthropogenic climate change has already elicited ecological responses from a wide range of organisms (Scheffers *et al.* 2016), including range restrictions of cold-adapted species and concomitant expansions of ranges of warm-adapted species (Smol *et al.* 2005, van Herk *et al.* 2002), earlier flowering times among temperate plants (Menzel & Dose 2005), earlier spawning of frogs (Walther *et al.* 2002), and local extinctions (Parmesan 2006, Urban 2015, Wiens 2016). Management of land and waterways will depend on our ability to adapt to and, if possible, predict the responses of biotas to continuing change.

The New World tetras (Ostariophysi: Characidae) are a family of primary freshwater fishes with origins in what is now South America (Calcagnotto *et al.* 2005, Novacek & Marshall 1976). The rise of the Isthmus of Panama allowed characids to expand their range into

North America over the last several million years (2.8-8 my, Bacon *et al.* 2015, Marko *et al.* 2015, Montes *et al.* 2012, O'Dea *et al.* 2016) Today, perhaps 30 species of characids now occur between western Panama to Texas, with characid diversity declining with increasing latitude (Berra 2007). Given that their colonization of North America is relatively recent, we ask whether the current northern distribution limits of characid lineages represents the limits of their ecological tolerances, or are simply the leading edges of ongoing expansions.

Focal taxa

We focused our efforts on three lineages of characid fishes with broad distributions in Central America and southern North America. *Brycon* is a genus of large, superficially trout-like fishes whose northern limit is the Usumacinta basin in southern Mexico (Miller *et al.* 2005). *Roeboides* is a group of small, mainly carnivorous tetras whose distribution likewise reaches as far north as the Usumacinta. *Astyanax* is among the most successful freshwater colonists of North America, reaching as far north as Texas, and includes forms such as the blind cave tetra of Mexico. With up to 19 species, *Astyanax* is the most diverse tetra lineage in North and Central America, evidently constituting a monophyletic group if Central American endemic species formerly assigned to *Bramocharax* are included (Ornelas-García *et al.* 2008, Schmitter-Soto 2017, Sosa unpublished data). *Bramocharax* spp. represent a small and highly localized fraction of *Astyanax* records, and frequently co-occur with congeners. As a result, their records do not appreciably affect spatial analyses that evaluate *Astyanax* in a more inclusive sense.

Methods

Locality data

We aggregated locality data from museum collections and new collections by TS and collaborators. We used FishNet2 (fishnet2.net, accessed 2016) to access collections of the Field Museum, Louisiana State University Museum of Natural Science, and Tulane University Museum of Natural History; and Web site of the University of Southern Mississippi Museum Ichthyology Collection (Schaefer 2004-2016). Raw data were curated and validated to exclude records with missing or erroneous locality data. For *Astyanax*, we aggregated 1244 occurrence records; for *Roeboides,* 390; and for *Brycon*, 181. We used the resulting locality data to construct genus-level distribution models.

Baseline models

Each genus distribution model was built in Maxent (Phillips *et al.* 2011); most default settings were used except: Random test percentage was set to 20; replicated run type was set to subsample; maximum number of iterations was set to 5000; random seed was turned on; sample radius set to -7; and threshold rule was set to 10th percentile training presence rule. Each model was replicated 15 times to verify robustness of results. The environmental layers for each variable, along with metadata files for each genus, were loaded into Maxent to create models for current distributions. Map extent was the entire contiguous landmass of North America as well as the extreme northwest of South America. We specifically excluded the Caribbean since islands such as Cuba and Hispaniola have what appear to be favorable climatic conditions but there is no plausible mechanism by which characid lineages could colonize them.

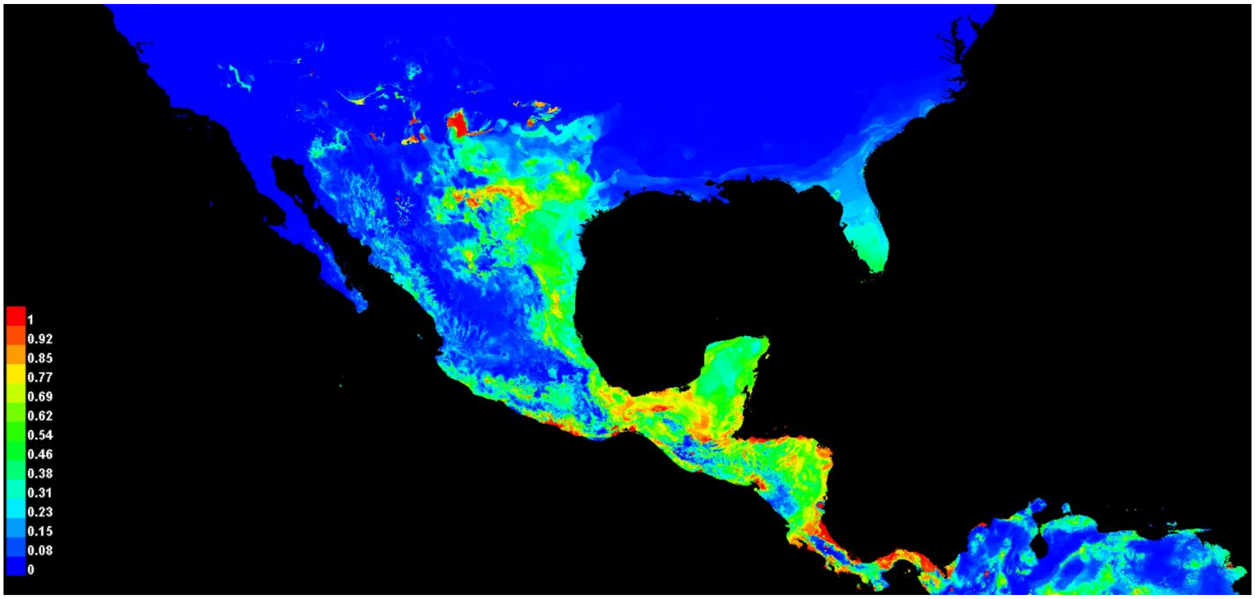

Figure 5.1: Current distribution model for *Astyanax*. The factors that most strongly affect the model are mean temperature of the coldest quarter, minimum temperature of the coldest month, and precipitation during the coldest month. *Astyanax* does not occur in Florida or western Mexico, although these regions appear hospitable.

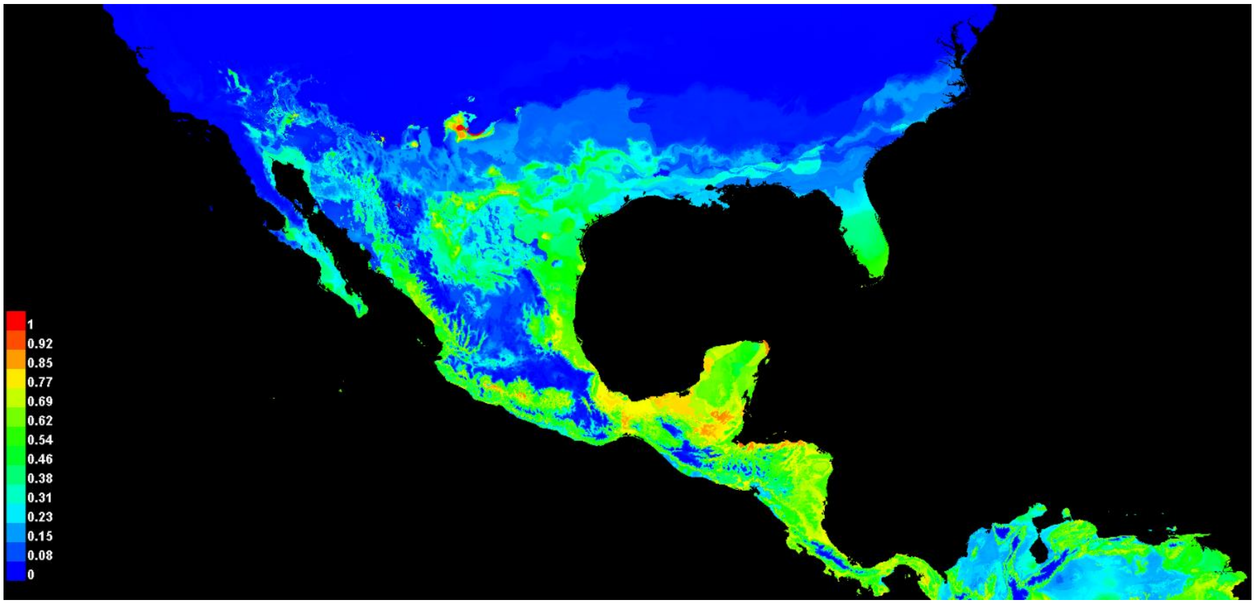

Figure 5.2: Projected 2050 distribution for *Astyanax*. Expansion includes the south-central and southeastern US and northwestern Mexico to the edge of the Sonora Desert.

Species distribution models were developed using the 18 bioclimatic variables included in WorldClim version 1.4 (Hijmans *et al*. 2014), with the addition of elevation data, to allow for the possibility that the lower partial pressure of oxygen at high elevations might affect species distributions independent of altitude-dependent temperature variation. The 19 variable files were loaded into QGIS and the rastersets were clipped and converted from GeoTIFF to ASCII files. Variables were chosen using a jackknifing approach; we retained variables which showed significant improvement to training gain, test gain, and area under the curve (AUC) in models which included them. Each taxon was allowed to fit its own set of explanatory variables.

Projection models

Using models generated from current distributions, we used projected values of WorldClim variables for the year 2050 under a business-as-usual representative concentration pathway as specified by the IPCC (IPCC 2013). These projections were used to generate estimates of the distribution of suitable habitat for each genus, assuming that each exhibits constant tolerances to the best-supported climate variables. As with the baseline models, each taxon was projected using its own set of variables.

Results

Astyanax

The best-supported model for the distribution of *Astyanax* was determined by mean

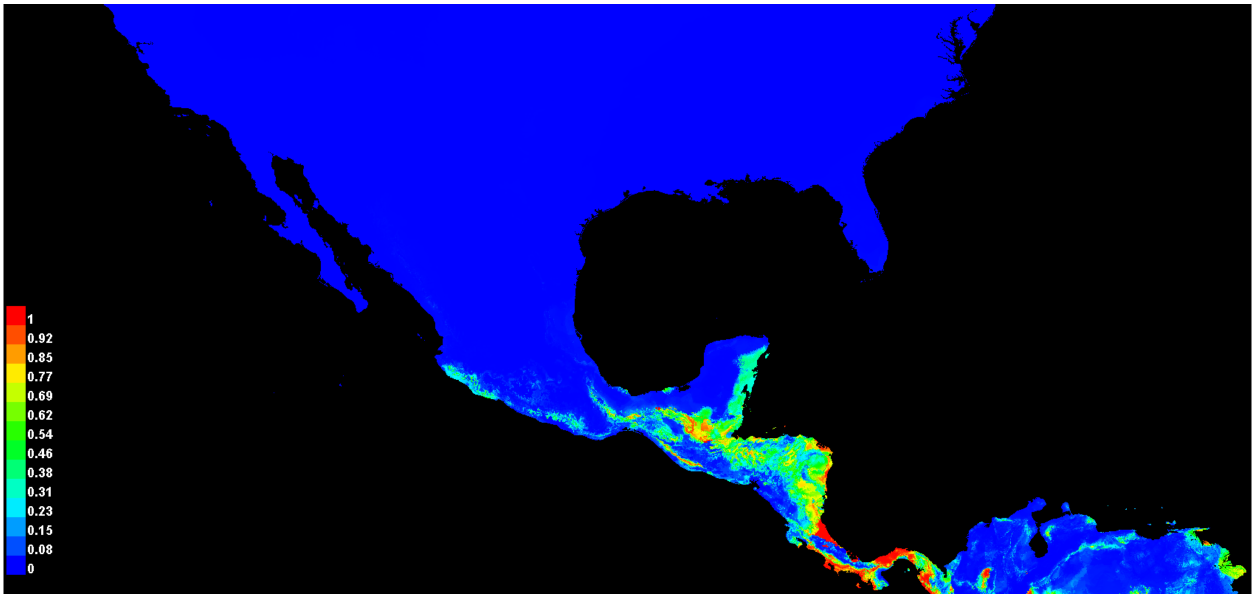

Figure 5.3: Current distribution model for *Brycon*. The factors that most strongly affect the model are precipitation during the driest month and precipitation seasonality.

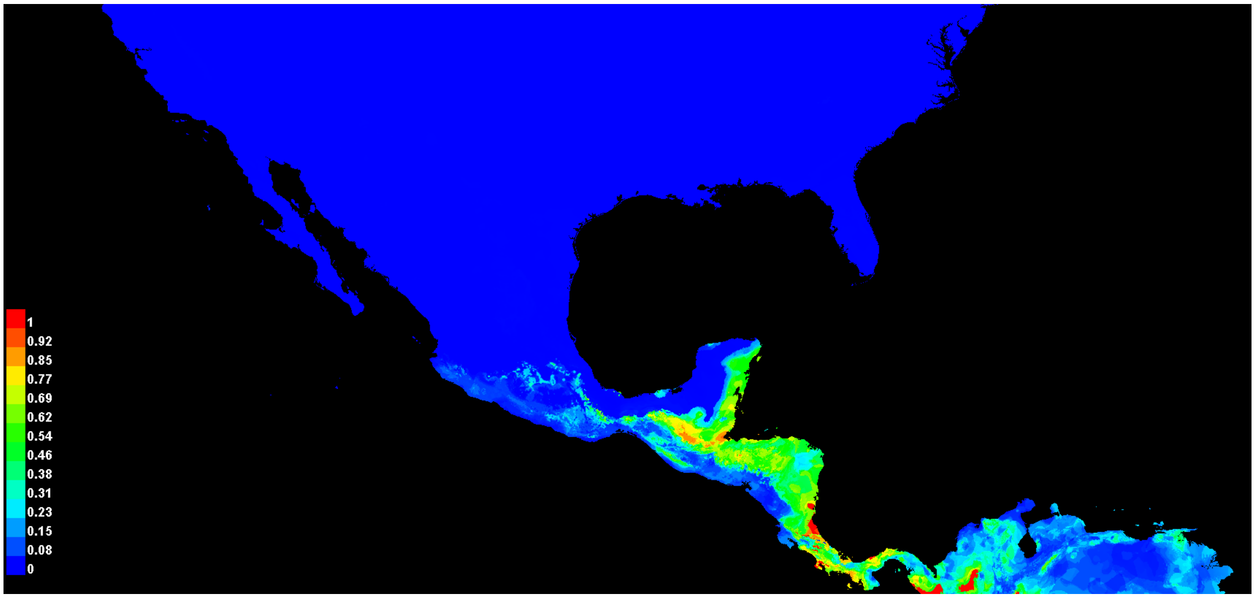

Figure 5.4: Projected 2050 distribution for *Brycon.* Expansion includes Pacific drainages in southern and central Mexico.

temperature of the coldest quarter, minimum temperature of the coldest month, and precipitation during the coldest month. All replicates returned the same rank order contributions; we present the percent contributions of the median model. This model found that some regions where there are no records of *Astyanax* are nevertheless favorable to their colonization: western Mexico, across the Sierra Madre from basins currently occupied; and southern Florida, which is separated from the current northeastern distribution limit by more than 1,500 kilometers (Fig. 5.1). The 2050 projection predicts significant expansion in most directions (Fig. 5.2). It finds a high probability of occurrence along most Pacific drainages of Mexico, extending into southernmost reaches of the Colorado River; a moderate probability of reaching as far as Mobile basin in Alabama; and a lower probability band connecting the current range to both the Carolina coastal plain and already favorable regions in Florida.

Variable	Percent contribution
Mean temp of coldest quarter	27.4
Min. temp of coldest month	24.9
Precipitation in wettest month	9.9

Table 5.1: Contributions of the three most important variables explaining current distribution of *Astyanax*.

Brycon

The best-supported model for the distribution of *Brycon* was determined by precipitation during the driest month and precipitation seasonality. All replicates returned the same rank order contributions; we present the percent contributions of the median model. The

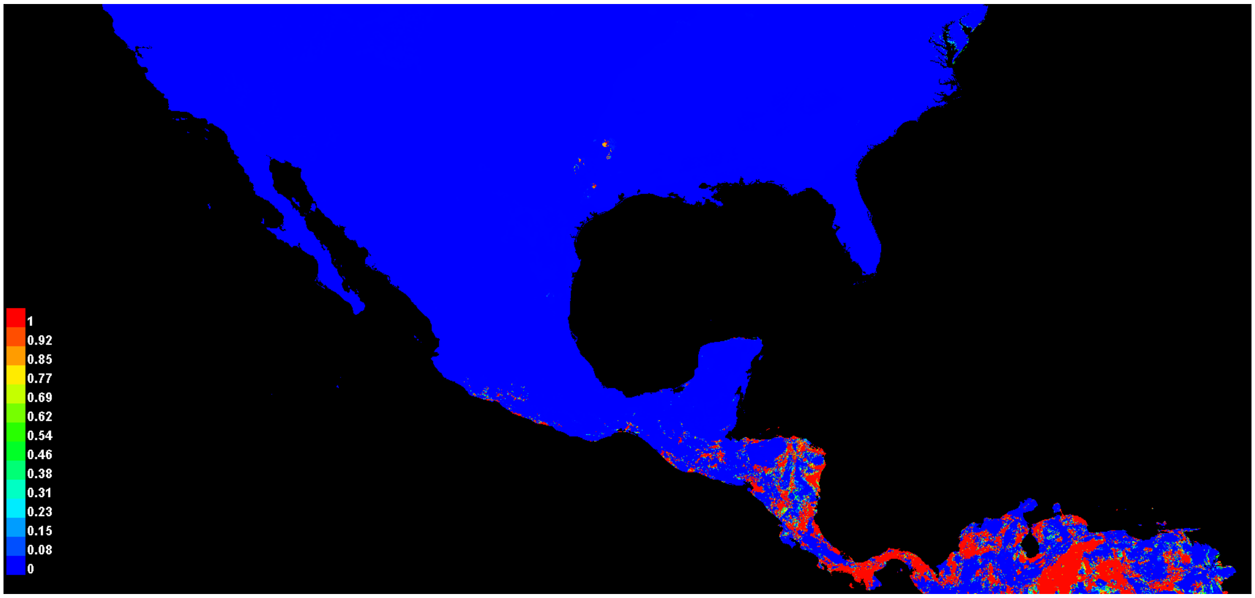

Figure 5.5: Current distribution model for *Roeboides*. The factors that most strongly affect the model are temperature seasonality, minimum temperature of the coldest month, and annual temperature range.

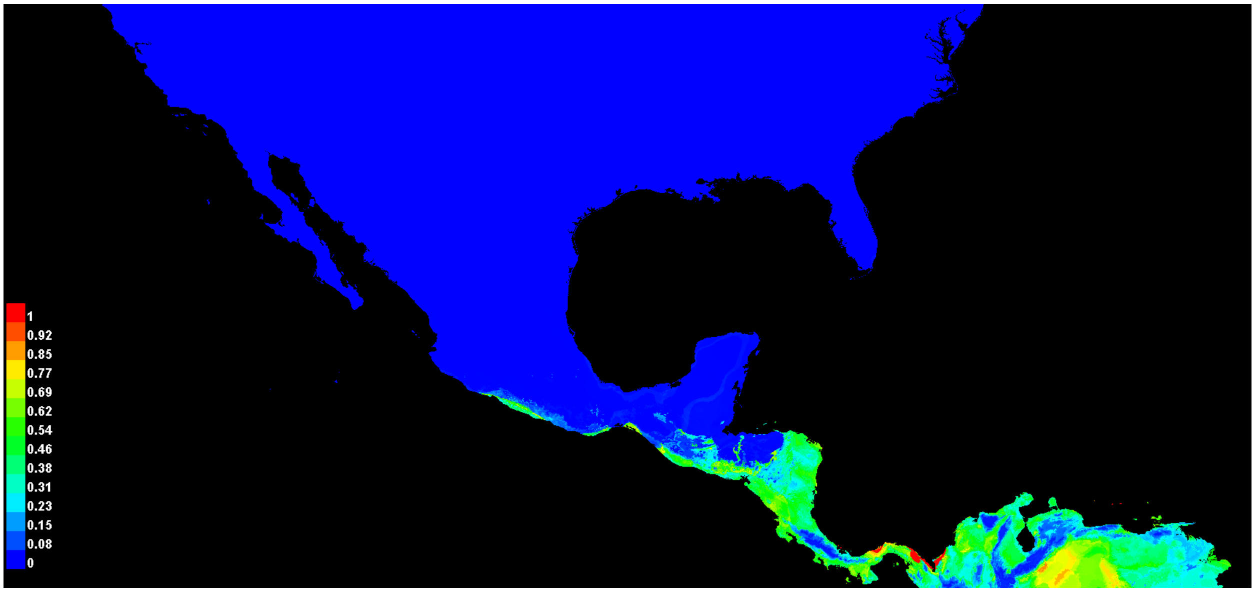

Figure 5.6: Projected 2050 distribution of *Roeboides*. Expansion is largely into Pacific drainages in southern Mexico.

distribution model closely matches the actual distribution, consisting of drainages on the Caribbean slope as far north as Quintana Roo, Mexico, in addition to the Usumacinta drainage in Chiapas and Oaxaca (Fig. 5.3). The 2050 projection predicts largely similar spatial extent of favorable conditions, but with perhaps higher average probabilities of occurrence (5.4). By comparison with *Astyanax*, the expansion predicted for *Brycon* is modest.

Variable	Percent contribution
Precipitation of driest month	44.0
Precipitation seasonality	38.2
Precipitation of coldest quarter	5.3

Table 5.2: Contributions of the three most important variables explaining current distribution of *Brycon*.

Roeboides

The best-supported model for the distribution of *Roeboides* was determined by temperature seasonality (*i.e.,* variability in monthly mean temperatures), minimum temperature of the coldest month, and annual temperature range. All replicates returned the same rank order contributions; we present the percent contributions of the median model. The current distribution model closely matched its actual distribution in southern Mexico (Fig. 5.5). The projection for 2050 predicts an expansion into upland reaches of Atlantic drainages, and expansion into new Pacific drainages. The reduction in warm colors on the map reflects lower confidence in the presence of fishes, an artifact of the model's caution rather than an explicit prediction of decline or range contraction.

Variable	Percent contribution
Temp seasonality	18.8
Min. temp, coldest month	10.0
Annual temp range	8.5

Table 5.3: Contributions of the three most important variables explaining current distribution of *Roeboides*.

Discussion

The three taxa in this study show markedly different projected responses to climate change. *Astyanax*, already the northernmost characid lineage, expands aggressively to the north, west, and east, reaching rivers draining into both the Sea of Cortez and the Atlantic Ocean in Florida. These models do not account for biotic interactions, and the possibility that *Astyanax* will be limited by competition with native fishes cannot therefore be excluded. Briggs (2005) suggested that characins and minnows (Cyprinidae) are "ecologically incompatible," a hypothesis based on the circumstantial observation that they do not often co-occur. This century will represent an explicit test of this hypothesis as climatic conditions for *Astyanax* improve across North America, which should in principle allow their expansion into areas where minnows are common now. These spatial models project a probability of occurrence; however, they are best thought of as a probability of sufficiently benign conditions, without assuming instantaneous occupation. Nevertheless, the apparently angler-mediated establishment of *Astyanax* in several new rivers in central

Texas (notably the Brazos, Colorado, and Sabine drainages) during the last few decades suggests that they will be able to occupy newly suitable habitat with relative ease (Edwards 2001, Hubbs *et al.* 2008). Moreover, climate models suggest that Gulf Coast flooding will become more frequent, which will intermittently connect river drainages to each other and permit dispersal between them (Hallegatte *et al.* 2013, Raabe & Stumpf 2016). At the same time, marine waters will intrude onto coastal communities. *Astyanax* is not known to tolerate salty or brackish water; however, the projected invasion path is sufficiently inland that marine intrusions are unlikely to interfere. The combination of an ample corridor and frequent flooding in lowland drainages creates ideal dispersal opportunities for an organism tolerant of turbidity, pollution, and low-oxygen conditions (Scarabotti *et al.* 2011).

The projected expansion of both *Brycon* and *Roeboides* is more modest. This is consistent with the narrower tolerances predicted by the distribution model. The parameters most closely associated with *Astyanax* occurrence probability are temperatures and rainfall in the most extreme quarter of the year. By contrast, in *Brycon*, the most extreme week is most predictive. The niche model for *Brycon* in particular is striking for its strong dependence on variation in precipitation. In *Roeboides*, overall seasonality is negatively associated with probability of occurrence. These models suggest that *Astyanax* tolerates the greatest range of climatic conditions.

Possible mediation of expansion by diet

Both *Brycon* and *Roeboides* are considerably more specialist in their diets than *Astyanax*. *Brycon guatemalensis*, the northernmost species, is insectivorous when young but almost exclusively herbivorous as an adult (Bussing 1987). In Mexico *B. guatemalensis* can reach a length of 50 cm, and may therefore require an environment with abundant plant matter, especially fruit, year-round. This is consistent with the niche model presented here, which predicts occurrence of *Brycon* only in areas with consistent high temperatures and abundant precipitation. *Roeboides* is a committed carnivore whose gut contents consist of insects and fish scales (Hildebrand 1925). Like *Brycon*, they are predicted to only occur in areas with low variance in both temperature and rainfall, but these predictions may be more closely related to the year-round availability of insect prey than temperature tolerance *per se*. The diet of *Astyanax*, by contrast, is more varied; one study of a Mexican population found gut contents consisting of 62% plant matter (including algae) and 22% animal matter, with detritus making up the remainder (Darnell 1962). Its broad tolerance to environmental conditions and its varied diet may explain why it is the most successful of all characid lineages to colonize North America, and suggests that it can continue to survive as the continent warms. The pattern described here, with *Astyanax* having both a larger current distribution and greater prospects for future expansion, is consistent with findings that generalists are better invaders under conditions of disturbance (Marvier *et al.* 2004).

It is not clear what impact the expansion of the northernmost species, *Astyanax mexicanus* (*A. argentatus sensu* Schmitter-Soto 2017), in particular might have on native freshwater communities. Hubbs *et al*. (2008) noted a decline in native fish diversity and abundance across Texas over the last decades of the 20th century and the beginning of the 21st. The decline was accompanied by an increase in nonnative fishes as well as an expansion of the range of *A. mexicanus*; however, the extent to which native declines are attributable to nonnative increases is unknown, and could instead be due to pollution, habitat fragmentation, or other factors. The projected invasion path of *A. mexicanus* crosses the lower reaches of the Mobile basin, which contains hundreds of endemic freshwater species of fishes, arthropods, and bivalves, among others (Burr & Mayden 1993, Near & Keck 2006, Gangloff *et al.* 2006, Helms *et al.* 2015). While fishes of Neotropical origin move north and east, no doubt native fishes will do so too, provided that there is sufficient habitat connectivity. The introduction of *Astyanax* to new rivers in Texas in the recent past is instructive, and part of a larger trend toward homogenization of fish faunas in temperate North America (Rahel 2000). It is unlikely that all endemics will survive in the face of warming, habitat disruptions, range expansions of a Neotropical vanguard, and human-mediated introductions of exotic species. Some will no doubt be able to persist by shifting to more northerly or higher elevation streams, but only where roads, dams, and other human-associated barriers do not hinder their movement.

Chapter 6: Discussion

The impacts of the Great American Interchange on the fish fauna of North America have been profound but spatially limited. The southern reaches of North America, from southern Mexico to western Panama, are dominated by fishes of South American extraction, including characins, southern catfish lineages, electric knifefishes, and cichlids. Consistent with the findings of Chapter 4, the distribution of these Neotropical emigrants suggests that climatic factors are a stronger determinant of their northern limits than biotic interactions are. The published Late Neogene and Quaternary fossil record for teleosts in Central America is almost entirely marine, and hence silent on the occupancy of freshwater ecosystems before the Interchange (Bussing 1985, Laurito *et al.* 2014). No doubt such ecosystems were occupied by a Nearctic fauna, but important northern lineages such as cyprinids (minnows), catostomids (suckers), ictalurids (bullhead catfishes and madtoms), salmonids (trout), and centrarchids (sunfishes) are not found south of Mexico or Guatemala, except where introduced for sport fishing (Berra 2007; Loppnow *et al.* 2013). This is in striking contrast to Interchange dynamics among mammals. The Miocene La Venta fauna from which I derived several of my fossil calibrations shows an ichthyofauna broadly similar to that found in the region today (Lundberg 1997). The mammal fauna, however, was dominated by xenarthrans, astrapotheres, hystricognath rodents, New World monkeys, and marsupials; with no evidence of now-abundant lineages such as carnivorans, myomorph rodents, or artiodactyls (Carrillo *et al.* 2015).

If the dates estimated in Chapter 1 are accurate, then this has been an extraordinarily rapid invasion; however, these dates may be to recent by hundreds of thousands or millions of years. It is nevertheless likely that the current northern limits of Neotropical lineages represent the vanguard of an invasion in progress, and that climate disruptions will prove advantageous to these warm-water fishes. There are multiple mechanisms unrelated to direct interspecific interactions that may affect the future distributions of freshwater fishes. One is direct temperature requirements, which may affect various life stages, especially in species with temperature-dependent sex determination. Another is increased flooding, which both serves to intermittently connect drainage basins, as well as temporarily deplete rivers of oxygen (Valett *et al.* 2005). Warmer waters are less able to accommodate dissolved oxygen than cold waters, and temperate fishes often have relatively high oxygen requirements (Elshout *et al.* 2013). Thus, rivers in subtropical North America are likely to become less hospitable to the native fish fauna even as they become more hospitable to Neotropical fishes.

Morphology and ecology

The similarity of extant characiform fishes to the Miocene La Venta Fauna, especially when contrasted with the very different mammal fauna, suggests a high degree of morphological conservatism. With the caveat that morphology is an imperfect proxy for ecology (Alfaro *et al.* 2005; Wainwright *et al.* 2005), the similarity of fossil to extant forms suggests that the ecological roles of characins have been roughly static over the last ten million years. This is also consistent with the finding that morphospace occupation in the northern Neotropics, and, now, the southern subtropical region of North America, are home to characins that are in large part a subsample of the South American fauna. The possible exception of *Bramocharax* may suggest that, given time, the potential for generation of new forms is possible. Also consistent with this is the evolution of blind cave fishes from surface tetras, although this is not detected by the geometric morphometric analysis used here (Espinasa & Borowsky 2001; Bradic *et al.* 2013). The significant variation elicited by ecological setting, in particular by elevation, suggests the mutually compatible possibilities that *Astyanax* in particular is either highly evolvable or highly plastic in terms of body profile. This flexibility, and its dietary flexibility, may underlie its success, as the most northerly characiform fish in the world, and may predict its future success as anthropogenic climate forcing heats the streams of North America.

Human dimensions

Whereas the presence of characiform fishes represents an ancient and natural invasion, it informs our understanding of human-mediated invasions. Human are, in a sense, a highly selective dispersal corridor, facilitating the range expansion of species with high fecundity, broad environmental tolerances, or obvious economic utility. The establishment of Mexican Tetra (*Astyanax mexicanus*) populations in new Texas rivers just since the mid-20th century due to angler releases is evidence of the rapidity of colonization that we permit. At the same time, by fragmenting the landscape with highways, dams, and other infrastructure, we inhibit the free movement of organisms with which we are less likely to interact directly (Warren & Pardew 1998; Bouska & Paukert 2010) . In order to respond to climate change, native fish faunas will either have to rapidly adapt, or move northward or upslope to more favorable conditions. Enabling their movement means enabling the movement of invasive species already present in the ecosystem; nevertheless, connectivity between currently separated parts of river drainages with high endemicity, such as the Mobile River, will likely offer the best chance for persistence of native faunas over the next century. Whereas specific management recommendations are beyond the scope of this thesis, the niche modeling results in particular suggest that conditions favorable to the expansion of invasive species will prove deleterious to native species, and that therefore taking no action is likely to consign many endemics to extinction. Some extinction is foregone; we must decide whether the remaining species are worth trying to conserve.

Future directions

The Characiformes *sensu lato* have proven a useful study system to generate and evaluate hypotheses about the dynamics of interchanges and invasions. There is considerable potential for further interrogation of this system. One of the most promising of these is the potential of plasticity to underlie variation. Captive rearing experiments could estimate the variation due to plasticity or inexorable genetics, enhancing understanding of the evolutionary dynamics of highly variable freshwater fishes. In terms of timing and history of the freshwater fish faunas of Central America, the nearly nonexistent fossil record is a major and crippling gap in our knowledge. Efforts to find paleontological or anthropological sites could prove one of the most useful ways to understand the history of the region, although there is no guarantee that adequate remains have even been preserved. As for the standing diversity, the recent discovery of a new genus and family of characin in Brazil demonstrates that there is significant basic taxonomic work remaining in

Neotropical freshwater fishes (de Pinna *et al.* 2017). Finally, this thesis explored only a fraction of the potential analyses possible with existing data. The morphometric data set is rich and broad, as is the genetic data set, and further study of these data will refine and clarify the tentative conclusions drawn herein.

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Appendix 1: List of specimens newly sequenced for this study.

Institution	Tissue #	Taxon	Family	Voucher
PUJ	111	*Brycon*	Bryconidae	111
PUJ	120	*Brycon*	Bryconidae	120
USM	TH07-519	*Brycon guatemalensis*	Bryconidae	34147
USM	WAM06-307	*Brycon guatemalensis*	Bryconidae	31832
USM	WAM06-308	*Brycon guatemalensis*	Bryconidae	31832
USM	WAM06-309	*Brycon guatemalensis*	Bryconidae	31832
USM	WAM06-344	*Brycon guatemalensis*	Bryconidae	31861
DePaul	Ast. sp. 205	*Astyanax festae*	Characidae	NA
DePaul	Ast. sp. 206	*Astyanax festae*	Characidae	NA
LSU	1295	*Astyanax aeneus*	Characidae	1295
LSU	1375	*Astyanax aeneus*	Characidae	1375
LSU	1504	*Astyanax aeneus*	Characidae	1504
LSU	1505	*Astyanax aeneus*	Characidae	1505
LSU	1616	*Astyanax aeneus*	Characidae	1616
LSU	1647	*Astyanax aeneus*	Characidae	1647
LSU	1663	*Astyanax aeneus*	Characidae	1663
LSU	1715	*Astyanax aeneus*	Characidae	1715
LSU	1857	*Astyanax aeneus*	Characidae	1857
LSU	1897	*Astyanax aeneus*	Characidae	1897
LSU	2056	*Astyanax aeneus*	Characidae	2056
LSU	2057	*Astyanax aeneus*	Characidae	2057
LSU	2148	*Astyanax aeneus*	Characidae	2148
LSU	2185	*Astyanax aeneus*	Characidae	2185
LSU	2195	*Astyanax aeneus*	Characidae	2195
LSU	2267	*Astyanax aeneus*	Characidae	2267
LSU	2389	*Astyanax aeneus*	Characidae	2389
LSU	2610	*Astyanax aeneus*	Characidae	2610
LSU	2611	*Astyanax aeneus*	Characidae	2611
LSU	2686	*Astyanax aeneus*	Characidae	2686
LSU	2687	*Astyanax aeneus*	Characidae	2687
LSU	2705	*Astyanax aeneus*	Characidae	2705
LSU	2706	*Astyanax aeneus*	Characidae	2706
LSU	2770	*Astyanax aeneus*	Characidae	2770
LSU	2771	*Astyanax aeneus*	Characidae	2771
LSU	3072	*Astyanax aeneus*	Characidae	3072

LSU	3091	*Astyanax aeneus*	Characidae	3091
LSU	3092	*Astyanax aeneus*	Characidae	3092
LSU	3101	*Astyanax aeneus*	Characidae	3101
LSU	3102	*Astyanax aeneus*	Characidae	3102
LSU	3166	*Astyanax aeneus*	Characidae	3166
LSU	3167	*Astyanax aeneus*	Characidae	3167
LSU	3202	*Astyanax aeneus*	Characidae	3202
LSU	3203	*Astyanax aeneus*	Characidae	3203
LSU	3277	*Astyanax aeneus*	Characidae	3277
LSU	3278	*Astyanax aeneus*	Characidae	3278
LSU	3432	*Astyanax aeneus*	Characidae	3432
LSU	3610	*Astyanax aeneus*	Characidae	3610
LSU	3611	*Astyanax aeneus*	Characidae	3611
PUJ	102	*Bryconamericus*	Characidae	102
PUJ	114	*Bryconamericus*	Characidae	114
PUJ	119	*Bryconamericus*	Characidae	119
PUJ	123	*Bryconamericus*	Characidae	123
PUJ	135	*Macropsobrycon*	Characidae	135
PUJ	140	*Moenkhausia*	Characidae	140
PUJ	148	*Bryconamericus*	Characidae	148
PUJ	467	*Bryconamericus*	Characidae	467
PUJ	474	*Bryconamericus*	Characidae	474
PUJ	653	*Bryconamericus*	Characidae	653
PUJ	680	*Creagrutus cf. magdalanae*	Characidae	680
PUJ	778	*Roeboides*	Characidae	778
PUJ	1656	*Bryconamericus*	Characidae	1656
PUJ	1662	*Hyphessobrycon*	Characidae	1662
PUJ	1663	*Creagrutus*	Characidae	1663
PUJ	1664	*Creagrutus*	Characidae	1664
PUJ	1665	*Creagrutus*	Characidae	1665
PUJ	1667	*Saccoderma*	Characidae	1667
PUJ	1672	*Parastrema*	Characidae	1672
PUJ	1672	*Parastrema*	Characidae	1672
PUJ	1674	*Gephyrocharax*	Characidae	1674
PUJ	1794	*Hemibrycon*	Characidae	1794
PUJ	1794	*Hemibrycon*	Characidae	1794
PUJ	1799	*Carlastyanax*	Characidae	1799
TAMU	15720.01	*Astyanax*	Characidae	15720
TAMU	15722.06	*Astyanax*	Characidae	15722
TAMU	15770.02	*Astyanax*	Characidae	15770

TOL	12	*Astyanax*	Characidae	12
TOL	18	*Astyanax*	Characidae	18
TOL	48	*Gephyrocharax*	Characidae	48
TOL	53	*Gephyrocharax*	Characidae	53
TOL	54	*Gephyrocharax*	Characidae	54
TOL	82	*Hyphessobrycon*	Characidae	82
TOL	85	Cheirodontinae	Characidae	85
TOL	88	*Hyphessobrycon*	Characidae	88
TOL	91	*Hyphessobrycon*	Characidae	91
TOL	93	Cheirodontinae	Characidae	93
TOL	94	Cheirodontinae	Characidae	94
TOL	96	Cheirodontinae	Characidae	96
TOL	100	Cheirodontinae	Characidae	100
TOL	142	*Roeboides*	Characidae	142
TOL	143	*Roeboides*	Characidae	143
TOL	147	*Phenagoniates*	Characidae	147
TOL	148	*Phenagoniates*	Characidae	148
TOL	149	*Roeboides*	Characidae	149
TOL	151	*Phenagoniates*	Characidae	151
TOL	153	*Phenagoniates*	Characidae	153
TOL	155	*Phenagoniates*	Characidae	155
TOL	188	*Astyanax*	Characidae	188
TOL	196	*Hyphessobrycon*	Characidae	196
TOL	200	*Astyanax*	Characidae	200
TOL	251	*Argopleura*	Characidae	251
USM	08-1616	*Astyanax aeneus*	Characidae	NA
USM	08-1617	*Astyanax aeneus*	Characidae	NA
USM	TH07-276	*Astyanax aeneus*	Characidae	34032
USM	TH07-277	*Astyanax aeneus*	Characidae	34032
USM	TH07-278	*Astyanax aeneus*	Characidae	34032
USM	TH07-347	*Astyanax aeneus*	Characidae	34095
USM	TH07-348	*Astyanax aeneus*	Characidae	34095
USM	TH07-349	*Astyanax aeneus*	Characidae	34095
USM	TH07-378	*Astyanax aeneus*	Characidae	34111
USM	TH07-445	*Astyanax aeneus*	Characidae	34131
USM	TH07-446	*Astyanax aeneus*	Characidae	34131
USM	TH07-49	*Astyanax aeneus*	Characidae	NA
USM	WAM06-05	*Roeboides*	Characidae	31648
USM	WAM06-270	*Astyanax aeneus*	Characidae	31809

USM	WAM06-271	*Astyanax aeneus*	Characidae	31809
USM	WAM06-272	*Astyanax aeneus*	Characidae	31809
USM	WAM06-274	*Astyanax aeneus*	Characidae	31809
USM	WAM06-311	*Astyanax aeneus*	Characidae	31837
TOL	169	*Ctenolucius*	Ctenoluciidae	169
PUJ	588	*Cynopotamus*	Cynodontidae	588
PUJ	579	*Hoplias malabaricus*	Erythrinidae	579
PUJ	1383	*Hoplias malabaricus*	Erythrinidae	1383
TOL	17	*Hoplias*	Erythrinidae	17
TOL	28	*Hoplias*	Erythrinidae	28
TOL	34	*Hoplias*	Erythrinidae	34
TOL	222	*Gasteropelecus*	Gasteropelecidae	222
TOL	223	*Gasteropelecus*	Gasteropelecidae	223
TOL	224	*Gasteropelecus*	Gasteropelecidae	224
PUJ	136	*Copella*	Lebiasinidae	136