Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Phylogenetics and Evolution 31 (2004) 754–764
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Temporal diversification of Mesoamerican cichlid fishes across a major biogeographic boundary

C. Darrin Hulsey,[a,*] Francisco J. García de León,[b] Yara Sánchez Johnson,[b] Dean A. Hendrickson,[c] and Thomas J. Near[a,1]

[a] *Center for Population Biology, Department of Evolution and Ecology, University of California-Davis, Davis, CA 95616, USA*
[b] *Laboratorio de Biología Integrativa, Instituto Tecnológico de Cuidad Victoria (ITCV), Mexico*
[c] *Section of Integrative Biology, University of Texas-Austin, Austin, TX 78712, USA*

Received 18 June 2003; revised 26 August 2003

Abstract

The Mexican Neovolcanic Plateau sharply divides the vertebrate fauna of Mesoamerica where the climate of both the neotropics and temperate North America gradually blend. Only a few vertebrate groups such as the Heroine cichlids, distributed from South America to the Rio Grande in North America, are found both north and south of the Neovolcanic Plateau. To better understand the geography and temporal diversification of cichlids at this geologic boundary, we used mitochondrial DNA sequences of the cytochrome *b* (cyt *b*) gene to reconstruct the relationships of 52 of the approximately 80 species of Heroine cichlids in Mesoamerica. Our analysis suggests several cichlids in South America should be considered as part of the Mesoamerican Heroine clade because they and the cichlids north of the Isthmus of Panama are clearly supported as monophyletic with respect to all other Neotropical cichlids. We also recovered a group containing species in *Paratheraps* + *Paraneetroplus* + *Vieja* as the sister clade to *Herichthys*. *Herichthys* is the only cichlid clade north of the Mexican Plateau and it is monophyletic. Non-parametric rate smoothing of cichlid cyt *b* sequence resulted in an estimated divergence time of approximately 6 million years for *Herichthys*. This temporal diversification is concordant with divergence times estimated for anurans in the genus *Bufo*, a group that exhibits a similar geographic distribution. Our results indicate the 5-million-year-old extension of the Mexican Neovolcanic Plateau to the Gulf Coast of Mexico has strongly influenced the current transition between the vertebrate faunas of the Neotropics and Nearctic.

1. Introduction

Along the eastern Gulf Coast of Mexico, where the climate and flora of the Neotropics and Nearctic gradually blend, the vertebrate fauna undergoes an abrupt transition. This region is a documented breaking point between major groups of tropical and temperate mammals (Pérez-Higaredera and Navarro, 1980), amphibians (Mulcahy and Mendelson, 2000), and reptiles (Pérez-Higaredera and Navarro, 1980). However, fish best exemplify the abruptness of this biotic break (Rosen, 1978). Fish communities change radically in composition at a site known as the Punta del Morro (PDM) (Obregon-Barboza et al., 1994), where the eastern-most extension of the Neovolcanic Plateau meets the Gulf of Mexico. At this point, the fauna changes from about 75% temperate species in the north to faunas in which 90% of fish species in the south have neotropical affinities (Miller, 1986). Only a few vertebrate groups occur on both sides of the Neovolcanic Plateau, and cichlids are perhaps the most species rich and ecologically diverse assemblage bridging this region. Understanding the geographic and temporal diversification of cichlids both north and south of the PDM could elucidate whether the abruptness of the temperate to tropical transition in this region's vertebrate fauna is primarily due to climate or to geology.

There are at least two alternative hypotheses that could explain the extensive shift in the fauna across the PDM. One hypothesis is that the faunistic replacement that occurs near the PDM could be due to a gradual

* Corresponding author. Fax: 1-530-752-1449.
E-mail address: cdhulsey@ucdavis.edu (C. Darrin Hulsey).
[1] Present address: Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996, USA.

1055-7903/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2003.08.024

change from a predominantly tropical to a more temperate climate in this region. If climatic patterns were the most important factor historically determining the distribution of vertebrate groups at the PDM, the divergence across this boundary might be recent for some groups (Fig. 1B) and relatively ancient for others (Fig. 1D). The current distribution of most taxa could be due to various clades slowly dispersing into the area (Figs. 1A and B) and simply reaching a limit along some abiotic gradient. When the timing of clade diversification is compared under this climatically driven scenario, there should be little concordance in divergence times for monophyletic groups of different clades distributed on both sides of the PDM.

Alternatively, the faunistic break at the PDM may be due to the formation of the Mexican Neovolcanic Plateau that reached the Gulf of Mexico coast approximately 5 mybp (million years before present) during the Miocene (Byerly, 1991). For many lineages of vertebrates, the formation of the Neovolcanic Plateau at the PDM could have provided a vicariant event, an extrinsic boundary responsible for the isolation of lineages previously distributed continuously (Rosen, 1978). This would have prevented groups that arrived after the event from transversing the PDM, and effectively split lineages distributed across the barrier prior to its formation (Figs. 1C and D). Importantly, the PDM does serve as the limit to the distribution of several Mesoamerican fish genera including *Dionda*, *Rivulus*, *Rhamdia*, *Belonesox*, *Ophisternon*, and *Poeciliopsis* (Obregon-Barboza et al., 1994). If a major geologic event did also isolate several lineages once continuously distributed on both sides of the PDM, divergence in these groups would likely be temporally congruent and should be at least 5 million years old.

Congruence in the phylogenetic branching pattern of clades found on either side of the PDM was central to the development of vicariance biogeography (Lydeard et al., 1995; Rosen, 1978). One of the greatest insights of the field of vicariance biogeography was the suggestion that topological congruence in the phylogeny of groups indicated similar evolutionary forces might have structured the distribution of entire clades (Page, 1988). However, these early studies largely depended on phylogenetic trees built using morphological data (Page, 1990; Rosen, 1978). Therefore, little emphasis was placed on evaluating whether clades with similar branching patterns exhibited temporal congruence (Page, 1990). Yet, by claiming a single vicariant mechanism is responsible for the distribution of groups ranging from toads to cichlid fishes, one is making an implicit, although untested, argument of temporal congruence in clade divergence.

With the greater availability of molecular sequence, estimation of temporal divergence has become increasingly tractable (Baldwin and Sanderson, 1998). Yet, despite its tractability, the only well-established assessment of a clade's divergence across the PDM is for toads

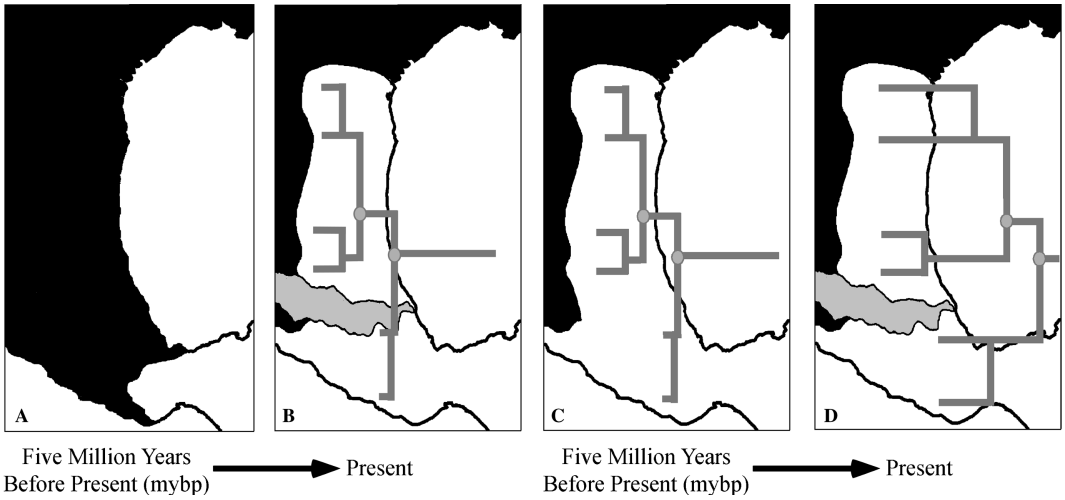

Fig. 1. Scenarios for the temporal diversification of Mesoamerican cichlids across the Punta Del Morro (PDM). In all four panels, cichlid presence in an area is indicated by white and putative sequence divergence within cichlids is represented by gray chronograms. The two panels (A) and (C) are hypothetical geographic distributions that could have existed prior to the extension of the Mexican Neovolcanic Plateau to the Gulf Coast of Mexico five million years before the present (5 mybp). The current range of both cichlids and the Mexican Neovolcanic Plateau, indicated by the gray shading, are shown in panels (B) and (D). If cichlid presence north of the PDM is due to recent dispersal around this boundary, then we would expect sequence divergence on either side of the PDM to be shallow (B) and might infer the northern limit to the distribution of cichlids 5 mybp to have been much further south (A). Alternatively, if cichlid divergence around the PDM is extensive and can be estimated at greater or equal to around 5 mybp (D) then we might infer the ancestral distribution and phylogenetic divergence of cichlids to have resembled what is depicted in (C). The second scenario (C and D) supports the hypothesis that the extension of the Mexican Neovolcanic Plateau to the Gulf Coast of Mexico provided a major vicariant event structuring both cichlid evolution and perhaps that of other vertebrates in the region.

in the genus *Bufo* (Mulcahy and Mendelson, 2000), calculated at 5.4 ± 0.4 mybp. This 5 mybp timetable for isolation caused by the formation of the Neovolcanic Plateau may seem too ancient to have influenced the distribution of a largely freshwater group such as cichlids that originated in South America. However, the best temporal estimate for the initiation of cichlid diversification in Mesoamerica is between 15~18 mybp (Martin and Bermingham, 1998). This places cichlids north of the Isthmus of Panama much earlier than many other Mesoamerican fish lineages that originated in South America (Bermingham et al., 1997; Perdices et al., 2002). Although cichlids north of the PDM were not included in Martin and Bermingham's (1998) diversification estimate, Mesoamerican cichlids clearly could have been distributed north of the PDM prior to the formation of the Neovolcanic Plateau. Determining whether cichlid lineages north of the PDM could have been split by the formation of the Mexican Neovolcanic Plateau is easily testable as all cichlids north of the PDM are classified into the single genus *Herichthys* (Kullander, 1998).

Generating a well-resolved phylogeny is necessary in order to use molecular divergence to estimate Mesoamerican cichlid divergence across the PDM and to assess both the monophyly and molecular diversity of the genus *Herichthys*. With a phylogeny, one can conservatively estimate divergence times (Magallon and Sanderson, 2001) across the PDM for both the most recent common ancestor of the cichlid clade(s) in Northeastern Mexico (crown group age) and the divergence of this group from its sister clade (stem group age). Nevertheless, the framework for estimating divergence time in cichlids at the PDM has only recently become feasible, because extensive hypotheses have now been proposed for relationships of major groups in the Cichlidae as a whole (Farias et al., 2001; Stiassny, 1991). Additionally, several investigators have initiated phylogenetic studies of cichlids in Mesoamerica using DNA sequence data (Farias et al., 1998; Farias et al., 2001; Martin and Bermingham, 1998; Roe et al., 1997). Previous work supports the hypothesis that aside from one species each in the distantly related genera *Aequidens* and *Geophagus* in Panama, all other cichlids in Mesoamerica may form a monophyletic group (Conkel, 1993). This putatively monophyletic clade of approximately 80 species of cichlids is nested within a larger group known as the Heroines (Kullander, 1998). An analysis of a large number of Mesoamerican Heroines would greatly contribute to our understanding of cichlid relationships and test the hypothesis of Mesoamerican Heroine monophyly. Furthermore, a well-sampled phylogeny would potentially identify the sister groups to cichlids in Northeastern Mexico as well as provide a test of the monophyly and temporal diversification of cichlids across the PDM.

Using a combination of all Mesoamerican cichlid species previously sequenced for cytochrome *b* (cyt *b*) and data for 19 species previously unsampled, we examine the support for monophyly of the Mesoamerican cichlids. By sampling cichlids from numerous aquatic systems on either side of the PDM, we also test whether the species in Northeastern Mexico form a monophyletic clade and determine which clade(s) are best supported as the sister group to *Herichthys*. Then, we assess the amount of molecular divergence in both the crown group and stem group of *Herichthys* in Northeastern Mexico using non-parametric rate smoothing (NPRS) of branch lengths (Sanderson, 1997). Finally, using estimates of the rate of cyt *b* divergence in fishes, we test whether the timing of cichlid diversification north of the PDM better supports a hypothesis of recent dispersal around the PDM or more ancient vicariant divergence.

2. Materials and methods

2.1. Specimen collection and sampling

A combination of new cyt *b* sequences (GenBank Accession Nos: AY323980–AY324031) and previously published sequences (Lydeard and Roe, 1997: U88856–U88863; Roe et al., 1997: U88853–U88857 and U97158–U97165; Roe and Lydeard, unpublished: AF141316–AF141319; Martin and Bermingham, 1998: AF009924–AF009938, AF009945–AF009952, and AF009993; Kumazawa et al., 1999: AB018985, AB018987, and AB018970; Farias et al., 2001: AF370644, AF370655, AF370657, AF370666–AF370668, AF370670, AF370671, and AF370673–AF370679; Rican et al., unpublished: AY050610, AY050613, AY050615, AY050616, AY050619–AY050621, and AY050623–AY050626) were used in this study. We focused our sampling along the Atlantic coast of Mexico from as far south as the Rio Tonala basin to as far north as the Rio Grande on the border of Texas and Mexico (Fig. 2 and Table 1).

We collected fish using a Smith & Root electrofisher, cast nets, and seines. Once collected, fish were placed on ice. Within 4 h after collection, axial muscle was removed from the left side of all fish and this tissue was preserved in 100% ethanol. Identification numbers were made on each collection tube and the corresponding number used to label the whole fish. After collection of tissues for genetic analysis, fish were preserved in buffered formalin and subsequently transferred to 70% ethanol for long-term preservation. All specimens were identified in the field and identifications were then confirmed in the laboratory. The species we sequenced, collection localities, and museum catalog numbers are listed in Table 1. We collected, exported, and imported samples under appropriate permits on a series of collecting expeditions in Mexico. All voucher specimens were deposited at the Texas Natural History Collection at the Texas Memorial Museum of

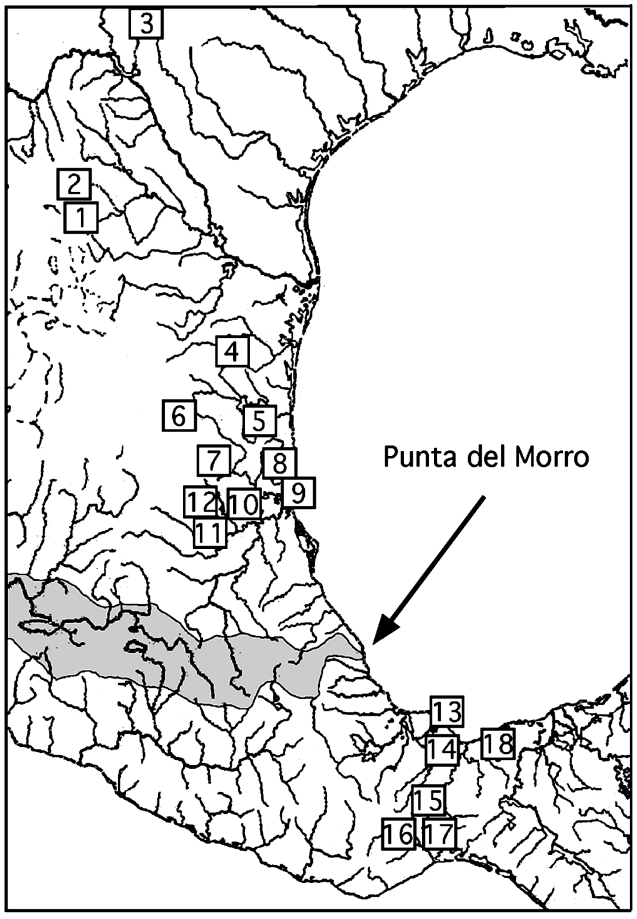

Fig. 2. Collection localities: 1. Cuatro Ciénegas, Coahuila; 2. Ocampo, Coahuila; 3. Devils River, Texas; 4. Rio San Fernando, Tamaulipas; 5. Soto la Marina, Tamaulipas; 6. Rio Tamesi, Tamaulipas; 7. Rio Guayalejo, Tamaulipas; 8. Rio Tigre, Tamaulipas; 9. Laguna Champayan, Tamaulipas; 10. Rio Gallinas, San Luis Potosi; 11. Rio Tamasopo, San Luis Potosi; 12. Media Luna, San Luis Potosi; 13. Lago Catemaco, Veracruz; 14. Rio Dos Caños, Veracruz; 15. Rio Sarabia, Veracruz or Rio Almoloya, Oaxaca; 16. Rio Tehauntepec, Oaxaca; 17. Rio de los Perros, Oaxaca; and 18. Rio Tonala, Veracruz. The Mexican Neovolcanic Plateau is depicted in gray. The Punta del Morro (PDM) along the eastern Coast of Mexico is indicated with an arrow.

the University of Texas at Austin. Four of the species sequenced had previously been sequenced, but either the entire cyt *b* gene had not been sequenced and/or there was no locality data available for the individuals sequenced (C. Lydeard, personal comment). We analyzed from one to six individuals for the 19 species we sequenced and included individuals from separate localities when possible (Table 1).

2.2. DNA isolation and sequencing

For sequencing, total genomic DNA was isolated at the Instituto Tecnológico de Cuidad Victoria using the Dneasy extraction kit or was isolated using Puregene 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 cyt *b* gene was PCR amplified using primers in Martin and Bermingham (1998) and the flanking primer (5′-AACCA CCGTTGTTATTCAACTA-3′). Amplifications were carried out in a Perkin–Elmer DNA thermocycler. The PCR volume was 50 μl [32 μl of H_2O, 5 μl of 10× $MgCl_2$ PCR buffer, 2.5 μl $MgCl_2$, 4 μl dNTPs (10 mM), 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 s), 55 °C (30 s), 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 5 min and destained in de-ionized 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 terminator cycle sequencing reactions). The PCR pirmers mentioned above and internal primers (5′-TAGTTTAGTTTAG AATTCTGGCTTTGG-3′, 5′-GAAACYGGNTCNAC MAACCC-3′, 5′-ATTGTTATGGCTGCRATGAT-3′, and 5′-AGGAAGTGGAANGCAAAGAATC-3′) designed for this study were used to sequence both strands of the cyt *b* gene. Sequences were read with an ABI377 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 3.1 (Gene Codes, Ann Arbor, MI).

2.3. Phylogenetic analysis

In our analysis, we included a total of 52 recognized species of the 83 named Heroine species from continental Mesoamerica (Kullander, 1998). All available sequences for species on GenBank were used. Sequences were aligned using Clustal X (Thompson et al., 1999). In both analyses, we included 20 species of cichlids from South America. Seventeen of these species represent a diversity of groups generally recognized as falling in the Heroines or Cichlasomines, the sister groups to the Mesoamerican Heroines (Farias et al., 1998; Farias et al., 2001; Kullander, 1998). Additionally, we used the South American cichlid *Cichla temensis* that in all previous analyses has been recovered as an outgroup to the Heroine cichlids (Farias et al., 2001) to polarize species in the Bayesian trees and to root the Maximum Parsimony tree for analyses.

ModelTest 3.06 (Posada and Crandall, 1998) was used to identify the best model of molecular evolution

Table 1
Collection localities

Species	Map	Collection locality	Museum number
Herichthys minckleyi (P)	1	Cuatro Ciénegas, Coahuila	29027
Herichthys minckleyi (M)	1	Cuatro Ciénegas, Coahuila	29029
Herichthys minckleyi (P)	1	Cuatro Ciénegas, Coahuila	29052
Herichthys cyanoguttatus	2	Ocampo, Coahuila	29068
Herichthys cyanoguttatus	3	Devils River, Texas	29024, 29025
Herichthys cyanoguttatus	4	Rio San Fernando, Tamaulipas	29062, 29063
Herichthys cyanoguttatus	5	Soto la Marina, Tamaulipas	29053
Herichthys pantostictus	6	Rio Tamesi, Tamaulipas	29060
Herichthys pantostictus	7	Rio Guayalejo, Tamaulipas	29022
Herichthys pantostictus	8	Rio Tigre, Tamaulipas	29061
Herichthys carpintis	8	Rio Tigre, Tamaulipas	29066
Herichthys carpintis	9	Laguna Champayan, Tamaulipas	29016
Herichthys steindachneri	10	Rio Gallinas, San Luis Potosi	29023
Herichthys steindachneri	11	Rio Tamasopo, San Luis Potosi	29040
Herichthys tamasopoensis	11	Rio Tamasopo, San Luis Potosi	29017, 29041
Herichthys labridens	11	Rio Tamasopo, San Luis Potosi	29033
Herichthys labridens	12	Media Luna, San Luis Potosi	29050, 29051
Herichthys labridens	12	Media Luna, San Luis Potosi	29034
Herichthys bartoni	12	Media Luna, San Luis Potosi	29048, 29049, 29047
Paratheraps fenestratus	13	Lago Catemaco, Veracruz	29020, 29021
Thorichthys ellioti	13	Lago Catemaco, Veracruz	29026, 29019
Thorichthys ellioti	14	Rio Dos Caños, Veracruz	29038
Thorichthys callolepis	15	Rio Sarabia, Veracruz	29036, 29028
Thorichthys callolepis	15	Rio Almoloya, Oaxaca	29030, 29031
Paratheraps fenestratus	14	Rio Dos Caños, Veracruz	29064
Paratheraps guttulatus	14	Rio Dos Caños, Veracruz	29065
Paratheraps guttulatus	15	Rio Sarabia, Veracruz	29054
'*Cichlasoma*' *salvini*	15	Rio Almoloya, Oaxaca	29035
'*Cichlasoma*' *salvini*	15	Rio Sarabia, Veracruz	29018
Paraneetroplus bulleri	15	Rio Sarabia, Veracruz	29055
Astatheros macracanthus	16	Rio Tehauntepec, Oaxaca	29032
Paratheraps guttulatus	17	Rio de los Perros, Oaxaca	29058, 29059
'*Cichlasoma*' *trimaculatum*	17	Rio de los Perros, Oaxaca	29056, 29057
Astatheros macracanthus	17	Rio de los Perros, Oaxaca	29039
'*Cichlasoma*' *salvini*	18	Rio Tonala, Veracruz	29067
'*Cichlasoma*' *octofasciatum*	18	Rio Tonala, Veracruz	29046
Astatheros robertsoni	18	Rio Tonala, Veracruz	29042, 29043
Thorichthys helleri	18	Rio Tonala, Veracruz	29044, 29045

for cyt *b* in these fish. A Bayesian analysis was executed to find approximations of the maximum likelihood tree using MrBayes 2.0 (Huelsenbeck and Ronquist, 2001) and employed a general time reversible (GTR) model with a flat prior probability distribution for all parameters. We ran five separate Bayesian analyses for 500,000 generations with five Markov chains. We sampled trees from the Markov Chain Monte Carlo (MCMC) search algorithm every 200 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 before 50,000 generations, and therefore, sample points prior to this equilibrium were discarded as "burn-in" samples. The remaining samples from each of the five runs were used to produce a majority rule consensus tree in PAUP* 4.0b10 (Swofford, 2002). The percentage of all trees that recovered a particular clade (the clade's posterior probability) was depicted on the single lowest likelihood tree found during the Bayesian analysis.

The aligned cyt *b* data set was also analyzed with maximum parsimony (MP) using PAUP* 4.0b10 (Swofford, 2002). All character state transformations were equally weighted. A full heuristic search with 200 sequence addition replicates and TBR branch-swapping was used to find the most parsimonious trees. Support for MP nodes was assessed using 1000 bootstrap pseudoreplicates (Felsenstein, 1985) with a heuristic tree search using a single addition sequence replicate.

Saturation of cyt *b*, multiple changes at homologous sites that accumulate over evolutionary time (Meyer, 1994), might influence our inferences concerning divergence at different depths in the phylogeny. Therefore, we plotted the maximum likelihood corrected pairwise sequence divergence versus transitions at third codon positions. This is the codon site and type of base pair changes in cyt *b* most frequently subject to saturation

(Farias et al., 2001; Meyer, 1994). We plotted these scores for all taxa in the Mesoamerican Heroines and also for the scores between *Acaronia* and all Mesoamerican Heroines. If there was close to a one to one relationship between estimated pairwise divergence and number of transitions, we interpreted this as a lack of saturation. If pairwise divergence increased without subsequent increase in number of transitions, this was interpreted as evidence for saturation.

2.4. Estimating divergence times

The GTR + I + gamma model, chosen by ModelTest 3.06 (Posada and Crandall, 1998), was used to determine likelihood adjusted sequence divergence in the cichlids examined. Divergence was measured as the length of branches connecting tip taxa to the nodes of interest. To estimate divergence times, we used a rate of 1.16% sequence divergence per million years for cyt *b* estimated for the temperate perciform black bass genus *Micropterus* (Near et al., 2003). This rate is similar to the other widely used rate for cyt *b* sequence divergence in fishes of 1–1.2% per million years (Bermingham et al., 1997). The error in our timing estimates was assessed using a non-parametric bootstrap procedure, as outlined in Baldwin and Sanderson (1998). We estimated divergence times on the branch lengths from the lowest likelihood topology obtained from the universe of Bayesian trees and with 100 bootstrap replicate data sets. The bootstrap replicate data sets were generated using the Seqboot program in Phylip (Felsenstein, 1993). The data sets were then individually imported into PAUP*. Branch lengths of each bootstrap replicate were inferred on the lowest likelihood topology and calculated using the optimal model of sequence evolution obtained from ModelTest. The trees were then individually transferred into TreeEdit where the branch lengths were transformed using Sanderson's (1997) method of NPRS to generate a mean and central 95% distribution of branch lengths connecting the crown and root nodes for the cichlid clade(s) in Northeastern Mexico.

3. Results

There were 1137 protein coding sites of the cyt *b* gene aligned in the analysis, for which 598 were variable. The five Bayesian runs all produced a different best tree. Each differed slightly in topology, but all had very similar likelihood scores. In the best trees of the first and fourth Bayesian runs, '*Cichlasoma octofasciatum*' was placed outside of the *Astatheros* group and placed as sister to '*Cichlasoma*' *festae* + '*Cichlasoma*' *atromaculatum*. The members of the genera *Archocentrus*, *Parachromis*, *Amphilophus* that form a clade sister to *Petenia splendida* + *Nandopsis uropthalmus* moved around topologically in the five best trees. The only other major topological incongruence between the five best Bayesian trees involved the South American taxon recovered as sister to this largely Mesoamerican Heroine clade.

In the parsimony analysis, 498 of the cyt *b* sites were considered parsimony informative. Of the 200 sequence addition replicates performed in the MP analysis, the same most-parsimonious tree island was recovered 12 times. This tree island had a tree score of 4661 steps and contained 112 most-parsimonious trees. Several aspects of the phylogenetic relationships recovered were robust to the utilization of both Bayesian and MP optimality criteria (Fig. 3). However, the two methods did differ on their placement of several Central American taxa including the three *Caquetaia* species, as well as '*Cichlasoma*' *umbriferus*, '*Cichlasoma*' *atromaculatum* + '*Cichlasoma*' *festae*, *Tomocichla underwoodii*, *Herotilapia multispinosa*, and '*Cichlasoma*' *octofasciatum* (Fig. 3). Based on either posterior probability or MP bootstrap scores, none of these species could be placed unambiguously with respect to other taxa in the phylogeny (Fig. 3).

Both phylogenetic analyses provided robust estimates for many relationships and the monophyly of all species sampled from Northeastern Mexico is perhaps best supported (Fig. 3). Our trees suggest that *Herichthys cyanoguttatus*, *H. carpintis*, and *H. tamasopoensis* form a monophyletic clade that is sister to the polymorphic species *H. minckleyi* (Node 82). The *H. labridens* sequence analyzed by Roe et al. (1997), does not group with the other *H. labridens* sequenced (Node 87). Within the *Herichthys* clade, there appears to be a well-supported split between a group containing *H. pantostictus* + *H. labridens* (Media Luna) + *H. bartoni* + *H. labridens* (Tamasopo) and another group containing *H. minckleyi* + *H. cyanoguttatus* + *H. carpintis* + *H. tamasopoensis* (Node 76). The species *H. labridens* as it has been interpreted in the past (Artigas-Azas, 1992; Taylor and Miller, 1983) appears to be paraphyletic with respect to *H. pantostictus*, *H. bartoni*, and *H. steindachneri* even when evaluating only the *H. labridens* sequenced here (Node 77). Regardless of the placement of *H. labridens*, this analysis suggests there is a clear split between two major groups of *Herichthys*, each containing several species, north of the PDM. This major split was used to estimate the crown group age or lower bound on the timing of *Herichthys* diversification in Northeastern Mexico.

Herichthys is strongly supported both in the Bayesian and MP analyses as sister to the group composed of *Paratheraps* + *Vieja* + *Paraneetroplus bulleri* (Fig. 3). However, the genus *Paraneetroplus* as defined by both *P. bulleri* and *P. seiboldii* (Bussing, 1975) is not monophyletic (Fig. 3). *P. seiboldii* is assignable to the large clade containing *Amphilophous citrenellus*. The strong support of (*Paratheraps* + *Vieja* + *Paraneetroplus*) as

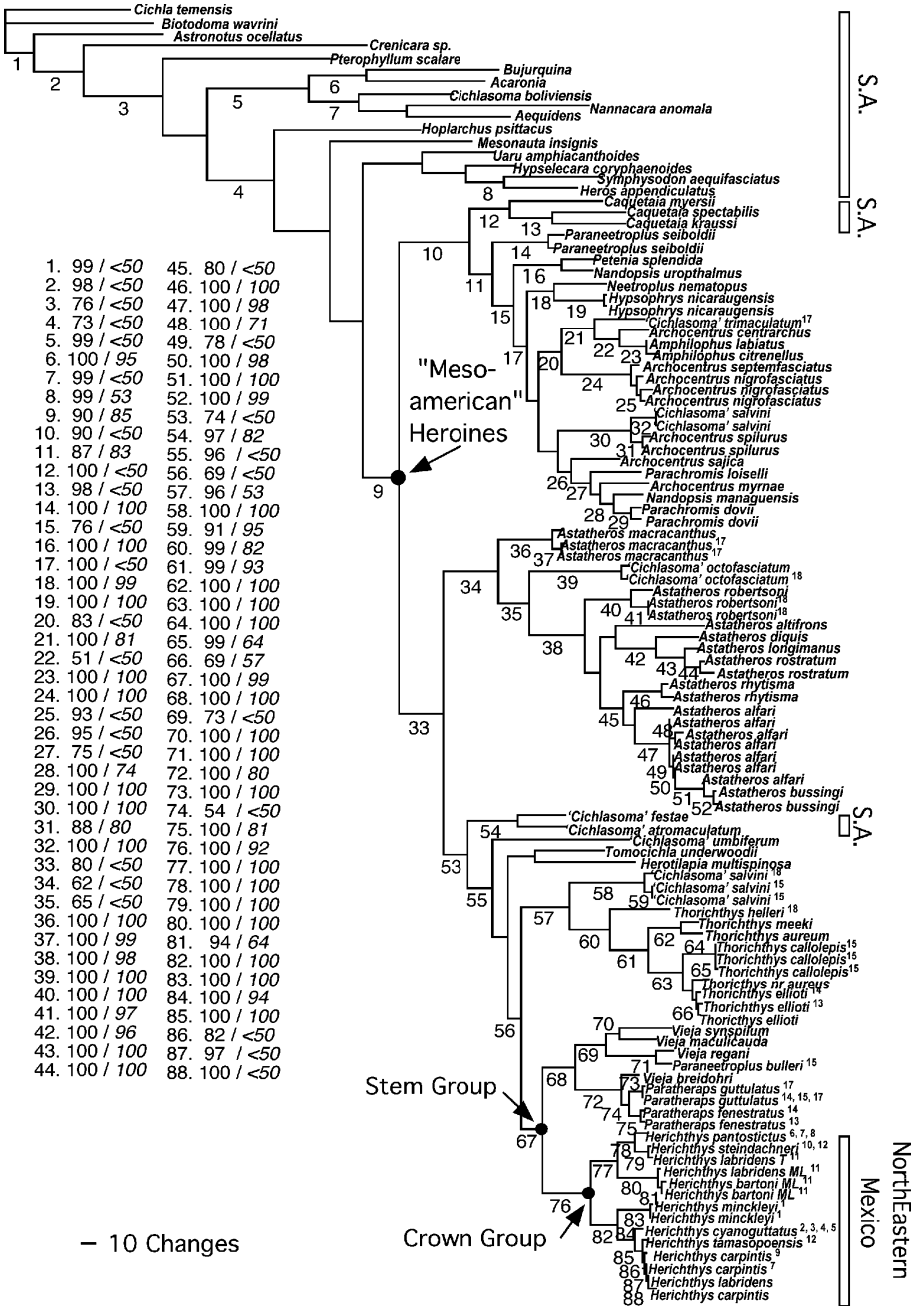

Fig. 3. The phylogeny with the lowest likelihood score from five Bayesian analyses is pictured with estimated branch lengths. Posterior probabilities were assessed from the concatenated trees of all five Bayesian tree searches remaining after the burn-in of 50,000 generations. Nodes receiving more than 50% posterior probability support are numbered. Posterior probability scores are followed by maximum parsimony bootstrap support in two columns to the left of the phylogeny. Specimens sequenced for this study are indicated by their collection localities numbered in Fig. 2. Species located in Northeastern Mexico and South America (S.A.) are identified on the phylogram by the labeled bars. The stem group and crown group nodes for the age estimates of *Herichthys* diversification across the PDM are also indicated.

sister to the *Herichthys* clade (Node 67) provided the phylogenetic comparison to approximate the stem group age or upper bound on diversification of *Herichthys* across the PDM.

There is little support for placing any of the other clades in Mesoamerica as the closest relative to (*Paratheraps* + *Vieja* + *Paraneetroplus*) + *Herichthys* (Fig. 3). But, as suggested by the study of Roe et al. (1997), *Thorichthys* forms a monophyletic group within Mesoamerica (Node 60) and our analyses provide an unambiguous set of phylogenetic relationships among the sampled species. *Thorichthys helleri* in the Rio Tonala basin is sister to the other five *Thorichthys* sequenced thus far. We repeatedly recovered *T. callolepis* as sister to *T. ellioti* and *T. aureus* + *T. meeki* grouping as monophyletic and sister to these other two (Node 61).

Only one named species of *Thorichthys*, of which we sequenced multiple individuals, may be paraphyletic. This potentially paraphyletic species was identified by Roe et al. (1997) as *Thorichthys* nr. *aureus* from Tabasco Mexico which in our analysis appears to nest with *T. ellioti* and *T. callolepis* (Node 63).

This analysis supports all previously reported relationships within the group *Astatheros* (Fig. 3). Although geographically quite distant, *A. robertsoni* in the Rio Tonala basin sequenced for this study is only 2% divergent at cyt *b* from the *A. robertsoni* present in the Sibun River of Belize. This is similar to the amount of divergence found for cichlid species in separate drainages in Costa Rica (Martin and Bermingham, 1998). We sequenced two individuals of *Astatheros macracanthus* from different drainages to fully assess the relationship of this species. As found in Roe et al. (1997), this species grouped outside of the rest of the *Asthatheros* group and may indeed be the sister to all other *Asthatheros* (Node 34). However, the monophyly of *Astatheros* will depend ultimately on the affinities of '*Cichlasoma*' *octofasciatum* that in some of the best trees recovered here was nested within *Asthatheros* (Node 35) while in others was placed well outside of this group.

The relationships of many cichlid species with close affinities to *A. citrenellus* have been previously identified (Farias et al., 2001; Martin and Bermingham, 1998), and this large clade containing numerous taxonomic groupings generally lacks resolution (Fig. 3). However, one very novel result of our analysis is the strong support for the cichlid '*Cichlasoma*' *trimaculatum* as belonging to a monophyletic clade containing *A. citrenellus*, *A. labiatus*, and *A. centrarchus* (Node 21). Another very interesting result is the paraphyly of the species commonly referred to as *Nandopsis salvini*. The individuals of this species sampled here consistently group with the *Thorichthys* clade (Node 57). However, the *N. salvini* sequenced by others (Rican et al., unpublished) appear to group closer within the clade of cichlids that include most of the other piscivorous *Nandopsis* species (Node 32). Nevertheless, '*Cichlasoma*' *atromaculatum* and '*Cichlasoma*' *festae* are commonly referred to as *Nandopsis* and they also group outside of the other *Nandopsis* and close to the *N. salvini* sequenced here (Node 54).

We obtained strong support for monophyly of what has been called the Mesoamerican Heroines (Farias et al., 2001). However, two non-Mesoamerican groups that consistently fell at the base of this radiation were the three species of *Caquetaia* (Node 12) and the clade represented by '*Cichlasoma*' *atromaculatum* + '*Cichlasoma*' *festae* (Node 54). The members of *Caquetaia* are distributed widely through tropical South America and the clade represented by '*Cichlasoma*' *atromaculatum* + '*Cichlasoma*' *festae* is common on the eastern slope of the Andes in northern South America (Kullander, 1998). The low posterior probability recovered for these clades makes it difficult to reject the possibility that all of the other cichlids in Central America form a monophyletic group. However, these South American species are clearly more closely aligned with the Mesoamerican cichlid radiation than with any other cichlid group in the Neotropics (Node 9).

The saturation plot (Fig. 4) suggests that saturation is not a critical consideration for the estimation of sequence divergence for the clades of interest on either side of the PDM. The average NPRS divergence along the branch leading to the node that separates the two major groups of *Herichthys* north of the PDM is 7.0%. This value is not greatly different from the average NPRS divergence of 7.8% along the branch leading to the node that separates *Herichthys* and its sister group south of the PDM. The average estimated divergence time between the crown group of the two major clades of *Herichthys* in Northeastern Mexico is therefore 6.0 mybp, with a 95% confidence interval of 3.9–8.1 mybp. The maximum likelihood divergence estimated for the stem group age is 6.7 mybp with a 95% confidence interval of 4.7–8.7 mybp.

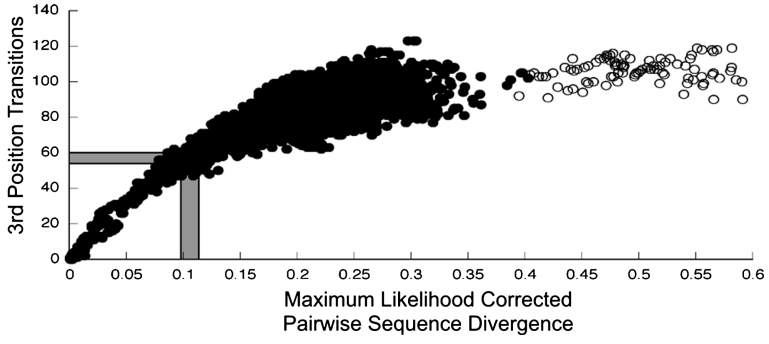

Fig. 4. Saturation plot of third position transitions versus maximum likelihood corrected pairwise sequence divergence. All comparisons between Mesoamerican Heroines are shown (●). The distances recovered between *Acaronia* and the Mesoamerican Heroines are also depicted (○). The sequence divergence for both the stem and crown group lineages used to measure temporal diversification across the PDM are bracketed in gray.

4. Discussion

The monophyly and temporal divergence of *Herichthys* north of the PDM robustly supports the hypothesis that the formation of the PDM served as a major biogeographic boundary. The relationships we recovered for Mesoamerican cichlids from both the Bayesian and MP analyses were largely congruent. The phylogenies suggest that some cichlids in South America should be considered as part of a clade containing the Mesoamerican Heroines. Additionally, the clade of eight named species of *Herichthys* sequenced from Northeastern Mexico were supported as monophyletic, and a group containing species in *Paratheraps* + *Paraneetroplus* + *Vieja* is strongly supported as sister to *Herichthys* (Fig. 3). Finally, our analyses of molecular divergence in *Herichthys* support the hypothesis that the PDM served as an important vicariant mechanism approximately 6 mybp.

To date, generating a well-resolved phylogeny for Mesoamerican cichlids has been hindered by the state of cichlid taxonomy, which for many years grew increasingly confused as a result of parallel, non-complementary attempts to provide nomenclature for these morphologically diverse fishes (Kullander, 1998; Miller, 1966; Regan, 1905). The problems with morphologically diagnosing species and monophyletic groups might be best illustrated by examining the confusion surrounding a species such as *H. minckleyi*. *H. minckleyi*, a single species in Northeastern Mexico that exhibits polymorphism in its jaw morphology, was at first thought to represent up to four distinct species (Minckley, 1969). Like all cichlid species in Mesoamerica, *H. minckleyi* was for many years placed in a large genus *Cichlasoma* (Miller, 1966), until this name was restricted to a small group of 12 species in South America (Kullander, 1983). *H. minckleyi* has also been classified in *Nandopsis* (Conkel, 1993) and only recently has it been definitively placed in the genus *Herichthys* (Kullander, 1998). Our analysis unambiguously provided a series of nested relationships for the entire group of *Herichthys* in Northeastern Mexico (Fig. 3). It also suggests only a few of the currently diagnosed genera in Mesoamerican cichlids are monophyletic. The placement of a large number of Mesoamerican cichlid species into an explicitly phylogenetic framework should help bring stability to this group's nomenclature and enhance the understanding of its evolutionary history.

Recovering the order of cladogenic events at the base of the Mesoamerican Heroine phylogeny is central to understanding phylogenetic relationships in this group. It is clear several species present in South America should likely be considered part of the "Mesoamerican cichlid" radiation (Fig. 3). However, our increased taxon sampling relative to earlier studies examining fewer species (Martin and Bermingham, 1998; Roe et al., 1997) did not provide any greater phylogenetic resolution for most of the basal nodes of the Mesoamerican Heroines. Inability to resolve the nodes at the base of the Mesoamerican Heroines could be due to processes such as gene saturation via multiple substitution (Slowinski, 2001). Saturation of cyt *b* at this level of inference for cichlid fishes has been reported in several studies (Meyer, 1994) including studies from which sequences used here were taken (Farias et al., 2001; Martin and Bermingham, 1998; Roe et al., 1997). We also found evidence of saturation (Fig. 4), especially past 25% estimated sequence divergence which is the minimum divergence found between taxa spanning the basal nodes of the Mesoamerican clade. However, it remains feasible that the short branch lengths in this part of the phylogeny could also be due to rapid cladogenesis of these fish initiated by their colonization of the otherwise cichlid free region north of the Panamanian Isthmus. Regardless, our analysis provided no reason to reject or disregard the timing estimates of between 15 and 18 mybp posited previously for the Heroine invasion of Mesoamerica (Martin and Bermingham, 1998). The difficulty we have rejecting the approximately 20 mybp timetable for the evolution of Heroines in Mesoamerica helps to bolster our surprisingly ancient estimates of cichlid divergence times across the PDM.

Without molecular divergence estimates of cichlids north of the PDM, there are several reasons one might suspect the invasion of cichlids into Northeastern Mexico to have occurred more recently than 5 mybp. First, although there is no evidence that cichlids in Northeastern Mexico are capable of effectively exploiting brackish conditions, tolerance to marine habitats has been postulated as a primary reason for the early cichlid colonization of Mesoamerica relative to other fish groups (Martin and Bermingham, 1998). Furthermore, there are less than 10 cichlid species north of this boundary. This is a low number of species considering the widely cited evidence that some groups of cichlids have diverged into hundreds of unique species in only a few million years (Meyer et al., 1990; Verheyen et al., 2003). Additionally, there has been repeated raising and lowering of sea level in this region during the Pleistocene (Beard et al., 1982), which could have allowed ancestral *Herichthys* to bypass any terrestrial boundary to dispersal. This documented sea level fluctuation was the main reason the timeframe postulated at the PDM for divergence of *Bufo* species, typically thought of as good dispersal agents (Mulcahy and Mendelson, 2000), was so surprising. Despite potential tolerance for brackish conditions, the relatively low number of species, and possible effects of climate, our results suggest both the cichlid colonization of Northeastern Mexico occurred only once and that as found for *Bufo*, it was not a recent event.

The estimated sequence divergence in *Herichthys* north of the PDM suggests cichlids could have been

diversifying in this region before the formation of the Neovolcanic Plateau. If one averages the crown and stem group estimates of temporal divergence together, the timing of the split between the *Herichthys* in Northeastern Mexico and this clade's ancestors to the south is estimated at approximately 6.35 mybp. Because we do not have sequences from all of the cichlids in Mesoamerica it is possible that further sampling could slightly influence our estimates of cichlid divergence across the PDM. However, with the addition of further taxa, it is unlikely the temporal estimate for diversification in this group would fall outside of the ages recovered here for the two nodes used to bracket cichlid divergence across the PDM. Importantly, the estimated divergence time across the PDM for *Bufo* is 5.4 mybp with the 95% confidence interval for divergence at the PDM ranging from 4.2 to 7.6 mybp (Mulcahy and Mendelson, 2000). These dates place the estimate of *Herichthys* divergence and *Bufo* divergence as roughly within the same period.

The temporal concordance of both *Bufo* and Mesoamerican Heroine diversification argue the abrupt faunal shift from a largely southern neotropical fauna to a primarily temperate fauna north of the PDM is not primarily due to climatic factors. Interestingly, cichlids in *Paratheraps* and *Thorichthys* are native to the region just south of the PDM, but do not occur north of this boundary. It is also notable that recent estimates of divergence in *Rhamdia*, two species of pimelodid catfishes which reach their northern limits at the PDM, suggest this group's ancestors were only crossing north of the South American Andes 8 mybp (Perdices et al., 2002). Likewise, a detailed phylogenetic study of *Poeciliopsis* (Mateos et al., 2002) indicates that this group of livebearing fish colonized rivers directly to the south of the PDM only 1 million years ago. This left little geologic time for these species to make their way north of the PDM in Mesoamerica, before the formation of the Neovolcanic Plateau. The extension of the Neovolcanic Plateau to the Gulf of Mexico coast likely sealed off further colonization north and south for lineages such as *Rhamdia* and *Poecilopsis* that appear to have arrived only recently in this region. This boundary also appears to have trapped lineages of organisms such as *Bufo* and *Herichthys* that were likely distributed on either side of the PDM prior to its formation. All available evidence supports the idea that the distribution of most vertebrate clades relative to the PDM is directly the result of the age and effectiveness of the Neovolcanic Plateau as a biogeographic barrier.

A more robust understanding of the evolutionary diversification of cichlids in Mesoamerica and the effects of the formation of the Neovolcanic Plateau on other taxa will hinge on successfully integrating various lines of evolutionary evidence. For the Mesoamerican Heroines, examination of other genes that evolve slowly and are less prone to saturation than cyt *b* would greatly enhance our understanding of when they invaded Mesoamerica. The identification of other major biogeographic breaks and vertebrate fossils in Mesoamerica would also be beneficial to further calibrate diversification in cichlids as well as diversification at the PDM. With the inclusion of both more independent data partitions and a greater number of Mesoamerican species into phylogenetic analyses, we will ultimately resolve the tempo and patterns of diversification at both the Punta del Morro and within Mesoamerican Heroines.

Acknowledgments

We thank the Wainwright and Shaffer labs at UC Davis for helpful comments and support. Aldo Guevara, Rocio Rodriguez, Andres Sandoval, Omar Torres, Liseth Martinez, Delladira Tirado, Celene Leal, and Anna Santos of the Instituto Tecnologico de Tamaulipas provided help collecting fish. Peter Wainwright, John Wares, David Collar, and the UC Davis Monte Carlo group provided helpful comments on the manuscript. Juan Miguel Artigas-Azaz provided help in the identification of some species. Chuck Lydeard provided unpublished information about some of the taxa sequenced. C.D. Hulsey was supported by a NSF Predoctoral Fellowship, the UCD Center for Population Biology, the UC Mexus Doctoral Fellowship, and a grant for Research and Exploration from the National Geographic Society. F. J. García de León was supported by the ITCV. T.J. Near was supported by the Center for Population Biology Postdoctoral Fellowship. The Texas Nature Conservancy provided access to the Devils River. We thank the Mexican government for providing us with permits (Permiso de Pesca de Fomento 230401-613-03 and DAN 02939) and permission to collect the organisms used in this study.

References

Artigas-Azas, J.M., 1992. The "*Cichlasoma*" *labridens*-complex. In: Konings, A. (Ed.), The Cichlid Yearbook, vol. 2. Cichlid Press, St. Leon-Rot, Germany, pp. 65–70.

Baldwin, B.G., Sanderson, M.J., 1998. Age and rate of diversification of the Hawaiian silversword alliance. Proc. Natl. Acad. Sci. USA 95, 9402–9406.

Beard, J.H., Sangree, J.B., Smith, L.A., 1982. Quaternary chronology, paleoclimate, depositional sequences, and eustatic cycles. Am. Assoc. Petroleum Geol. Bull. 66, 158–169.

Bermingham, E., McCafferty, S.S., Martin, A., Kocher, P., 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. In: Thomas, D., Stepien, C.A. (Eds.), Molecular Systematics of Fishes. Academic Press, London, pp. 113–128.

Bussing, W.A., 1975. Taxonomy and biological aspects of the Central American cichlid fish *Ciclasoma seiboldii* and *C. tuba*. Rev. Biol. Trop. 23, 189–211.

Byerly, G.R., 1991. Igneous activity. In: Salvador, A. (Ed.), The Gulf of Mexico Basin: The Geology of North America. J. Geol. Soc. Am., Boulder, pp. 91–108.

Conkel, D., 1993. Cichlids of North and Central America. T.F.H. Publications, Neptune, NJ.

Farias, I.P., Scneider, H., Sampaio, I., 1998. Molecular phylogeny of neotropical cichlids: the relationships of cichlasomines and heroines. In: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, Z.M., Lucena, C.A.S. (Eds.), Phylogeny and Classification of Neotropical Fishes. Edipucrs, Puerto Alegre, Brazil, pp. 499–508.

Farias, I.P., Orti, G., Sampaio, I., Schneider, H., Meyer, A., 2001. The cytochrome *b* gene as a phylogenetic marker: the limits of resolution for analyzing relationships among cichlid fishes. J. Mol. Evol. 53, 89–103.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 9, 783–791.

Felsenstein, J., 1993. PHYLIP (Phylogeny Inference Package) Version 3.57, University of Washington, Seattle.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

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

Kumazawa, Y., Yamaguchi, M., Nishida, M., 1999. Mitochondrial molecular clocks and the origin of euteleostean biodiversity: Familial radiation of perciforms may have predated the Cretaceous/Tertiary boundary. In: Ato, M. (Ed.), The Biology of Biodiversity. Springer-Verlag, Tokyo, pp. 35–52.

Kullander, S.O., 1983. A revision of the South American cichlid genus *Cichlasoma* (Teleostei: Cichlidae). Naturhistoriska Riksmuseet, Stockholm.

Lydeard, C., Roe, K.J., 1997. The phylogenetic utility of the mitochondrial cytochrome *b* gene for inferring relationships among actinopterygian fishes. In: Thomas, D., Stepien, C.A. (Eds.), Molecular Systematics of Fishes. Academic Press, New York, pp. 285–303.

Lydeard, C., Wooten, M.C., Meyer, A., 1995. Molecules, morphology, and area cladograms: a cladistic and biogeographic analysis of *Gambusia*(Teleostei: Poeciliidae). Syst. Biol. 44, 221–236.

Magallon, S., Sanderson, M.J., 2001. Absolute diversification rates in Angiosperm Clades. Evolution 55, 1762–1780.

Martin, A.P., Bermingham, E., 1998. Systematics and evolution of lower Central American cichlids inferred from analysis of cytochrome *b* gene sequences. Mol. Phylogenet. Evol. 9, 192–203.

Mateos, M., Sanjur, O.I., Vrijenhoek, R.C., 2002. Historical biogeography of the livebearing fish genus *Poeciliopsis* (Poeciliidae: Cyprinodontiformes). Evolution 56, 972–984.

Meyer, A., Kocher, T.D., Basasibwaki, P., Wilson, A.C., 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. Nature 347, 550–553.

Meyer, A., 1994. Shortcomings of the cytochrome *b* gene as a molecular marker. TREE 9, 278–280.

Miller, R.R., 1966. Geographical distribution of Central American freshwater fishes. Copeia, 773–802.

Miller, R.R., 1986. Origin and geography of fishes of Central Mexico. In: Hocutt, C.H., Wiley, E.O. (Eds.), The Zoogeography of North American Freshwater Fishes. Wiley, New York, pp. 487–517.

Minckley, W., 1969. Environments of the Bolsón of Cuatro Cienegas, Coahuila, Mexico, with special reference to the aquatic biota. Univ. of Texas El Paso Sci. Ser. 2, 1–65.

Mulcahy, D.G., Mendelson III, J.R., 2000. Phylogeography and speciation of the morphologically variable, widespread species *Bufo valliceps*, based on molecular evidence from mtDNA. Mol. Phylogenet. Evol. 17, 173–189.

Near, T.J., Kassler, T.W., Koppleman, J.B., Dillman, C.B., Phillip, D.P., 2003. Speciation in North American black basses, *Micropterus* (Actinopterygii: Centrarchidae). Evolution 57, 1610–1621.

Obregon-Barboza, H., Contreras-Balderas, S., Lozano-Vilano, M.D.L., 1994. The fishes of northern and central Veracruz, Mexico. Hydrobiologia 286, 79–95.

Page, R.D.M., 1988. Quantitative cladistic biogeography: constructing and comparing area cladograms. Syst. Zool. 37, 254–270.

Page, R.D.M., 1990. Temporal congruence and cladistic analysis of biogeography and cospeciation. Syst. Zool. 39, 205–226.

Perdices, A., Bermingham, E., Montilla, A., Doadrio, I., 2002. Evolutionary history of the genus *Rhamdia* (Teleostei: Pimelodidae) in Central America. Mol. Phylogenet. Evol. 25, 172–189.

Pérez-Higaredera, G., Navarro, L.D., 1980. The faunistic districts of the low plains of Veracruz, Mexico, based on reptilian and mammalian data. Bull. Maryland Herpetol. Soc. 16, 54–69.

Posada, D., Crandall, K.A., 1998. MODEL TEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Regan, C.T., 1905. A revision of the fishes of the American cichlid genus *Cichlasoma* and allied genera. Ann. Mag. Nat. Hist. (Ser. 7) 16, 60–77, 225–243, 316–340, 433–445.

Roe, K.J., Conkel, D., Lydeard, D.C., 1997. Molecular systematics of Middle American cichlid fishes and the evolution of trophic-types in 'Cichlasoma (*Amphilophus*)' and 'C. (*Thorichthys*)'. Mol. Phylogenet. Evol. 7, 366–376.

Rosen, D.E., 1978. Vicariant patterns and historical explanation in biogeograpy. Syst. Zool. 27, 159–188.

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

Slowinski, J.B., 2001. Molecular polytomies. Mol. Phylogenet. Evol. 19, 114–120.

Stiassny, M.L.J., 1991. Phylogenetic relationships of the family Cichlidae: an overview. In: Keenleyside, M.H.A. (Ed.), Cichlid Fishes: Behavior, Ecology, and Evolution. Chapman & Hall, London, pp. 1–18.

Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods) Beta Version 4.0. Sinauer, Sunderland, MA.

Taylor, J.N., Miller, R.R., 1983. Cichlid fishes (Genus *Cichlasoma*) of the Rio Pánuco basin, Eastern Mexico, with description of a new species. Occas. Pap. Mus. Nat. His. Univ. Kansas 104, 1–24.

Thompson, J.D., Plewniak, F., Poch, O., 1999. A comprehensive comparison of multiple sequence alignment programs. Nucleic Acids Res. 27, 2682–2690.

Verheyen, E., Salzburger, W., Snoeks, J., Meyer, A., 2003. The origin of the superflock of cichlid fishes from Lake Victoria, East Africa. Science 300, 325–329.