The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2012, **105**, 382–394. With 6 figures

Tracing the origins of widespread highland species: a case of Neogene diversification across the Mexican sierras in an endemic lizard

ROBERT W. BRYSON JR* and BRETT R. RIDDLE

School of Life Sciences, University of Nevada, Las Vegas, 4505 Maryland Parkway, Las Vegas, NV 89154-4004, USA

Received 27 July 2011; revised 22 August 2011; accepted for publication 23 August 2011

The evolutionary history of the Mexican sierras has been shaped by various geological and climatic events over the past several million years. The relative impacts of these historical events on diversification in highland taxa, however, remain largely uncertain owing to a paucity of studies on broadly-distributed montane species. We investigated the origins of genetic diversification in widely-distributed endemic alligator lizards in the genus *Barisia* to help develop a better understanding of the complex processes structuring biological diversity in the Mexican highlands. We estimated lineage divergence dates and the diversification rate from mitochondrial DNA sequences, and combined divergence dates with reconstructions of ancestral geographical ranges to track lineage diversification across geography through time. Based on our results, we inferred ten geographically structured, well supported mitochondrial lineages within *Barisia*. Diversification of a widely-distributed ancestor appears tied to the formation of the Trans-Mexican Volcanic Belt across central Mexico during the Miocene and Pliocene. The formation of filter barriers such as major river drainages may have later subdivided lineages. The results of the present study provide additional support for the increasing number of studies that suggest Neogene events heavily impacted genetic diversification in widespread montane taxa.

ADDITIONAL KEYWORDS: ancestral area reconstruction – biogeography – divergence dating – diversification rate – Mexico – phylogeography.

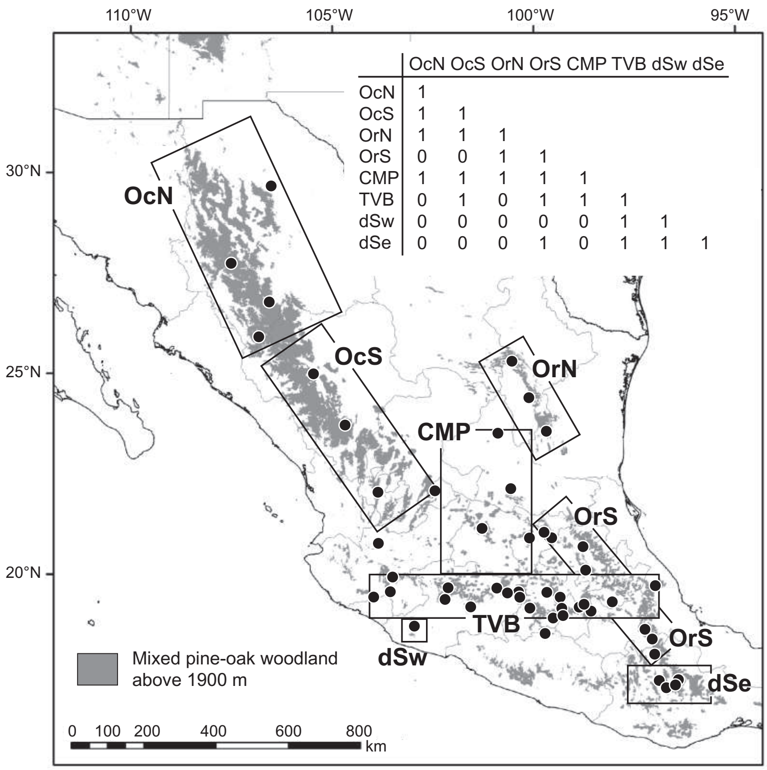
INTRODUCTION

Central to our understanding of diversification is recognition of the mechanisms that impact differentiation of species across a landscape. Many questions remain about the processes of diversification, particularly in understudied regions of high biodiversity. One such region, the Mexican highlands, is a globally important biodiversity hotspot (Ramamoorthy *et al*., 1993; Mittermeier *et al*., 2005), yet a fundamental understanding of processes driving the origins of diversity in this region lags behind that of temperate counterparts in North America (Soltis *et al*., 2006; Shafer *et al*., 2010).

The Mexican highlands consist primarily of four major mountain ranges and a large plateau spanning most of mainland Mexico (Fig. 1). These include the north-to-south trending Sierra Madre Occidental and Sierra Madre Oriental of northern Mexico, the east-west trending Trans-Mexican Volcanic Belt and Sierra Madre del Sur in central and southern Mexico, and an elevated Central Mexican Plateau. The topography and biota of the Mexican highlands have been shaped by various geological and climatic events over the past several million years (Ramamoorthy *et al*., 1993; Bryson *et al*., 2011a). The relative impact of these historical events on the diversification of co-distributed montane taxa remains uncertain. Only recently have studies begun to thoroughly examine diversification in widespread Mexican montane taxa (McCormack, Zellmer & Knowles, 2010; Bryson *et al*., 2011a, b; Bryson & García-Vázquez, 2011). An emerging picture reveals evolutionary complexity characterized by strong geographical structuring. The relatively recent Neogene formation of the

---

*Corresponding author. E-mail: brysonjr@unlv.nevada.edu



	OcN	OcS	OrN	OrS	CMP	TVB	dSw	dSe
OcN	1							
OcS	1	1						
OrN	1	1	1					
OrS	0	0	1	1				
CMP	1	1	1	1	1			
TVB	0	1	0	1	1	1		
dSw	0	0	0	0	0	1	1	
dSe	0	0	0	1	0	1	1	1

Figure 1. Map depicting the highlands of Mexico. Localities of genetic samples used in study are overlaid on mixed pine-oak forests above 1900 m. Biogeographical areas include the northern Sierra Madre Occidental (OcN), southern Sierra Madre Occidental (OcS), northern Sierra Madre Oriental (OrN), southern Sierra Madre Oriental (OrS), Central Mexican Plateau (CMP), Trans-Mexican Volcanic Belt (TVB), eastern Sierra Madre del Sur (dSe), and western Sierra Madre del Sur (dSw). For ancestral area reconstructions, an area adjacency matrix was used (upper right corner) to restrict ranges to geographically proximate areas. '1' indicates area connectivity; '0' indicates no connectivity.

Trans-Mexican Volcanic Belt in the Miocene and Pliocene appears to be a strong driver of diversification in several taxa associated with this volcanic mountain range (Bryson & García-Vázquez, 2011). Causal mechanisms driving diversification across the remaining Mexican sierras is less clear, although they may be associated with a number of filter barriers differentially subdividing lineages through time (Bryson *et al.*, 2011a). Geographical patterns of genetic diversification across the Mexican highlands can be strengthened by examination of additional co-distributed species. In the present study, we provide additional insight from a widely-distributed highland lizard.

Alligator lizards in the genus *Barisia* are widespread across the Mexican highlands (Figs 1, 2), and inhabit mixed pine-oak woodlands from Chihuahua south to Oaxaca (Guillette & Smith, 1982; Zaldivar-Riverón & Nieto-Montes de Oca, 2002). Previous phylogenetic work (Zaldivar-Riverón, Nieto-Montes de Oca & Laclette, 2005) postulated this genus to be composed of at least five distinct species (*Barisia levicollis*, *Barisia planifrons*, *Barisia rudicollis*, *Barisia herrerae*, and *Barisia imbricata*) that likely diverged from one another during the Pliocene. Furthermore, several deep genetic lineages are embedded within the two subspecies of *B. imbricata*: *Barisia imbricata imbricata* and *Barisia imbricata ciliaris*. Investigating the tempo and mode of diversification in *Barisia* will add considerably to a fuller understanding of the complex processes structuring biological diversity in the Mexican highlands. Patterns of genetic structuring across geography shared with co-distributed highland taxa can help to highlight some of the potential mechanisms underlying diversification. Novel patterns in *Barisia* may alternatively provide insight into biogeographical scenarios that might not otherwise be apparent.

During the past decade, an increasingly integrative biogeography has developed tools allowing researchers to gain new insight into historical diversification processes across a landscape (Riddle *et al.*, 2008). In the present study, we take advantage of this recent methodological toolbox to reconstruct the evolution-

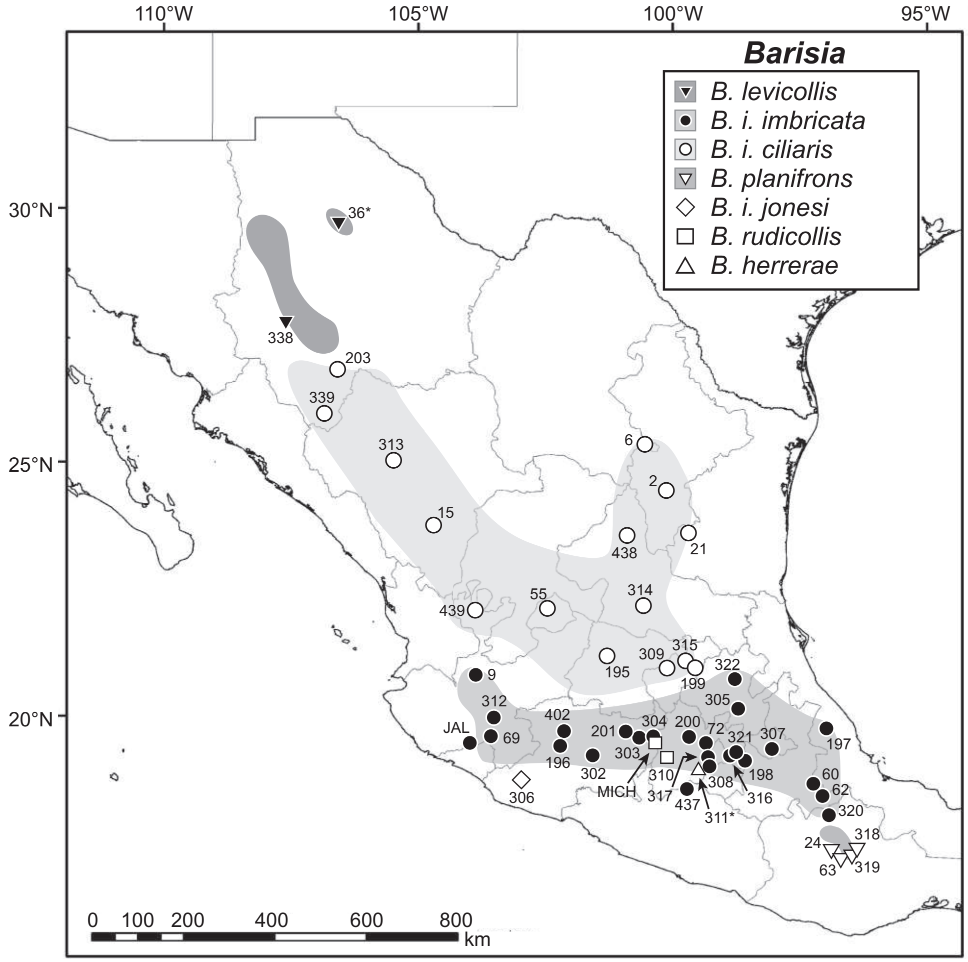


Figure 2. Localities of genetic samples used and distribution (adapted from Zaldivar-Riverón *et al*., 2005) of taxa in the genus *Barisia*. Asterisks denote multiple samples used from the same locality. The prefixes 'MX' and 'MZFC' (for samples JAL and MICH) were omitted from sample numbers for clarity.

ary history of *Barisia*. We estimated lineage divergence dates and diversification rate from mitochondrial DNA (mtDNA) sequences, and combined divergence dates with reconstructions of ancestral geographical ranges to track lineage diversification across geography through time. This integrative approach represents an important step towards developing a more thorough evaluation of the range of potential histories underlying divergence patterns across the Mexican highlands.

MATERIAL AND METHODS

TAXONOMIC SAMPLING AND DNA SEQUENCING

We obtained tissues from 50 *Barisia* (Table 1) spanning the distributions of all putative taxa within the genus (Fig. 2). Sequences from an additional two samples were obtained from GenBank (Table 1) to fill in small gaps in the distribution. We used *Elgaria kingii* and *Abronia graminea* as outgroups (Macey *et al*., 1999; Conroy *et al*., 2005).

We sequenced two mtDNA gene regions, including NADH dehydrogenase subunit 4 and flanking tRNAs (ND4) and a continuous region encompassing ATPase subunits 8 and 6 (ATPase 8, ATPase 6), in accordance with previously described methods Bryson, García-Vázquez & Riddle (2011c). These gene regions have been previously shown to be informative at different levels of divergence within lizards (Leaché & Mulcahy, 2007; Lindell, Méndez-de la Cruz & Murphy, 2008). Although it has been demonstrated that single locus genetic studies have some limitations (Edwards & Bensch, 2009), mtDNA nonetheless remains useful in exploring geographical relationships among closely-related species (Pyron & Burbrink, 2009; Daza, Castoe & Parkinson, 2010; Kuriyama *et al*., 2011). Primer sequences for ATPase were specifically designed for this project (C2LF4,

Table 1. Collection and voucher data for genetic samples used in the present study

Sample ID (MX)	Taxon	Locality	Voucher number
2	*Barisia imbricata ciliaris*	Mexico: Nuevo León: Pablillo	LVT 10795
6	*Barisia imbricata ciliaris*	Mexico: Coahuila: Santa Rita	LVT 10796
9	*Barisia imbricata imbricata*	Mexico: Jalisco: Volcán Tequila	LVT 10797
15	*Barisia imbricata ciliaris*	Mexico: Durango: Rancho Santa Barbara	LVT 10798
21	*Barisia imbricata ciliaris*	Mexico: Tamaulipas: Aserradero	LVT 10799
24	*Barisia planifrons*	Mexico: Oaxaca: Sierra Monte Flor	LVT 10800
36	*Barisia levicollis*	Mexico: Chihuahua: Sierra del Nido	LVT 10801
55	*Barisia imbricata ciliaris*	Mexico: Aguascalientes: Sierra Fría	LVT 10802
60	*Barisia imbricata imbricata*	Mexico: Veracruz: Xometla	AMCC 118392
62	*Barisia imbricata imbricata*	Mexico: Puebla: Sierra Negra	AMCC 118355
63	*Barisia planifrons*	Mexico: Oaxaca: Cerro San Felipe	AMCC 117877
69	*Barisia imbricata imbricata*	Mexico: Jalisco: Volcán del Fuego	LVT 10803
72	*Barisia imbricata imbricata*	Mexico: Estado de México: Chapa de Mota	LVT 10804
195	*Barisia imbricata ciliaris*	Mexico: Guanajuato: Sierra Santa Rosa	LVT 10805
196	*Barisia imbricata imbricata*	Mexico: Michoacán: Cerro Tancítaro	LVT 10806
197	*Barisia imbricata imbricata*	Mexico: Veracruz: Las Vigas	LVT 10807
198	*Barisia imbricata imbricata*	Mexico: Puebla: Volcán Iztaccihuatl	LVT 10808
199	*Barisia imbricata ciliaris*	Mexico: Querétaro: Nuevo San Joaquin	LVT 10809
200	*Barisia imbricata imbricata*	Mexico: Estado de México: Atlacomulco	LVT 10810
201	*Barisia imbricata imbricata*	Mexico: Michoacán: Parque José María Morelos	LVT 10811
202	*Barisia levicollis*	Mexico: Chihuahua: Sierra del Nido	LVT 10812
203	*Barisia imbricata ciliaris*	Mexico: Chihuahua: Mesa de Agostadero	LVT 10813
302	*Barisia imbricata imbricata*	Mexico: Michoacán: Tacambaro	LVT 10814
303	*Barisia imbricata imbricata*	Mexico: Michoacán: Mil Cumbres	LVT 10815
304	*Barisia imbricata imbricata*	Mexico: Michoacán: SE Aporo	LVT 10816
305	*Barisia imbricata imbricata*	Mexico: Hidalgo: Calicanto	LVT 10817
306	*Barisia imbricata jonesi*	Mexico: Michoacán: Dos Aguas	LVT 10818
307	*Barisia imbricata imbricata*	Mexico: Tlaxcala: Apizaco	LVT 10819
308	*Barisia imbricata imbricata*	Mexico: Morelos: Zempoala	LVT 10820
309	*Barisia imbricata ciliaris*	Mexico: Querétaro: Cerro Zamorano	LVT 10821
310	*Barisia rudicollis*	Mexico: Estado de México: Valle de Bravo	LVT 10822
311	*Barisia herrerae*	Mexico: Estado de México: Ocuilan	LVT 10823
312	*Barisia imbricata imbricata*	Mexico: Jalisco: Tapalpa	LVT 10824
313	*Barisia imbricata ciliaris*	Mexico: Durango: Mesa de las Navar	LVT 10825
314	*Barisia imbricata ciliaris*	Mexico: San Luis Potosí: Alvarez	LVT 10826
315	*Barisia imbricata ciliaris*	Mexico: Querétaro: Rancho Los Velázquez, Pinal de Amoles	LVT 10827
316	*Barisia imbricata imbricata*	Mexico: Estado de México: Llano Grande	LVT 10828
317	*Barisia imbricata imbricata*	Mexico: Distrito Federal: Sierra Ajusco	LVT 10829
318	*Barisia planifrons*	Mexico: Oaxaca: Ixtlán de Juárez	LVT 10830
319	*Barisia planifrons*	Mexico: Oaxaca: Yuvila	LVT 10831
320	*Barisia imbricata imbricata*	Mexico: Oaxaca: Peña Verde	LVT 10832
321	*Barisia imbricata imbricata*	Mexico: Estado de México: Rio Frio	MVZ 191048
322	*Barisia imbricata imbricata*	Mexico: Hidalgo: Eloxochitlán	LVT 10833
338	*Barisia levicollis*	Mexico: Chihuahua: Creel	LVT 10834
339	*Barisia levicollis*	Mexico: Chihuahua: Ejido Zorillo	LVT 10835
402	*Barisia imbricata imbricata*	Mexico: Michoacán: Cerro Angahuan	LVT 10836
437	*Barisia imbricata imbricata*	Mexico: Guerrero: Sierra Taxco	LVT 10837
438	*Barisia imbricata ciliaris*	Mexico: San Luis Potosí: Real de Catorce	LVT 10838
439	*Barisia imbricata ciliaris*	Mexico: Jalisco: Sierra Huichol	LVT 10839
440	*Barisia herrerae*	Mexico: Estado de México: Ocuilan	LVT 10840
MZFC JAL	*Barisia imbricata imbricata*	Mexico: Jalisco: Manantlán	GenBank AY605116
MZFC MICH	*Barisia rudicollis*	Mexico: Michoacán: El Pinal, Mpo. Tuxpan	GenBank AY605121
–	*Elgaria kingii*	Mexico: Chihuahua: Sierra del Nido	LVT 10841
–	*Abronia graminea*	Mexico: Veracruz: Puerto del Aire	LVT 10842

All samples deposited in the Las Vegas Tissue Collection (LVT), Ambrose Monell Cryo Collection (AMCC), or Museum of Vertebrate Zoology, University of California, Berkeley (MVZ).

5′-CAATGCTCAGARATYTGYGG-3′; C3LR, 5′-GCG TGTGYTTGGTGGGTCAT-3′), and required an annealing temperature of 50 °C. Forward and reverse sequences for each individual were edited and manually aligned using SEQUENCHER, version 4.2 (Gene Codes Corporation).

PHYLOGENETIC INFERENCE

Phylogenies for the mtDNA data were reconstructed using Bayesian inference (BI) and maximum likelihood methods. MRMODELTEST, version 2.1 (Nylander, 2004) was used to select a best-fit model of evolution, based on Akaike information criteria (AIC), for each gene region (ND4, tRNAs, ATPase 8, ATPase 6). Bayesian inference analyses were conducted on a partitioned-by-gene dataset using MrBayes, version 3.1 (Ronquist & Huelsenbeck, 2003). Bayesian settings included a variable rate prior, a mean branch length exponential prior of 75, and heating temperature of 0.05. All parameters except branch length and topology were unlinked between partitions. Analyses consisted of four runs (using the nruns = 4 command) for 4×10^6 generations using three heated and one cold Markov chain sampling every 100 generations. Output parameters were visualized using TRACER, version 1.4 (Rambaut & Drummond, 2007) to ascertain stationarity and convergence. Convergence occurred during the first 400 000 generations of each run; subsequently, we conservatively discarded all samples obtained during the first one million (25%) generations as burn-in. A 50% majority-rule consensus phylogram with nodal posterior probability support was estimated from the combination of the four runs post-burn-in. Maximum likelihood analyses were conducted using RAxML, version 7.0.3 (Stamatakis, 2006) with the same partitioning scheme used for the BI analyses. The GTRGAMMA model was used, and 1000 nonparametric bootstrap replicates were performed to assess nodal support. We considered those nodes with ≥ 95% Bayesian posterior probability and ≥ 70% bootstrap support as strongly supported (Hillis & Bull, 1993; Felsenstein, 2004).

TIMING AND TEMPO OF DIVERSIFICATION

Divergence dates were estimated from a partitioned-by-gene dataset of ingroup samples using BEAST, version 1.6.1 (Drummond & Rambaut, 2007). To calibrate our tree, we used rate calibrations for mtDNA employed previously to estimate divergences in anguid lizards (0.65–0.69% change/lineage/million years; Macey *et al.*, 1999) and within *Barisia* (0.85% change/lineage/million years; Zaldivar-Riverón *et al.*, 2005). We unlinked best-fit models of sequence evolution across partitions, implemented an uncorrelated lognormal clock with a Yule tree prior, and gave the ulcd.mean parameter a uniform distribution with the lower bound set to 6.5×10^{-3} substitutions/site/million years, and the upper bound set to 8.5×10^{-3} substitutions/site/million years. Analyses consisted of two independent runs each of 4×10^7 million generations, with samples retained every 1000 generations. A Yule tree prior does not include a model of coalescence to account for intraspecific data represented in our heterogeneous dataset with both inter- and intraspecific diversity, so we ran additional analyses on a reduced dataset with only one representative from each lineage for comparison. Results were displayed in TRACER to confirm acceptable mixing and likelihood stationarity, appropriate burn-in, and adequate effective sample sizes (> 200 for each estimated parameter). After discarding the first four million generations (10%) as burn-in, the trees and parameter estimates from the two runs were combined using LOGCOMBINER, version 1.6.1 (Drummond & Rambaut, 2007). The parameter values of the samples from the posterior distribution were summarized on the maximum clade credibility tree using TREEANNOTATOR, version 1.6.1 (Drummond & Rambaut, 2007), with the posterior probability limit set to zero and mean node heights summarized.

We analyzed temporal shifts in diversification rates using maximum likelihood-based diversification-rate analysis (Rabosky, 2006a) and divergence dates estimated in BEAST. The fit of different birth–death models implementing two constant rates (pure-birth and birth–death) and four variable rates (exponential and logistic density-dependent and two-rate and three-rate pure-birth) was computed with LASER, version 2.3 (Rabosky, 2006b). Model fit was measured using AIC scores. Significance of the change in AIC scores (ΔAICrc) between the best rate-constant and best rate-variable model was determined by creating a null distribution for ΔAICrc. This was done by simulating 1000 trees using yuleSim in LASER with the same number of nodes and the same speciation rate as that estimated under the pure-birth model. We additionally generated a lineage-through-time plot using the plotLtt function in LASER to visualize the pattern of accumulation of log-lineages over time.

ANCESTRAL AREA RECONSTRUCTION

Ancestral areas for *Barisia* were reconstructed using a stochastic model of geographical range evolution (dispersal–extinction–cladogenesis; DEC) implemented in LAGRANGE, version 2.0.1 (Ree & Smith, 2008). Although the DEC model is unable to account for phylogenetic uncertainty, it can uniquely incorporate temporal and spatial constraints on range evolution that may result in more plausible area range histories (Clark *et al*., 2008; Ree & Smith, 2008). The maximum clade-credibility tree from BEAST analyses was used in DEC analyses, and each taxon in the genealogy was coded as present or absent in one of eight biogeographical areas based on distribution (Fig. 1): northern Sierra Madre Occidental, southern Sierra Madre Occidental, northern Sierra Madre Oriental, southern Sierra Madre Oriental, Trans-Mexican Volcanic Belt, western Sierra Madre del Sur, and eastern Sierra Madre del Sur. The region south of the Trans-Mexican Volcanic Belt in Puebla, Veracruz, and northern Oaxaca has a complex geological history, and contains geological and biotic elements of the Sierra Madre Oriental, Trans-Mexican Volcanic Belt, and Sierra Madre del Sur (Marshall & Liebherr, 2000; Salinas-Moreno *et al*., 2004; Corona, Toledo & Morrone, 2007; León Paniagua & Morrone, 2009). For our analyses, we considered this region as part of the Sierra Madre Oriental based on faunal affinities (León Paniagua & Morrone, 2009). Range sizes were constrained to the maximum number of biogeographical regions presently inhabited by any single lineage. We additionally constrained widespread ancestors to spatially adjacent areas (Fig. 1) to exclude unlikely ranges (e.g. northern Sierra Madre Occidental + Sierra Madre del Sur).

RESULTS

SEQUENCE CHARACTERISTICS AND PHYLOGENETIC ESTIMATE

The final dataset consisted of 1714 aligned nucleotide positions. Models of sequence evolution selected for partitions in both BI and BEAST analyses were GTR + I + G (ND4, ATPase 8, ATPase 6) and GTR + I (tRNA). All sequences were deposited in GenBank (accession numbers JN885202–JN885305).

From our phylogenetic analyses, we inferred ten strongly supported lineages within three geographical clades of *Barisia* (Figs 3, 4). Four lineages corresponded to the species *B. levicollis*, *B. planifrons*, *B. rudicollis*, and *B. herrerae*. Two subspecies of *B. imbricata*, *B. i. ciliaris* and *B. i. imbricata*, appeared to each be a composite of three distinct lineages. Our sample of *B. i. jonesi* was similar to geographically adjacent samples of *B. i. imbricata*. The composition of clades was:

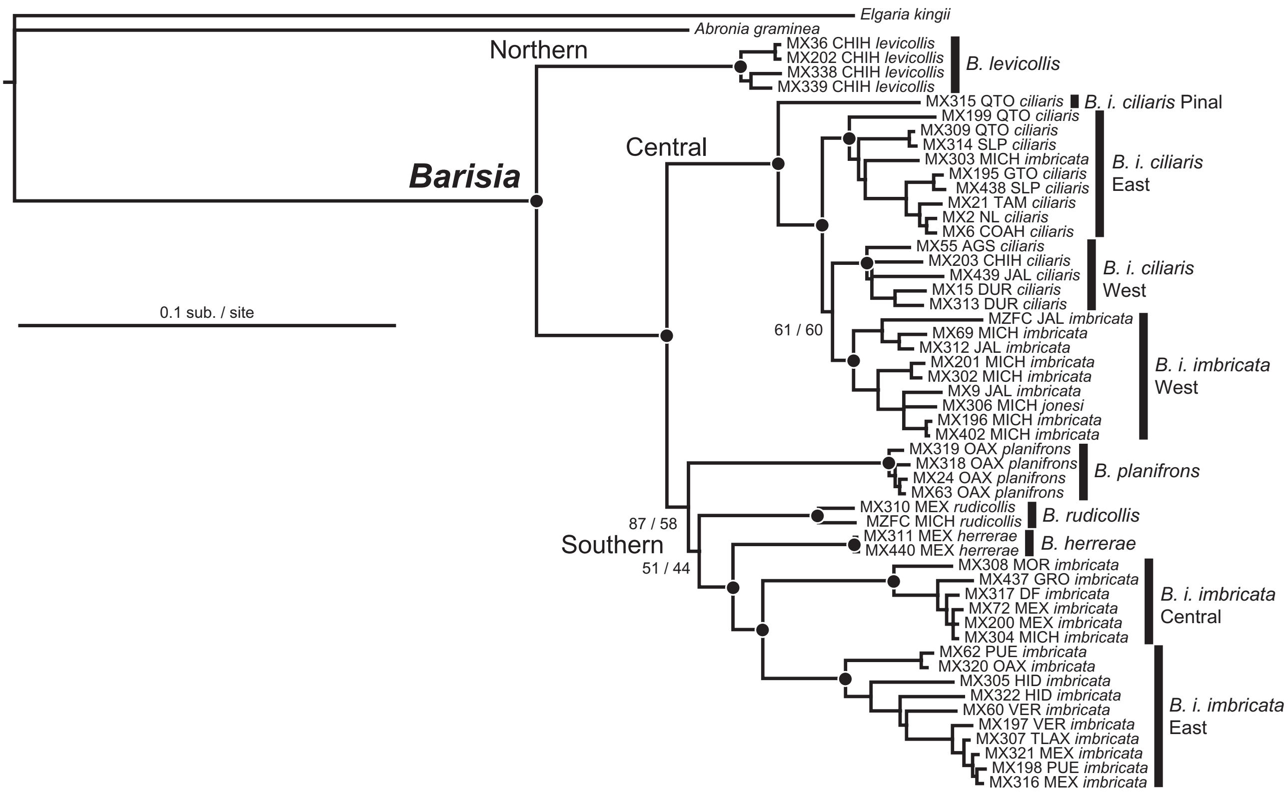


Figure 3. Maternal genealogy of *Barisia* based on mixed-model Bayesian inference (tree shown) and maximum likelihood analyses of mitochondrial DNA sequence data. Numbers at nodes indicate support values (Bayesian posterior probability followed by maximum likelihood bootstrap). Nodes that received ≥ 95% Bayesian posterior probability and ≥ 70% bootstrap support are depicted with black dots.

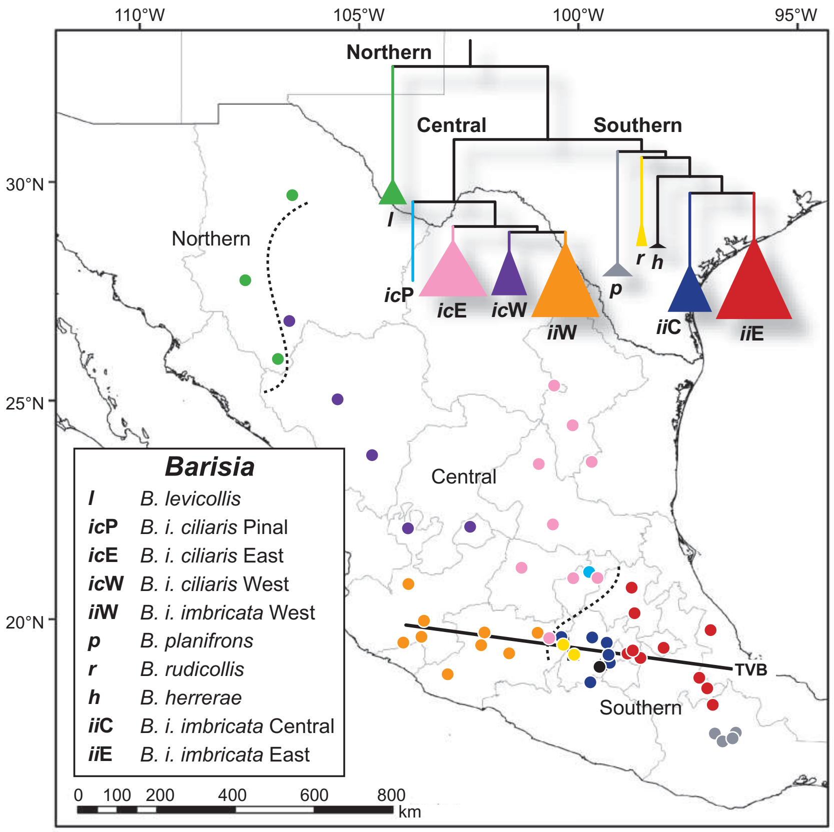


Figure 4. Geographical distribution of inferred mitochondrial lineages within *Barisia*. Lineages are colour-coded to correspond to haplotypes plotted on the map. Dotted lines denote approximate breaks between northern, central, and southern clades. Note midpoint of the Trans-Mexican Volcanic Belt (TVB) in relation to lineage distributions.

1. Northern clade: one lineage, *B. levicollis*, from the northern Sierra Madre Occidental.
2. Central clade: comprising four lineages. Samples from the southern Sierra Madre Occidental and one sample from southern Chihuahua formed a lineage, here termed '*B. i. ciliaris* West'. A second lineage was distributed from the northern Sierra Madre Oriental across the Central Mexican Plateau ('*B. i. ciliaris* East'). This lineage also contained one sample from the southern Sierra Madre Oriental and one sample of *B. i. imbricata* from the Trans-Mexican Volcanic Belt. We referred to a single divergent sample from Pinal de Amoles, Querétaro as '*B. i. ciliaris* Pinal', the third lineage. Samples from the western half of Trans-Mexican Volcanic Belt, including the sample of *B. i. jonesi*, formed the fourth lineage '*B. i. imbricata* West'.
3. Southern clade: comprising five lineages. Samples from western Michoacán east to Distrito Federal formed a '*B. i. imbricata* Central' lineage. The second lineage, '*B. i. imbricata* East', comprised samples distributed from Hidalgo south through Tlaxcala, Puebla, Veracruz, and northern Oaxaca, spanning the southern Sierra Madre Oriental and eastern portion of the Trans-Mexican Volcanic Belt. The remaining three lineages represent *B. planifrons* from the eastern Sierra Madre del Sur, and *B. rudicollis* and *B. herrerae* from the central portion of the Trans-Mexican Volcanic Belt.

Relationships among lineages were generally well supported with three exceptions (Fig. 3). The node subtending *B. i. ciliaris* West and *B. i. imbricata* West received low support (61% posterior probability and 60% bootstrap). Weak support was also inferred for the basal divergence of *B. planifrons* from *B. rudicollis*, *B. herrerae*, *B. i. imbricata* Central, and *B. i. imbricata* East (87% posterior probability and 58% bootstrap), and the divergence of *B. rudicollis* from *B. herrerae*, *B. i. imbricata* Central, and *B. i. imbricata* East (51% posterior probability and 44% bootstrap).

DIVERGENCE TIMES AND TEMPO OF DIVERSIFICATION

Results from analyses on the full and reduced datasets were nearly identical; means varied less

Table 2. Estimated divergence dates within *Barisia* derived from Bayesian relaxed clock estimates

Node	Posterior mean age (95% HPD)	Ancestral area (relative probability)
1	11 (8.4–13.7)	Northern SMOc/northern SMOc + southern SMOc + southern SMOr + TVB (0.33)
2	8.9 (7.2–10.7)	Northern SMOc + southern SMOc + southern SMOr + TVB/TVB (0.39)
3	5.1 (3.9–6.3)	Southern SMOr/northern SMOc + southern SMOc + southern SMOr + TVB (0.34) Southern SMOr/northern SMOc + southern SMOc + CMP + TVB (0.21) Southern SMOr/northern SMOc + southern SMOc + TVB (0.15)
4	3.7 (2.9–4.5)	Southern SMOr/northern SMOc + southern SMOc + TVB (0.36) Northern SMOr + southern SMOr + CMP + TVB/TVB (0.26) TVB/northern SMOc + southern SMOc + TVB (0.12)
5	3.4 (2.7–4.1)	Northern SMOc + southern SMOc/TVB (0.56) Southern SMOc/TVB (0.36)
6	8 (6.4–9.7)	TVB/TVB (0.50) Eastern SMS/TVB (0.47)
7	7.7 (6.2–9.4)	TVB/TVB (0.99)
8	6.7 (5.4–8.2)	TVB/TVB (0.97)
9	6.1 (4.8–7.4)	TVB/TVB (0.88)

Numbers refer to nodes identified in Fig. 5. Posterior mean ages and 95% highest posterior density intervals (HPD) are provided in millions of years ago. Ancestral areas reconstructed for each node are followed by relative probability values. Area reconstructions at each node are the split of areas inherited by the two descendent branches (upper branch followed by lower branch). The optimal ancestral areas with the highest probabilities were selected among the alternatives and presented in Fig. 5. SMOc, Sierra Madre Occidental; SMOr, Sierra Madre Oriental; CMP, Central Mexican Plateau; TVB, Trans-Mexican Volcanic Belt; SMS, Sierra Madre del Sur.

than 0.5 Mya at each node. Dating estimates based on analyses of the full dataset suggested that diversification in *Barisia* probably began in the Late Miocene (Fig. 5, Table 2) with an initial split between *B. levicollis* and the remaining lineages of *Barisia*. Divergences among the nine remaining lineages appeared to have followed later in the Neogene during the Late Miocene and Pliocene. Diversification within lineages likely began near the Pliocene–Pleistocene boundary and into the Pleistocene.

Birth–death likelihood analyses rejected the null hypothesis of rate-constancy ($P = 0.01$). The rate-variable model that best fit the dataset was the logistic density-dependent model. Under this model, diversification rate in *Barisia* has gradually decreased through time, with diversification rate estimated at 0.8 divergences per million years (Fig. 6).

Ancestral area reconstruction

The maximum number of biogeographical regions inhabited within an extant lineage was four in *B. i. ciliaris* East. Optimal ancestral areas with the highest likelihood scores and probabilities were identified at each node (Fig. 5). Alternative ancestral areas within two log-likelihood units were summarized in Table 2. One node with alternative ancestral areas (node 6) appeared unresolved; probabilities between alternative reconstructions differed by less than 10%. Initial diversification within *Barisia* (node 1) probably occurred between the northern Sierra Madre Occidental and a widespread common ancestor distributed across the Sierra Madre Occidental, southern Sierra Madre Oriental, and Trans-Mexican Volcanic Belt. This widespread ancestor was later fragmented within the Trans-Mexican Volcanic Belt (node 2), and the ancestor within the Trans-Mexican Volcanic Belt subsequently split four times across this mountain range (nodes 6–9) and dispersed into the eastern Sierra Madre del Sur. The remaining ancestor distributed across the Sierra Madre Occidental, southern Sierra Madre Oriental, and Trans-Mexican Volcanic Belt later diverged within the southern Sierra Madre Oriental (node 3) before splitting apart (nodes 4 and 5). Colonization of the western isolate of the Sierra Madre del Sur occurred relatively recently from the Trans-Mexican Volcanic Belt (node not shown).

DISCUSSION

Based on the results of the present study, alligator lizards in the genus *Barisia* have had a long history in the Mexican highlands. Diversification likely began in the Late Miocene in a wide-ranging ancestor distributed across the Sierra Madre Occidental, Trans-Mexican Volcanic Belt, and southern Sierra Madre

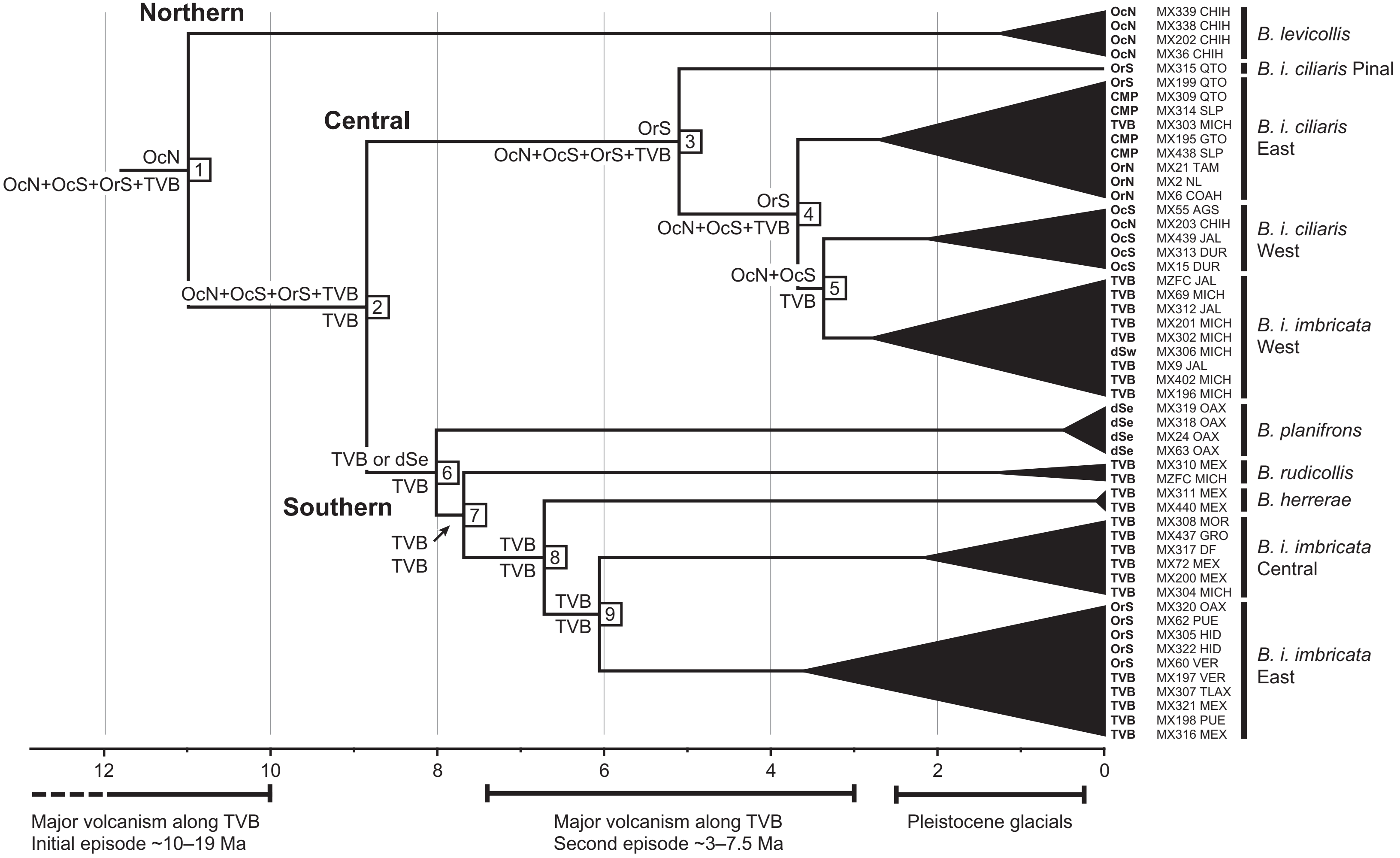


Figure 5. Chronogram in millions of years (Ma) for lineage divergences within *Barisia* and maximum likelihood reconstruction of geographical range evolution. Optimal ancestral areas with the highest likelihood scores and the highest probabilities are presented at each node. Alternative ancestral areas within two log-likelihood units and estimated divergence dates for numbered nodes are provided in Table 2. Time frames spanning two distinct volcanic episodes across the Trans-Mexican Volcanic Belt and the Pleistocene period are shown. Biogeographical areas are delineated in Figure 1 and are: northern Sierra Madre Occidental (OcN), southern Sierra Madre Occidental (OcS), northern Sierra Madre Oriental (OrN), southern Sierra Madre Oriental (OrS), Central Mexican Plateau (CMP), Trans-Mexican Volcanic Belt (TVB), eastern Sierra Madre del Sur (dSe), and western Sierra Madre del Sur (dSw).

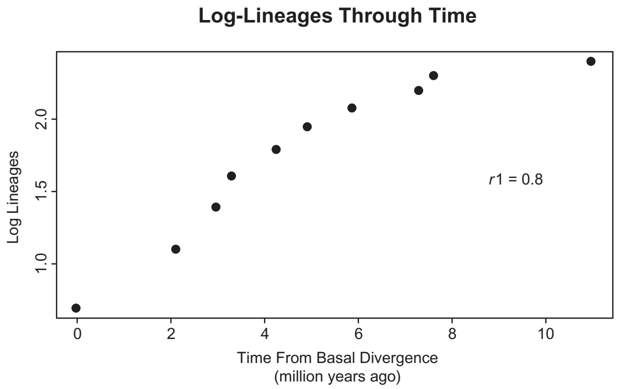


Figure 6. Lineage through time plot derived from Bayesian relaxed clock estimates of divergence dates within *Barisia*. Birth–death likelihood analyses suggest a gradual decrease in diversification rate though time, with diversification rate (r) estimated at 0.8 divergences per million years.

Oriental (Fig. 5). Following this initial split approximately 11 Mya, a second divergence occurred within this wide-ranging ancestor approximately 9 Mya, and two geographical clades subsequently emerged. Divergences within these regional clades appear to have happened during distinctly different temporal periods. A southern clade distributed across the Trans-Mexican Volcanic Belt and into the eastern Sierra Madre del Sur split rapidly between 6–8 Mya into five lineages (*B. planifrons*, *B. herrerae*, and *B. i. imbricata* Central and East). Afterwards, diversification across a broader landscape approximately 3–5 Mya led to the formation of the four lineages within the northern clade (*B. i. ciliaris* Pinal, *B. i. ciliaris* East and West, and *B. i. imbricata* West).

Lineage diversification in *Barisia* during the Neogene appears linked with the development of the Trans-Mexican Volcanic Belt (Fig. 5). The formation of this mountain range across south-central Mexico impacted diversification in a range of highland and lowland species (Bryson *et al.*, 2011c). Uplift of the Trans-Mexican Volcanic Belt created new geographical barriers and united previously isolated highland biotas (Anducho-Reyes *et al.*, 2008). Five lineages of *Barisia* are broadly distributed across the Trans-Mexican Volcanic Belt (Fig. 4). Mean estimated divergence dates among all five of these lineages (Table 2) fall near or within a second major episode of widespread volcanism along the Trans-Mexican Volcanic Belt in the Neogene (Fig. 5) (Gómez-Tuena, Orozco-Esquivel & Ferrari, 2007). Because of the relatively young geological age of this second episode approximately 3–7.5 Mya, the extensive uplifting likely left a large evolutionary imprint on the genetic structures of extant lineages that has not yet eroded through time. Indeed, a variety of co-distributed taxa associated with the Trans-Mexican Volcanic Belt demonstrate mean genetic divergences temporally congruent with this second period of Trans-Mexican Volcanic Belt formation, including fish (Hulsey *et al.*, 2004), amphibians (Mulcahy & Mendelson, 2000), reptiles (Bryson *et al.*, 2011a, c; Bryson & García-Vázquez, 2011), and birds (McCormack *et al.*, 2008, 2010). Similarity in spatio-temporal pattern of divergence between *Barisia* and taxonomic groups that differ in ecology, demography, and life history suggest a common underlying mechanism of diversification that appears attributable to uplift of the Trans-Mexican Volcanic Belt. Although this finding is not necessarily novel (Mulcahy, Morrill & Mendelson, 2006; Bryson *et al.*, 2011c), diversification tied to a specific period of uplift, specifically the second widespread episode approximately 3–7.5 Mya, emerges as a testable biogeographical hypothesis for future studies.

The diversification rate within *Barisia* appears to be slowly declining (Fig. 6). This is likely attributable to early diversification linked to the formation of the Trans-Mexican Volcanic Belt followed by diversification linked to the formation of filter barriers. A period of initial widespread uplifting of the Trans-Mexican Volcanic Belt between approximately 10–19 Mya (Gómez-Tuena *et al.*, 2007) may have created a highland corridor that promoted diversification through dispersal between the montane biotas of the Sierra Madre Occidental and Sierra Madre Oriental. This Late Miocene linkage could explain our reconstruction of initial fragmentation approximately 11 Mya of a widespread common ancestor distributed across the Sierra Madre Occidental, Trans-Mexican Volcanic Belt, and southern Sierra Madre Oriental. Similar patterns in co-distributed highland taxa of deep divergences between largely northern and southern highland clades (e.g. bunchgrass lizards; Bryson & García-Vázquez, 2011) might also be attributed to an early connection of highland biotas across the Trans-Mexican Volcanic Belt. A second episode of widespread uplifting across the Miocene–Pliocene boundary may have subsequently divided *Barisia* distributed along the Trans-Mexican Volcanic Belt. *Barisia* to the north were later likely fragmented during the Pliocene, perhaps as filter barriers such as major river drainages (Bryson *et al.*, 2011a) formed and subdivided lineages. Most notably, the Lerma-Santiago and Rio Pánuco Basins across the Central Mexican Plateau may have isolated lineages of *Barisia*, a pattern observed in several co-distributed highland species (Anducho-Reyes *et al.*, 2008; McCormack *et al.*, 2008; Moreno-Letelier & Piñero, 2009; Bryson *et al.*, 2011a). However, estimated dates of divergences within these taxa across these river basins differ by several million years. This suggests that these and presumably other filter barriers may be differentially affecting lineage splitting through time.

Diversification within several widespread lineages of *Barisia* such as *B. i. ciliaris* West and East and *B. i. imbricata* East appear to date to near the Pliocene–Pleistocene boundary. However, an overall lack of clear geographical partitioning of genetic diversity within these and other lineages (Figs 3, 4) makes inferences on the historical processes responsible for within-lineage diversification difficult. Expansions of pine-oak woodlands during Pleistocene glacial cycles (Bryson *et al*., 2011c; Gugger *et al*., 2011) may have promoted dispersal and periodic bouts of gene flow that could have erased or obscured previously acquired signals of historical isolation in *Barisia*. Similar patterns of deep genetic subdivisions blurred by overlapping lineage boundaries have been observed in other co-distributed highland taxa (Moreno-Letelier & Piñero, 2009; Ornelas, Ruiz-Sánchez & Sosa, 2010; Bryson *et al*., 2011c). Future studies utilizing multilocus data are needed to better discern patterns of incomplete lineage sorting and gene flow within and between major lineages of *Barisia*.

The results from the present study are broadly congruent with results from a previous study (Zaldivar-Riverón *et al*., 2005) with few notable exceptions. Our range-wide sampling not only helped further delineate distributions of mtDNA lineages, but also elucidated the discovery of a deeply divergent lineage, *B. levicollis*. Zaldivar-Riverón *et al*. (2005), lacking molecular data for *B. levicollis* and relying on morphology, suggested a close relationship between this species and northern populations of *B. i. ciliaris* (Zaldivar-Riverón *et al*., 2005). Our data suggest that *B. levicollis* might instead be a paleoendemic restricted to the northern Sierra Madre Occidental, sister to all other *Barisia*. Furthermore, based on our results, localities of two samples used previously appear to be reversed ('*B. i. imbricata*2' and '*B. i. imbricata*8'). This is additionally confirmed by our comparison of ND4 sequences from identical (Mil Cumbres, Michoacán) and nearby (Pico de Orizaba and Xometla, Veracruz) localities. Reversing operational taxonomic units in the combined phylogenetic analyses of Zaldivar-Riverón *et al*. (2005) might change their results on the evolution of dorsal pattern in these lizards.

As with other taxa restricted to the highlands of Mexico, *Barisia* are threatened by an ever-expanding human population and habitat destruction. At least two species (*B. rudicollis* and *B. herrerae*) are confined to a relatively small region adjacent to one of the world's most populated areas, and both species may already be in decline (Zaldivar-Riverón & Nieto-Montes de Oca, 2002). *Barisia rudicollis* and *B. herrerae* are considered endangered by the International Union for Conservation of Nature (Flores-Villela & Canseco-Márquez, 2007). Another distinct lineage inferred in the present study from near Pinal de Amoles, Querétaro may also have a small range. Future studies should focus on further delineating evolutionary distinct taxa within *Barisia*. In particular, the currently recognized subspecies *B. i. imbricata* and *B. i. ciliaris* each appear to be a composite of distinct taxa. These findings viewed in concert with previous phylogeographical studies may fuel new interest in elucidating potentially hidden yet critically threatened components of a unique Mexican highland biota.

ACKNOWLEDGEMENTS

We dedicate the present study to the late Fernando Mendoza-Quijano. Without his enthusiasm and ample support through the years, this study would not have been possible. We thank the following people, curators, and institutions for providing tissue samples: Ambrose Monell Cryo Collection and D. R. Frost (AMNH); A. Kardon (San Antonio Zoo); J. Alvarado-Díaz (Universidad Michoacána de San Nicolás de Hidalgo, Michoacán); O. Flores-Villela and A. Nieto-Montes (MZFC, Universidad Nacional Autónoma de México); D. Lazcano (Universidad Autónoma de Nuevo León); J. A. Campbell, C. Franklin, and E. N. Smith (University of Texas at Arlington); U. O. García-Vázquez, C. Grüenwald, J. Jones, J. Meik, E. Mociño-Deloya, K. Setser, and J. Reyes-Velasco. We thank the numerous people that assisted in the field, including E. García-Padilla, C. Grüenwald, R. W. Hansen, C. Harrison, J. Jones, G. Quijano-Manila, F. R. Mendoza-Paz, F. Mendoza-Quijano, S. A. Neiswenter, M. Price, S. Ruane, B. T. Smith, B. Thomason, and M. Torocco. This project was funded in part through grants from the American Museum of Natural History (Theodore Roosevelt Memorial Fund), Southwestern Association of Naturalists (Howard McCarley Student Research Award), and UNLV (Graduate and Professional Student Association and Major Research Instrumentation grant DBI-0421519). Collecting was conducted under permits granted by SEMARNAT to R.W.B., D. Lazcano, and F. Mendoza-Quijano. For additional support and advice, we thank M. R. Graham, J. Jones, J. Klicka, D. Lazcano, A. Nieto-Montes, J. Pastorini, B. T. Smith, A. Zaldívar-Riverón, and the UNLV Systematics and SaBR Groups.

REFERENCES

Anducho-Reyes MA, Cognato AI, Hayes JL, Zuniga G. 2008. Phylogeography of the bark beetle *Dendroctonus* *mexicanus* Hopkins (Coleoptera: Curculionidae: Scolytinae). *Molecular Phylogenetics and Evolution* **49:** 930–940.

Bryson RW, García-Vázquez UO, Riddle BR. In press. Relative roles of Neogene vicariance and Quaternary climate change on the historical diversification of bunchgrass lizards (*Sceloporus scalaris* group) in Mexico. *Molecular Phylogenetics and Evolution*.

Bryson RW, García-Vázquez UO, Riddle BR. 2011c. Phylogeography of Middle American gophersnakes: mixed responses to biogeographical barriers across the Mexican Transition Zone. *Journal of Biogeography* **38:** 1570–1584.

Bryson RW, Murphy RW, Graham MR, Lathrop A, Lazcano-Villareal D. 2011b. Historical diversification of the *Crotalus intermedius* group and Pleistocene pine-oak woodland connections between Mexico's Sierra Madre Occidental and Sierra Madre Oriental. *Journal of Biogeography* DOI: 10.1111/j.1365-2699.2011.02565.x.

Bryson RW, Murphy RW, Lathrop A, Lazcano-Villareal D. 2011a. Evolutionary drivers of phylogeographical diversity in the highlands of Mexico: a case study of the *Crotalus triseriatus* species group of montane rattlesnakes. *Journal of Biogeography* **38:** 697–710.

Clark JR, Ree RH, Alfaro ME, King MG, Wagner WL, Roalson EH. 2008. A comparative study in ancestral range reconstruction methods: retracing the uncertain histories of insular lineages. *Systematic Biology* **57:** 693–707.

Conroy CJ, Bryson RW, Lazcano D, Knight A. 2005. Phylogenetic position of the pygmy alligator lizard based on mitochondrial DNA. *Journal of Herpetology* **39:** 142–147.

Corona AM, Toledo VH, Morrone JJ. 2007. Does the Trans-Mexican Volcanic Belt represent a natural biogeographic unit? An analysis of the distributional patterns of Coleoptera. *Journal of Biogeography* **34:** 1008–1015.

Daza JM, Castoe TA, Parkinson CL. 2010. Using regional comparative phylogeographic data from snake lineages to infer historical processess in Middle America. *Ecography* **33:** 343–354.

Drummond AJ, Rambaut A. 2007. *Beast*: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7:** 214.

Edwards S, Bensch S. 2009. Looking forwards or looking backwards in avian phylogeography? A comment on Zink and Barrowclough 2008. *Molecular Ecology* **18:** 2930–2933.

Felsenstein J. 2004. *Inferring phylogenies*. Sunderland, MA: Sinauer Associates.

Flores-Villela O, Canseco-Márquez L. 2007. *Barisia herrerae*. *IUCN 2010. IUCN Red List of Threatened Species*, Version 2010.4. Available at: http://www.iucnredlist.org. Accessed 4 April 2011.

Gómez-Tuena A, Orozco-Esquivel MaT, Ferrari L. 2007. Igneous petrogenesis of the Trans-Mexican Volcanic Belt. *Geological Society of America, Special Paper* **422:** 129–181.

Gugger PF, Gonzalez-Rodriguez A, Rodriguez-Correa H, Sugita S, Cavender-Bares J. 2011. Southward Pleistocene migration of Douglas-fir into mexico: phylogeography, ecological niche modeling, and conservation of 'rear edge' populations. *New Phytologist* **189:** 1185–1199.

Guillette LJ, Smith HM. 1982. A review of the Mexican lizard *Barisia imbricata*, and the description of a new subspecies. *Transactions of the Kansas Academy of Sciences* **85:** 13–33.

Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42:** 182–192.

Hulsey CD, García de León FJ, Johnson YS, Hendrickson DA, Near TJ. 2004. Temporal diversification of Mesoamerican cichlid fishes across a major biogeographic boundary. *Molecular Phylogenetics and Evolution* **31:** 754–764.

Kuriyama T, Brandley MC, Katayama A, Mori A, Honda M, Hasegawa M. 2011. A time-calibrated phylogenetic approach to assessing the phylogeography and colonization history of snakes in the Japanese Izu Islands. *Journal of Biogeography* **38:** 259–271.

Leaché AD, Mulcahy DG. 2007. Phylogeny, divergence times, and species limits of spiny lizards (*Sceloporus magister* species group) in western North American deserts and Baja California. *Molecular Ecology* **16:** 5216–5233.

León Paniagua L, Morrone JJ. 2009. Do the Oaxacan Highlands represent a natural biotic unit? A cladistic biogeographical test based on vertebrate taxa. *Journal of Biogeography* **36:** 1939–1944.

Lindell J, Méndez-de la Cruz FR, Murphy RW. 2008. Deep biogeographical history and cytonuclear discordance in the black-tailed brush lizard (*Urosaurus nigricaudus*) of Baja California. *Biological Journal of the Linnean Society* **94:** 89–104.

Macey JR, Schulte JA II, Larson A, Tuniyev BS, Orlov N, Papenfuss TJ. 1999. Molecular phylogenetics, tRNA evolution, and historical biogeography in anguid lizards and related taxonomic families. *Molecular Phylogenetics and Evolution* **12:** 250–272.

Marshall CJ, Liebherr JK. 2000. Cladistic biogeography of the Mexican transition zone. *Journal of Biogeography* **27:** 203–216.

McCormack JE, Peterson AT, Bonaccorso E, Smith TB. 2008. Speciation in the highlands of Mexico: genetic and phenotypic divergence in the Mexican jay (*Aphelocoma ultramarina*). *Molecular Ecology* **17:** 2505–2521.

McCormack JE, Zellmer AJ, Knowles LL. 2010. Does niche divergence accompany allopatric divergence in *Aphelocoma* jays as predicted under ecological speciation?: Insights from tests with niche models. *Evolution* **64:** 1231–1244.

Mittermeier RA, Gil PR, Hoffman M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J, da Fonseca GAB. 2005. *Hotspots revisited: earth's biologically richest and most endangered terrestrial ecoregions*. Washington, DC: Conservation International.

Moreno-Letelier A, Piñero D. 2009. Phylogeographic structure of *Pinus strombiformis* Engelm. across the Chihuahuan Desert filter-barrier. *Journal of Biogeography* **36:** 121–131.

Mulcahy DG, Mendelson JR III. 2000. Phylogeography and speciation of the morphologically variable, widespread species *Bufo valliceps*, based on molecular evidence from mtDNA. *Molecular Phylogenetics and Evolution* **17:** 173–189.

Mulcahy DG, Morrill BH, Mendelson JR III. 2006. Historical biogeography of lowland species of toads (*Bufo*) across the Trans-Mexican Neovolcanic Belt and the Isthmus of Tehuantepec. *Journal of Biogeography* **33:** 1889–1904.

Nylander JAA. 2004. *Mrmodeltest*, Version 2. Software distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.

Ornelas JF, Ruiz-Sánchez E, Sosa V. 2010. Phylogeography of *Podocarpus matudae* (Podocarpaceae), pre-Quaternary age relicts in the northern Mesoamerican cloud forests. *Journal of Biogeography* **37:** 2384–2396.

Pyron RA, Burbrink FT. 2009. Lineage diversification in a widespread species: roles for niche divergence and conservatism in the common kingsnake, *Lampropeltis getula*. *Molecular Ecology* **18:** 3443–3457.

Rabosky DL. 2006a. Likelihood methods for inferring temporal shifts in diversification rates. *Evolution* **60:** 1152–1164.

Rabosky DL. 2006b. LASER: a maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenies. *Evolutionary Bioinformatics Online* **2:** 257–260.

Ramamoorthy T, Bye R, Lot A, Fa J. 1993. *Biological diversity of Mexico: origins and distribution*. Oxford: Oxford University Press.

Rambaut A, Drummond AJ. 2007. *TRACER*, Version 1.4. Available at: http://beast.bio.ed.ac.uk/Tracer

Ree RH, Smith SA. 2008. Maximum-likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* **57:** 4–14.

Riddle BR, Dawson MN, Hadly EA, Hafner DJ, Hickerson MJ, Mantooth SJ, Yoder AD. 2008. The role of molecular genetics in sculpting the future of integrative biogeography. *Progress in Physical Geography* **32:** 173–202.

Ronquist F, Huelsenbeck JP. 2003. *MrBayes 3*: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Salinas-Moreno Y, Mendoza-Correa G, Barrios MA, Cisneros R, Macías-Sámano J, Zúñiga G. 2004. Areography of the genus *Dendroctonus* (Coleoptera: Curculionidae: Scolytinae) in México. *Journal of Biogeography* **31:** 1163–1177.

Shafer ABA, Cullingham CI, Côté SD, Coltman DW. 2010. Of glaciers and refugia: a decade of study sheds new light on the phylogeography of northwestern North America. *Molecular Ecology* **19:** 4589–4621.

Soltis DE, Morris A, McLachlan JS, Manos PS, Soltis PS. 2006. Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology* **15:** 4261–4293.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Zaldivar-Riverón A, Nieto-Montes de Oca A. 2002. Variation in the rare lizard *Barisia rudicollis* (Anguidae) (Wiegmann) with the description of a new species from Central Mexico. *Herpetologica* **58:** 313–326.

Zaldivar-Riverón A, Nieto-Montes de Oca A, Laclette JP. 2005. Phylogeny and evolution of dorsal pattern in the Mexican endemic lizard genus *Barisia* (Anguidae: Gerrhonotinae). *Journal of Zoological Systematics and Evolutionary Research* **43:** 243–257.