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ORIGINAL ARTICLE

Asynchronous diversification of snakes in the North American warm deserts

Edward A. Myers[1,2]*, Michael J. Hickerson[1,3,4] and Frank T. Burbrink[5]

[1]Department of Biology, The Graduate School, City University of New York, New York, NY 10016, USA, [2]Department of Biology, College of Staten Island, 2800 Victory Boulevard, 6S-143, Staten Island, NY 10314, USA, [3]Biology Department, City College of New York, New York, NY 10016, USA, [4]Division of Invertebrate Zoology, The American Museum of Natural History, Central Park West and 79th Street, New York, NY 10024, USA, [5]Department of Herpetology, The American Museum of Natural History, Central Park West and 79th Street, New York, NY 10024, USA

*Correspondence: Edward A. Myers, Department of Biology, The Graduate School, City University of New York, New York, NY 10016, USA.
E-mail: eddie.a.myers@gmail.com

ABSTRACT

Aim We quantify the degree to which co-distributed snakes across the Cochise Filter Barrier (CFB) have a shared history of population divergence and estimate the timing of divergence for each taxon pair.

Location North America.

Methods A single locus dataset was collected ($n = 747$ individuals) for 12 snake taxon pairs. Phylogeographical structure was estimated for each taxon. Redundancy analyses were used to assess the importance of geographical distance, climate and putative barriers to gene flow in structuring genetic diversity. Hierarchical approximate Bayesian computation was used to estimate the magnitude of synchronicity in divergence times across a well-documented phylogeographical barrier. Lastly, gene divergence and population divergence times were estimated using multiple methods.

Results There is substantial phylogeographical structure in many of the snake taxa, particularly at the CFB. A model containing distance, climate and barriers explained the greatest amount of genetic variation in nearly all taxa. When each variable was examined separately, climate explained the most variation. The hABC model testing indicates that there is overwhelming support for asynchronous phylogeographical histories within these co-distributed taxa. Estimated divergence times range throughout the Quaternary and Neogene.

Main Conclusions We demonstrate that the 12 snake taxon pairs studied here have diversified within the desert Southwest forming distinct Sonoran and Chihuahuan populations, illustrating the importance of this region in driving diversification in North American taxa. Although these groups exhibit the same pattern of lineage formation, there is strong support for asynchronous diversification and little concordance in divergence time estimates.

Keywords
biogeography, divergence time estimation, geographical barrier, lineage formation, Pleistocene speciation, redundancy analysis, reptiles, vertebrates

INTRODUCTION

Climate and associated habitat change have acted as drivers of species diversification and altered community composition, particularly during the glacial cycles of the Pleistocene (Rand, 1948; Arbogast & Slowinski, 1998). The Pleistocene species pump hypothesis (PSP) suggests that glacial advances restricted sister populations in allopatric refugia resulting in species divergence (Knowles, 2000; Weir & Schluter, 2004). However, some studies suggest that species diversification preceded the Pleistocene (Klicka & Zink, 1997; Zink *et al.*, 2004). Despite the timing of species diversification, many taxa were displaced from much of their current distributions while tracking suitable habitat as glaciers repeatedly advanced and retreated during the Pleistocene (Hewitt, 2000). Therefore, it might be expected that there are concerted phylogeographical patterns within co-distributed species (Arbogast & Kenagy, 2001). These concordant patterns might also extend to a single pulse of diversification across multiple population pairs spanning the same barrier to gene flow. Evidence

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against this has been reported, where divergence times are not shared but rather the observed phylogeographical patterns are the result of various processes occurring at different times (Soltis *et al.*, 2006).

The Cochise filter barrier (CFB, a region that coincides with the Western Continental Divide; Fig. 1), which is an ecotonal area between the Sonoran and Chihuahuan Deserts, has been recognized as a region promoting lineage divergence, a suture zone between recently diverged taxa, and a filter barrier between the two deserts (Remington, 1968; Morafka, 1977; Zink *et al.*, 2001). Palaeo-niche models and fossil pollen data suggest this region would have been inhospitable to desert-adapted taxa during the Last Glacial Maximum (Thompson & Anderson, 2000; Rebernig *et al.*, 2010; Zink, 2014). The PSP hypothesis would be supported if glacial-pluvial cycles were driving ecologically-mediated divergence (Riddle & Hafner, 2006; Pyron & Burbrink, 2010). However, it is possible that divergence at the CFB is older than the Pleistocene, particularly given the initial desertification in the Pliocene and the uplift of the Sierra Madre Occidental during the Miocene (Riddle & Hafner, 2006; Wilson & Pitts, 2010a).

Despite numerous phylogeographical studies at the CFB (Zink *et al.*, 2001; Pyron & Burbrink, 2010), it remains unclear how the spatial genetic structure of organisms across the community was formed by the combined effects of isolation by distance, environmental heterogeneity, or other barriers to gene flow. Geographic features might explain spatial genetic structure (Sexton 2014); across the southwestern deserts we might expect divergence to be correlated with sampling locality east or west of the CFB or for genetic diversity to be negatively correlated with longitude. Alternatively, genetic similarity might be a function of geographical distance between populations, resulting in a pattern of isolation by distance (Wright, 1943). Population differentiation could also be due to local adaptation to abiotic factors such as climate (Sexton *et al.*, 2014). Therefore, it is important to understand how each of these variables influence patterns of gene flow and maintain divergent lineages across communities. A comparative phylogeographical approach provides the necessary data to address both the PSP hypothesis while also addressing what variables correlate with genetic diversity.

Herein, we investigate the phylogeographical history of 12 species of snakes co-distributed across arid western North

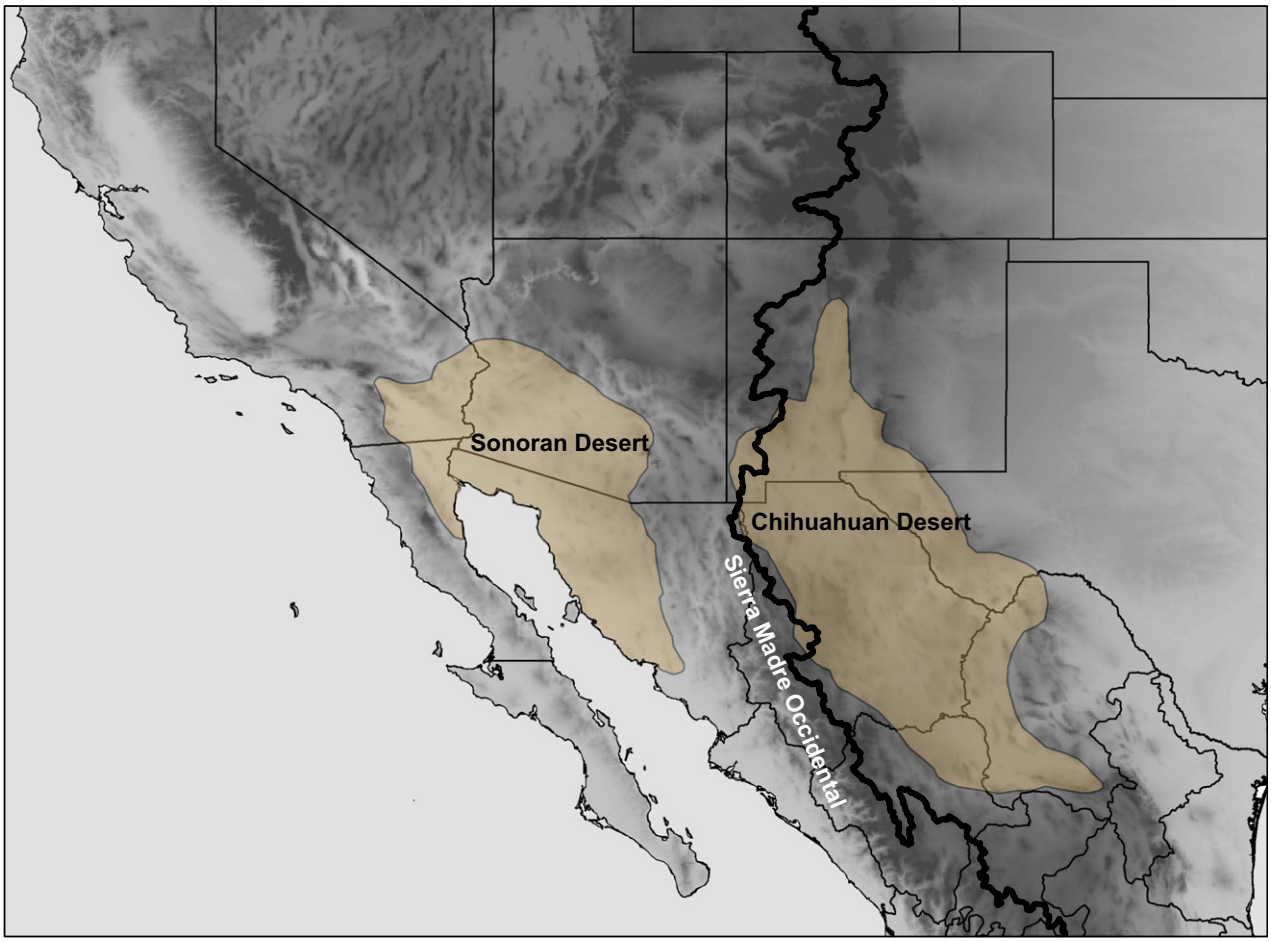


Figure 1 Map of focal region illustrating the Sonoran and Chihuahuan Deserts in tan and the Sierra Madre Occidental. The Western Continental Divide is highlighted in black.

America. The focal taxa occupy similar environments, however, they differ in important ecological characteristics such as body size, dispersal capabilities and microhabitat preferences (Ernst & Ernst, 2003). Five of these species groups have structured populations at the CFB, and many of these lineages have been elevated to species-level status (Devitt, 2006; Castoe *et al.*, 2007; Mulcahy, 2008; Pyron & Burbrink, 2009; Bryson *et al.*, 2011; Anderson & Greenbaum, 2012; Schield *et al.*, 2015). We ask if all 12 species groups have the same determinants of spatial genetic diversity and if genetic diversity is correlated with particular environmental variables, given this scenario we might also expect that sister populations within these species have clustered divergence times. Alternatively, if genetic diversity among taxa is correlated with different variables, then we would expect that they have heterogeneous divergence times and pseudo-congruent phylogeographical histories. We use hierarchical approximate Bayesian computation (hABC) to explicitly model the stochasticity associated with mutation and gene-tree coalescence while allowing species-specific parameters to vary to assess support for a clustering of divergence times. Divergence times based on gene-tree divergence and population divergence are then estimated using a number of methods.

MATERIALS AND METHODS

Study taxa and genetic data

Our focal community consists of 12 snake species that span the CFB (Table 1: the use of nominate species names does not indicate that we disagree with previous species delimitation analyses). Samples were taken from across the distribution of these taxa; however, collecting was focused largely in the Sonoran and Chihuahuan Deserts within the USA. In several instances, sequences from previous studies were downloaded from Genbank (Devitt, 2006; Mulcahy, 2008; Pyron & Burbrink, 2009; Anderson & Greenbaum, 2012) and incorporated into this project. DNA was extracted from tissue with Qiagen DNeasy kits, and the mitochondrial cytochrome *b* (cytb) locus was amplified via PCR using primers H16064 and L14910 (Burbrink *et al.*, 2000). PCR products were cleaned using Exo-Sap-IT (USB Corporation, Santa Clara, CA, USA) and sequenced in the forward direction with the L14919 primer (Burbrink *et al.*, 2000). All sequences were aligned in Geneious using MUSCLE with default parameters and translated to amino acid sequences to ensure an open-reading frame.

Gene-tree estimation

To assess phylogeographical structure and gene divergence times within each taxon, gene trees were generated using Bayesian inference in BEAST 1.8.0 (Drummond *et al.*, 2012). Each tree was rooted with an appropriate outgroup (see Appendix S1 in Supporting Information). The best-fit model of sequence evolution based on AIC was determined using jModeltest2 (Darriba *et al.*, 2012). Best-fit models were implemented in gene-tree estimation, with a constant population size prior, and a molecular clock rate of 1.34×10^{-8}. This mutation rate was chosen based on fossil calibrated divergence time estimates for both cytb and ND4 in colubroid snakes (Daza *et al.*, 2009); importantly, the 95% CI of this estimate broadly overlaps with other estimated mtDNA mutation rates in snakes and therefore is not significantly different from other estimated rates (Zamudio & Greene, 1997; Burbrink *et al.*, 2011). Analyses were run for between 10^6 and 25×10^7 generations, with the first 10% of samples discarded as burn-in. Two BEAST runs were conducted with random starting seeds to ensure Markov chain Monte Carlo (MCMC) chains were converging on similar parameter estimates.

Tests of association with genetic structure

We used redundancy analyses (RDA), a method that tests how much variation in a set of variables is predicted by the variation in another set of variables allowing us to test for correlation between genetic diversity and several abiotic variables. This method can be used to test for correlations while bypassing statistical problems with distance measures using standard Mantel tests (Kierepka & Latch, 2015). A normalized genetic distance matrix was created from sequence data for each taxon, which was then subjected to a principal coordinate analysis (PCoA). Current climate variables interpolated at 2.5 arc-min resolution were obtained from the WorldClim database (Hijmans *et al.*, 2005), and data at sampling localities were extracted. Isothermality and temperature annual range were excluded because they were derived from combinations of existing variables in this dataset. The Western Continental Divide (WCD) is often identified as the barrier responsible for lineage formation in this region (Castoe *et al.*, 2007); we use this distinction here and determine populations based on sampling localities of individuals east or west of the WCD (USGS, 2002). PCoA matrices of genetic distance were used as response variables, where geographical distances between collecting localities, current climate and location east or west of the WCD were used as explanatory variables. Each of these variables could be confounded by the others, thus analyses of single variables were conditioned on the remaining variables (e.g., when a correlation between genetic variation and distance is estimated, climate and the effect of the barrier are controlled). We conducted seven analyses, where predictions of genetic structure were tested using geographical distance, current climate, a geographical barrier, or all combinations of these and a full model with all variables. These analyses return an r^2 value where statistical significance can be assessed using an ANOVA. To conduct these analyses, we used the R packages 'raster', 'rworldmap', 'rgdal', 'ape' and 'vegan' (Paradis *et al.*, 2004; Keitt *et al.*, 2011; South, 2011; Hijmans & van Etten, 2012; Oksanen *et al.*, 2007; R scripts and an example have been deposited on Dryad: doi:10.5061/dryad.74mn5).

Table 1 Total number of base pairs, the model of sequence evolution, total number of individuals sampled and priors used in Bpp analyses for each taxon group. Also presented are the results from five different estimates of divergence time. WCD = Western Continental Divide, GT = gene-tree monophyly.

Taxon	Locus (bp)	Model of sequence evolution	Number of samples	Bpp priors	π_{net}/Mutation rate estimate based on WCD	π_{net}/Mutation rate estimate based on GT	Mode divergence time estimates from Bpp based on WCD (95% HPD)	Mode divergence time estimates from Bpp based on GT (95% HPD)	Mode gene divergence time estimates from beast (95% HPD)
Arizona elegans (Kennicott, 1859)	cytb (1103)	HKY+G	51	Θ~G (2, 50) τ~G (2, 1500)	4.14 Ma	7.22 Ma	1.01 Ma (318 Ka–1.86 Ma)	2.83 Ma (1.01–5.17 Ma)	3.96 Ma (2.58 Ma–5.99 Ma)
Crotalus atrox Baird & Girard, 1853	cytb (963)	GTR+G	62	Θ~G (2, 110) τ~G (2, 1515)	418 Ka	1.43 Ma	828 Ka (368 Ka–1.42 Ma)	2.65 Ma (1.11 Ma–4.90 Ma)	2.87 Ma (1.07 Ma–6.49 Ma)
Crotalus molossus Baird & Girard, 1853	cytb (1114)	HKY+G	50	Θ~G (2, 65) τ~G (2, 317)	4.22 Ma	4.60 Ma	1.21 Ma (420 Ka–2.01 Ma)	5.33 Ma (1.35 Ma–14.0 Ma)	3.04 Ma (1.58 Ma–4.30 Ma)
Crotalus scutulatus (Kennicot, 1861)	cytb (1103)	HKY+I	73	Θ~G (2, 170) τ~G (2, 1500)	1.50 Ma	1.50 Ma	2.09 Ma (758 Ka–4.06 Ma)	2.07 Ma (743 Ka–4.04 Ma)	1.47 Ma (752 Ka–2.39 Ma)
Hypsiglena torquata (Günther, 1860)	ND4 (797)	GTR+I+G	132	Θ~G (2, 41) τ~G (2, 189)	2.83 Ma	4.47 Ma	3.58 Ma (3.05 Ma–4.15 Ma)	18.21 Ma (14.76 Ma–22.31 Ma)	6.99 Ma (4.04 Ma–11.6 Ma)
Lampropeltis getula (Linnaeus, 1766)	cytb (1117)	GTR+I+G	92	Θ~G (2, 100) τ~G (2, 952)	579 Ka	1.47 Ma	768 Ka (323 Ka–1.27 Ma)	3.98 Ma (1.64 Ma–7.18 Ma)	2.19 Ma (1.11 Ma–3.93 Ma)
Masticophis flagellum (Shaw, 1802)	cytb (1117)	HKY+I+G	42	Θ~G (2, 48) τ~G (2, 800)	3.83 Ma	5.13 Ma	1.95 Ma (891 Ka–3.19 Ma)	3.94 Ma (1.64 Ma–7.52 Ma)	6.23 Ma (4.26 Ma–8.86 Ma)
Pituophis catenifer (Blainville, 1835)	cytb (898)	GTR+I	97	Θ~G (2, 86) τ~G (2, 528)	1.08 Ma	3.74 Ma	821 Ka (367 Ka–1.43 Ma)	6.07 Ma (1.56 Ma–11.45 Ma)	2.97 Ma (2.19 Ma–3.80 Ma)
Rhinocheilus lecontei Baird & Girard, 1853	cytb (966)	GTR+I+G	62	Θ~G (2, 92) τ~G (2, 1000)	1.65 Ma	2.08 Ma	1.31 Ma (562 Ka–2.25 Ma)	3.26 Ma (1.21 Ma–6.72 Ma)	1.62 Ma (1.15 Ma–2.28 Ma)
Salvadora hexalepis (Cope, 1867)	cytb (930)	HKY+I	27	Θ~G (2, 83) τ~G (2, 5000)	115 Ka	4.58 Ma	201 Ka (45.5 Ka–437 Ka)	490 Ka (171 Ka–982 Ka)	2.10 Ma (502 Ka–7.08 Ma)
Thamnophis marcianus (Baird & Girard, 1853)	cytb (978)	HKY+G	21	Θ~G (2, 270) τ~G (2, 2000)	206 Ka	475 Ka	698 Ka (206 Ka–1.38 Ma)	1.34 Ma (423 Ka–2.64 Ma)	432 Ka (175 Ka–875 Ka)
Trimorphodon biscutatus (Duméril, Bibron & Duméril)	ND4 (813)	HKY+I	38	Θ~G (2, 313) τ~G (2, 2500)	17.0 Ka	Not monophyletic	456 Ka (119 Ka–940 Ka)	Not monophyletic	Not monophyletic

Test of selection

Any correlation between genetic variation and the variables tested above might suggest that the locus is under selection. We tested for a signature of positive selection using Tajima's *D* (Tajima, 1989) in DnaSP 5.10.1 (Rozas *et al.*, 2003), by calculating this summary statistic for both synonymous and non-synonymous sites separately. Purifying selection is expected to result in significantly negative values of non-synonymous sites only, whereas a result of negative values for both synonymous and non-synonymous sites is suggestive of recent population expansion (Hahn *et al.*, 2002).

Test of synchronous divergence

The hABC software pipeline msBayes was used to test for synchronous divergence among the 12 population pairs (Huang *et al.*, 2011). This analysis was conducted using two different population assignments: (1) individuals were assigned to populations based on gene-tree monophyly regardless of sampling location and (2) population assignment based on sampling locality with respect to the WCD rather than based on the criteria of strict reciprocal monophyly. For both analyses, 1000 samples from the posterior distribution of the hyperparameter Ω, the ratio of variance to the mean in divergence times, were obtained using rejection sampling 3×10^6 simulated draws from the prior generated by msBayes followed by a post acceptance adjustment using local linear regression (Beaumont *et al.*, 2002).

The power of msBayes in detecting variation in divergence times has been called into question (Oaks *et al.*, 2012, 2014) even though empirical inferences of synchronous divergence is rarely a result (Hickerson *et al.*, 2014). These papers demonstrate that msBayes can underestimate the number of co-divergence events (Ψ) at shallow time-scales. First, this bias occurs due to insufficient prior sampling arising from the use of unnecessarily wide priors, resulting in an under-sampling of non-simultaneous divergence histories within the time-scale of divergence times with higher likelihood (Hickerson *et al.*, 2014). Second, this bias becomes negligible if one uses estimates of the dispersion index of population divergence times (Ω) rather than Ψ (Hickerson *et al.*, 2014). Although Ψ is used to structure the hABC model, unlike Ω, it is not well correlated with the variability in divergence times. For example, $\Psi = 8$ could generate less variability than a history of $\Psi = 2$ if the former consists of eight tightly clustered divergence times, while the latter consists of two pulses of divergence separated by millions of years. Furthermore, the reported bias is towards a result of synchronous divergence such that an inference of asynchronous divergence across taxon pairs, especially at recent time-scales, is likely a conservative result. Lastly, power analyses with priors on population divergence times informed by gene divergence times has demonstrated that msBayes correctly rejects simultaneous divergence with low error rates even with narrowly spaced divergence times, thus analyses should not suffer from model misspecification (Hickerson *et al.*, 2014).

Demographical inference based on single locus data can be precarious because of coalescent variance (Edwards & Beerli, 2000). Hierarchical models can incorporate such stochasticity across taxa by combining datasets into a single analysis, thus gaining 'borrowing strength' (Xue & Hickerson, 2015). Therefore, power is gained by making inferences across species by pooling the data without making the assumption that these data come from the same population sizes, divergence times or population size changes (Beaumont & Rannala, 2004). This allows for estimation across species parameter congruence while borrowing strength from the entire phylogeographical sample, effectively increasing the sample size.

Divergence time estimation

In addition to the above beast divergence time estimates, a point estimate of divergence time was calculated by dividing π_{net} by a rate of 1.34×10^{-8} mutations/site/year (Daza *et al.*, 2009), where π_{net} was calculated from the msBayes pipeline. Furthermore, estimates of divergence time between taxon pairs were estimated using Bpp 3.1 (Yang & Rannala, 2014). Individuals were again divided in to populations based on (1) sampling locality and (2) on gene-tree monophyly. This method uses the multispecies coalescent to estimate parameters in a Bayesian framework accounting for incomplete lineage sorting (Yang & Rannala, 2014). Priors on the population size parameters (θ's) were given a gamma distribution with shape parameters from Watterson's θ summary statistic calculated using msBayes. Divergence time (τ) for each taxon pair was also assigned a gamma prior. Where possible, these were based on previous estimates from phylogeographical studies (Table 1: Castoe *et al.*, 2007; Mulcahy & Macey, 2009; Pyron & Burbrink, 2009; Bryson *et al.*, 2011; Anderson & Greenbaum, 2012). However, if prior information was unavailable, we selected priors by conducting preliminary runs; here each population pair was run with τ priors of G(2, 500) (deeper divergence) with a mean of 0.004 and then with G(2, 2000) (shallower divergence) with a mean of 0.001. These τ estimates were checked to ensure that the prior means were realistic for the data and a reasonable prior was then selected for longer runs (Table 1). Analyses were run for 1×10^6 generations following a burn in of 1×10^5 with a sampling frequency of 5. Each analysis was run three separate times to ensure consistency among parameter estimation. τ was converted to absolute time following Burgess & Yang (2008) with a mutation rate of 1.34×10^{-8} mutations/site/year. Tracer 1.6 (Rambaut *et al.*, 2014) was used to examine the trace plots of τ and Θ parameters to ensure that stationarity had been reached. Additionally, the sensitivity of our results to assumptions on mutation rates was explored using a range of mutation rates from the literature (Appendix S2).

RESULTS

Genetic data and gene-tree estimates

The mean number of samples/species was 62.25 (range: 20–132; Table 1). The new DNA sequence data consisted of 445 cytb sequences, plus an additional 302 sequences from Genbank (two of these datasets were ND4; Devitt, 2006; Mulcahy, 2008) for a total of 747 sequences. All new sequences have been accessioned to Genbank (No. KX835536 - KX835995, see Appendix S1).

All ESS values were > 200 for all BEAST gene-tree estimates. The multiple runs within each taxon converged on similar topologies and divergence times; tree files have been submitted to Dryad (accession: doi:10.5061/dryad.74mn5). Our gene-tree estimates revealed a division in nearly all taxa at the CFB, with lineages largely found east or west of the WCD (Fig. 2). The only exception to this is the *Trimorphodon biscutatus* species complex, where the two deserts are not reciprocally monophyletic (Devitt, 2006). The topologies of these gene-tree estimates are similar to studies examining the phylogeography of some of these taxa (Devitt, 2006; Castoe *et al.*, 2007; Mulcahy, 2008; Pyron & Burbrink, 2009; Anderson & Greenbaum, 2012; Schield *et al.*, 2015). Here, we show that *Arizona elegans*, *Crotalus scutulatus*, *Rhinocheilus lecontei*, *Salvadora hexalepis* and *Thamnophis marcianus* have also diverged across the CFB. The only species for which there was geographical overlap in the two lineages are *Crotalus atrox* and *Lampropeltis getula* (Fig. 2).

Several taxa with wider geographical distributions reveal additional phylogeographical structure associated with geography (Fig. 2). *Crotalus scutulatus* shows shallow population structure east and west of the CFB with an additional lineage in the Mexican states of Tamaulipas and Veracruz. There are four mtDNA lineages within *A. elegans* corresponding to an eastern lineage throughout Texas, Oklahoma and Kansas, a lineage in New Mexico south to Coahuila, a lineage west of the CFB through to California and a lineage distributed in Baja California. *Rhinocheilus lecontei* shows population structure from western Texas into Kansas, a western Texas and New Mexico clade, a lineage endemic to the CFB region, a lineage west of the CFB into California and a lineage in the Central Valley of California. There are also four lineages within *Pituophis catenifer*, an eastern lineage occurring within most of Texas and Oklahoma, a lineage east of the CFB in central Mexico and New Mexico north into Colorado, a Sonoran lineage west of the CFB and a widespread group in peninsular Baja California, California, Utah and Oregon. We do not always find that the lineages east and west of the CFB are most closely related, yet lineage divergence across this barrier indicates that this diversification event would have occurred earlier than subsequent divergence events (e.g. in *A. elegans*).

The mode divergence times from BEAST ranged from 1.47 to 6.99 Ma. When including the 95% highest posterior densities (HPDs), these results suggest that diversification across the CFB has occurred multiple times ranging from the late Miocene to the late Pleistocene. Importantly, the 95% HPDs of the most recently diverged populations do not overlap with more ancient population divergence times (Table 1). For example, *T. marcianus* (95% HPD: 0.18–0.88 Ma), *C. scutulatus* (0. 752–2.39 Ma) and *R. lecontei* (1.15–2.28 Ma) do not overlap with *A. elegans* (2.58–5.99 Ma), *H. torquata* (4.04–11.6 Ma) and *M. flagellum* (4.26–8.86 Ma).

Explanatory variables of genetic variation

The RDA analyses show that in nearly all taxa, a full model incorporating geographical distance, climatic conditions and sampling locality captures the greatest amount of genetic variance (Appendix S1). Two species deviate from this pattern: *R. lecontei*, where genetic diversity is most highly correlated with climate, and *T. marcianus*, which does not show any correlation with the three variables. The amount of genetic variance that is contributed to the full model ranges from 3.24% to 78.7%, geographical distance explains up to 18.4%, climate contributes to 1.2–62.7% and sampling locality explains up to 13.7% of the genetic variation.

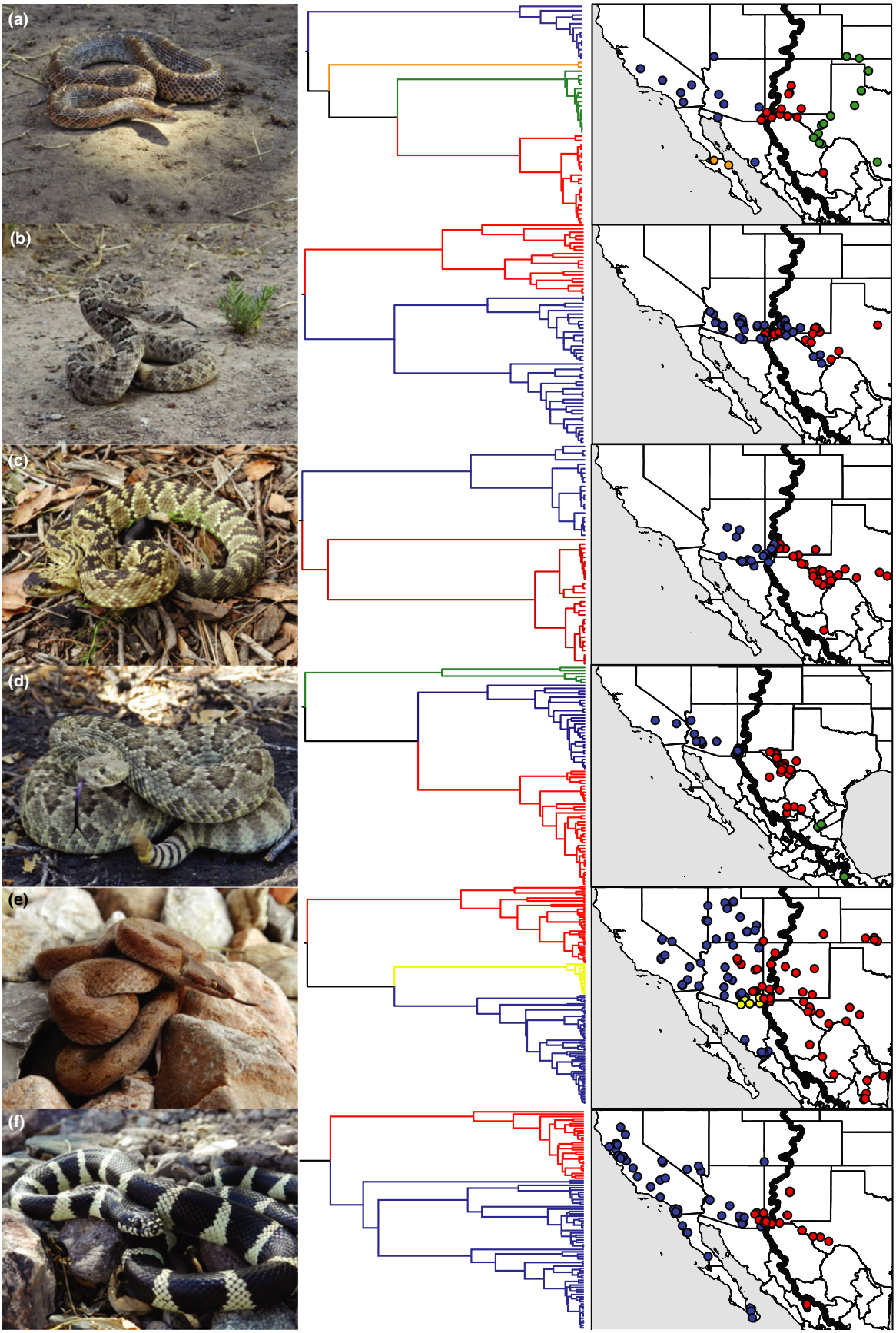
Test of synchronous divergence

There is no posterior support for synchronous divergence, suggesting that diversification at the CFB can be attributed to multiple historical events. The estimated dispersion index of divergence times, Ω, for both msBayes analyses does not sample 0, a value indicative of complete co-occurring divergence. The mean of this estimate where populations are based on locality is 0.41 (highest posterior density, HPD = 0.29–0.53), whereas the mean estimate based on monophyly is 0.30 (HPD = 0.21–0.40). There is uncertainty in the estimate of Ψ, the number of divergence events, with the highest posterior support for two divergences times (PP = 0.34) when based on locality, and eight divergence times (PP = 0.22) when based on monophyly. However, the posterior never samples a $\Psi = 1$ in either analysis; thus, there is strong support for multiple historical events driving divergence between the Sonoran and Chihuahuan Desert population pairs.

Tests of selection

Both *Crotalus atrox* and *C. scutulatus* have significantly negative Tajima's *D* values at non-synonymous sites, suggesting

Figure 2 Representative photos, rooted Bayesian inference gene-trees and geographical distributions of lineages within North America. Species are as follows: (a) *Arizona elegans*, (b) *Crotalus atrox*, (c) *Crotalus molossus*, (d) *Crotalus scutulatus*, (e) *Hypsiglena torquata*, (f) *Lampropeltis getula*, (g) *Masticophis flagellum*, (h) *Pituophis catenifer*, (i) *Rhinocheilus lecontei*, (j) *Salvadora hexalepis*, (k) *Thamnophis marcianus*, (l) *Trimorphodon biscutatus* (photo credits: EAM).



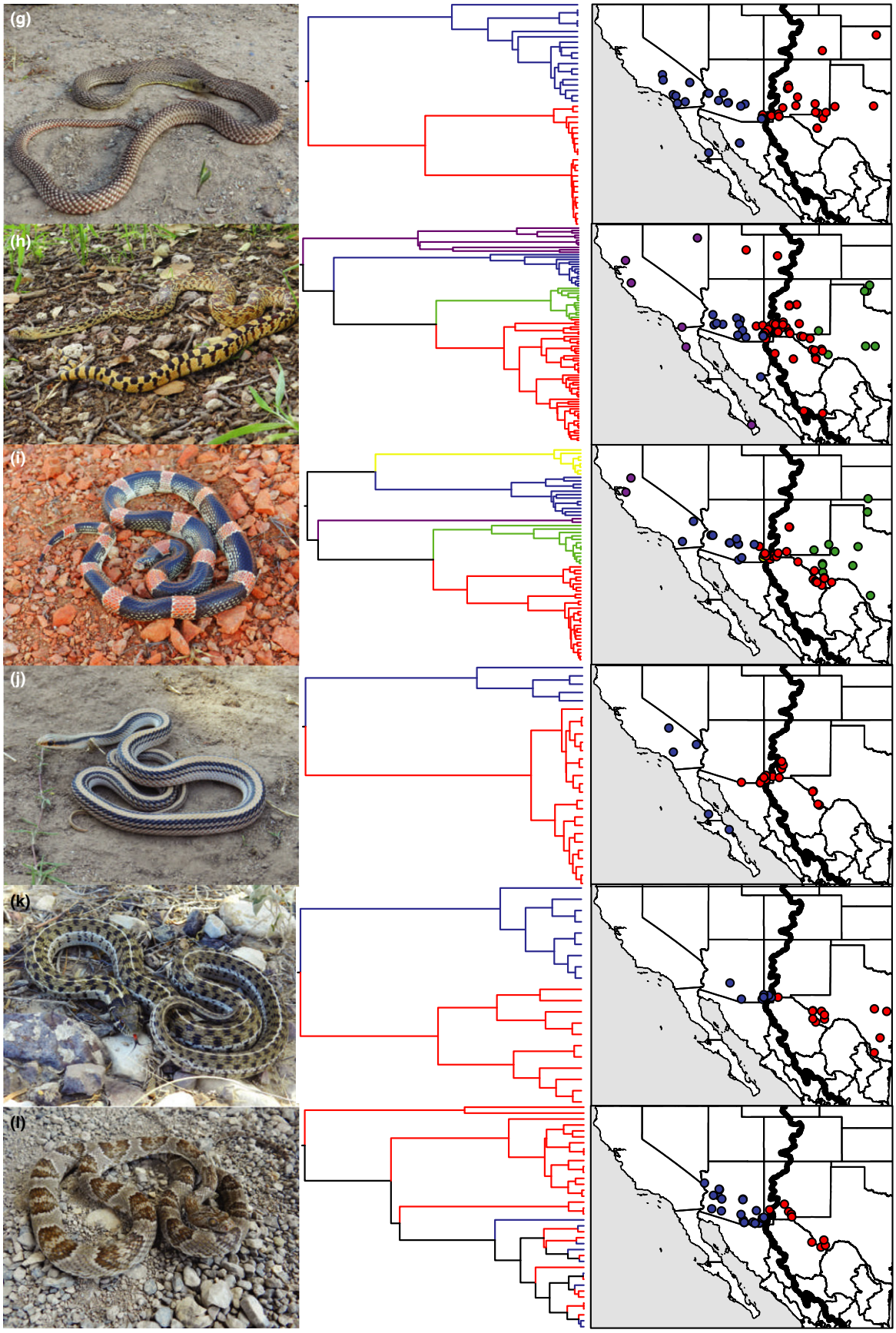


Figure 2 Continued.

that the cytb locus has experienced purifying selection. Two other taxa (*H. torquata* and *L. getula*) have negative Tajima's *D* values at non-synonymous sites with low but non-significant *P*-values. All other estimated Tajima's *D* values are non-significant at both synonymous and non-synonymous sites (Appendix S1).

Divergence time estimates

Parameter estimates were congruent among the replicate Bpp runs (coefficient of variation in mode divergence time estimates ranged from 0.003 to 0.10) with moderate to large ESS values (range = 30–5552) suggesting the MCMC analyses had converged. Furthermore, plots of τ and Θ in Tracer v1.6 (Rambaut *et al.*, 2014) indicated that stationarity had been reached. The mode divergence times of the population pairs based on geographical sampling ranged from 270 Ka to 4.9 Ma with considerable variance in each estimate and overlap in most, but not all, of the 95% HPDs (Fig. 3; Table 1). For example, the *Salvadora hexalepis* and *Hypsiglena torquata* species complexes do not overlap in the distributions of estimated divergence times. Estimated divergence times from Bpp runs where populations are based on monophyly range from 490 Ka to 18.5 Ma; again there are non-overlapping HPD distributions that do not overlap in estimated divergence times (Table 1). The estimates based on monophyly are generally older than those based on geographically assigned populations, and in some cases substantially older (i.e. *H. torquata*; Table 1). Point estimates based on the π_{net} summary statistic were generally within the 95% HPDs of the Bpp and Beast divergence time estimates and ranged from 17 Ka to 4.22 Ma when populations are based on geography and 475 Ka–7.22 Ma when individuals are assigned to populations based on monophyly. The taxon pairs that have been elevated to full species status are not older than species pairs that are classified as the same species. The oldest diverged populations are found within *Hypsiglena* and have been elevated to species (Mulcahy, 2008), and the most recently diverged populations found within *Trimorphodon*, have also been split into distinct species (Devitt *et al.*, 2008). These results are consistent with msBayes suggesting that diversification across the CFB is the result of multiple historical events.

DISCUSSION

Populations of most species of snakes are structured at the CFB into the Chihuahuan and Sonoran Deserts; we found reciprocally monophyletic lineages in 11 of the 12 study taxa (not *Trimorphodon*, Fig. 2; Devitt, 2006). However, there is likely not a single cause driving this diversification. After rejecting a history of simultaneous divergence, we can discount

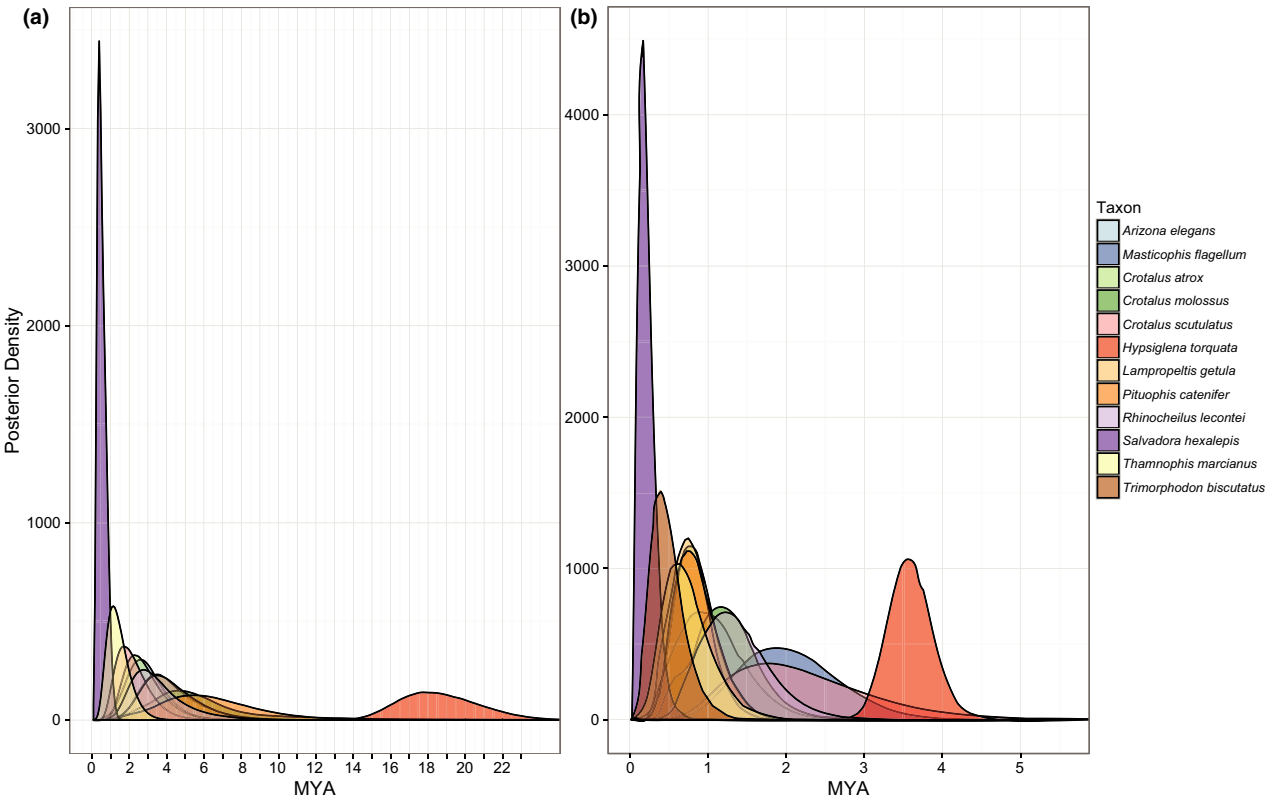


Figure 3 Divergence time estimates. (a) Posterior distribution of divergence times for all taxa from Bpp when populations are defined by gene-tree monophyly (not that *T. biscutatus* is missing from this analysis because this taxon is not monophyletic between the two deserts); (b) posterior distribution of divergence times from Bpp when populations are defined by sampling locality east or west of the WCD.

that variation arose by purely stochastic means; multiple variables (e.g., distance, climate and geographical barriers) explain genetic divergence, although these vary in magnitude across co-distributed species. Similarly, over broad geographical scales both climate and distance explain species turnover and phylogenetic diversity of snake communities (Burbrink & Myers, 2015). Locality, east or west of the WCD, and geographical distance explained approximately equal proportions of genetic diversity, whereas current climate explains the greatest amount of pairwise genetic divergence in 10 of the 12 study species (excluding *Thamnophis marcianus* and *Crotalus molossus*). It has been suggested that most genetic structure between populations is due to isolation by ecology, a pattern that could arise via natural selection leading to non-random gene flow (Sexton *et al.*, 2014; Zink, 2014). It is possible that divergent natural selection is maintaining these lineages at the CFB in their respective desert biome. However, by testing for the signature of purifying selection, we find that only two species (*C. atrox* and *C. scutulatus*) exhibit a signature of selection within the locus examined here; the other 10 species do not show this pattern.

Consistent with climate and distance being the main factors structuring genetic diversity and there being no parallel response in being located east or west of the CFB, we found no support for synchronous divergence among taxon pairs based on two different population assignments. Instead, there are heterogeneous patterns in population divergence, where multiple historical events have been responsible for promoting divergence and maintaining isolation between the Sonoran and Chihuahuan Deserts. By using an approach that accommodates coalescent stochasticity and demographical variability across taxa, we find strong support for multiple divergence events, where $\Psi = 1$ is never sampled in the posterior. We exercise caution and do not overinterpret Ψ beyond simply inferring asynchronous divergence (Hickerson *et al.*, 2014), which is also reflected in the estimate of the dispersion index of divergence times (Ω) and is more accurately estimated by msBayes (Stone *et al.*, 2012). The posterior sample of Ω ranged from 0.29 to 0.53 when populations were partitioned by the WCD and 0.21–0.40 when populations were defined by gene-tree monophyly, indicating that the divergence events have occurred across a wide range of time.

Asynchronous diversification is supported within this assemblage at the CFB, although the timing of each event is uncertain. The summary statistic π_{net} was used to derive a point estimate of population divergence time, BEAST estimated gene divergence times, and Bpp was used to model the multi-species coalescent. This resulted in five estimates of divergence time, including both population and gene divergences, for the 12 taxon pairs. When considering all methods, the times of gene and population divergence both range from the late Pleistocene to the mid-Miocene, yet each method resulted in a different estimate for each taxon pair (Table 1). This is unsurprising given that the underlying assumptions of the models are very different: the π_{net} method estimates population divergence time while accounting for ancestral polymorphism from estimates of extant polymorphism (Nei & Li, 1979), BEAST estimates gene divergence times, while Bpp accounts for incomplete lineage sorting under a likelihood-based statistical model. The confidence intervals of our estimates do overlap with previous divergence time estimates in *C. atrox* (Castoe *et al.*, 2007), *L. getula* (Pyron & Burbrink, 2009), *Pituophis* (Bryson *et al.*, 2011), *Hypsiglena* (Mulcahy & Macey, 2009) and *C. molossus* (Anderson & Greenbaum, 2012). Because increasing the number of loci reduces confidence intervals around parameter estimates (Felsenstein, 2006; Robinson *et al.*, 2014), future studies should increase the sampling of the genome to better determine the timing of divergence events across the CFB.

The discrepancies observed in our estimated divergence times could arise from the assumed mutation rate and/or migration as well as the fact that gene and population divergence times are expected to be different due to ancestral polymorphism under an isolation model (Edwards & Beerli, 2000). Finding an applicable mutation rate across taxa is difficult because of rate heterogeneity (Gillooly *et al.*, 2005). The rate we implemented was derived from the same loci used in this study and was estimated from a fossil calibrated phylogeny that included representatives of many of the major Colubroid families (Daza *et al.*, 2009), and broadly overlaps with other estimated rates within snakes (Zamudio & Greene, 1997; Burbrink *et al.*, 2011; see Appendix S2 for uncertainty in mutation rates). Furthermore, gene flow between these lineages could be high within some taxon pairs. For example, the *C. atrox* lineages that overlap across a large geographical region might show extensive gene flow, however, none of the methods parameterize migration and using a single locus will provide poor estimates of this parameter (Robinson *et al.*, 2014). If migration had occurred and was ignored, the estimated divergence times would be younger than the actual timing (Leaché *et al.*, 2013).

The CFB is a broad region associated with population divergence across multiple taxonomic groups, and therefore, the mesquite-grassland ecotone between the Chihuahuan and Sonoran Deserts may not represent a single specific area driving divergence. Using the WCD as the barrier to divide samples into populations may be too strict, resulting in populations of mixed ancestry. We avoided this potential pitfall by analysing the data in a two ways, where populations were based on sampling locality and where populations were based on gene-tree monophyly. Irrespective of population assignment strategy, our model-based inference for a single pulse of divergence resulted in a strong signal of multiple waves of diversification. The same is true for divergence time estimates from Bpp and BEAST, where the confidence intervals of the most recent and ancient divergence times do not overlap, strongly suggesting that there have been multiple vicariance events between the Chihuahuan and Sonoran Deserts (Fig. 3). It is also possible that the CFB is maintaining divergences caused by unrelated geologic events; for example in *A. elegans*, lineage divergence might have been associated

with the development of the Baja peninsula and these non-sister lineages are now in contact at the CFB, a similar situation could have occurred in the diversification of the *R. lecontei* group.

It is likely that the cyclic nature of climatic fluctuations during the Quaternary as well as the secondary uplift of the Sierra Madre Occidental during the Neogene (Wilson & Pitts, 2010a) were responsible for lineage formation within this region. Climatic cycles with large-scale fluctuations in global and regional temperatures have occurred throughout Earth's history (Dynesius & Jansson, 2000), resulting in restructuring of habitats. Palaeontological studies indicate that composition of biological communities during the Pleistocene has no modern analogues and it is likely that individual species responded independently to environmental changes (Whittaker, 1967), further highlighting species-specific ecological tolerances and historical distributions supported in comparative phylogeographical studies (e.g. Papadopoulou & Knowles, 2015).

The absence of a single time of divergence is evident from other comparative phylogeographical studies that have tested for shared vicariance among co-distributed taxon pairs. A common finding is asynchronous divergence across a large number of taxon pairs (Chan *et al.*, 2011), but when subsets of taxa are analysed, a result of simultaneous divergence is sometimes recovered (Chan *et al.*, 2011; Papadopoulou & Knowles, 2015). However, these studies neglect to focus on Ω, the dispersion index of divergence times, a hyperparameter that has been shown to robustly characterize variability in population divergence times (Hickerson *et al.*, 2014). Second, smaller taxon sampling will inherently involve sets of taxa with less variability in divergence times because of smaller sample sizes. However, there may be less statistical power to reject the incorrect history given that smaller samples sizes yield a weaker statistical borrowing strength (Xue & Hickerson, 2015).

Concordance inferred from smaller subsets of species assemblages could be mediated by species-specific ecological traits, for example, particular habitat associations may allow for persistence of local populations across a landscape thus influencing population connectivity (Papadopoulou & Knowles, 2015). This has been demonstrated in Neotropical birds where ecology may predict population history, with understory species having older divergence times than canopy taxa (Smith *et al.*, 2014). Ecologically mediated vicariance likely acts periodically through time and influences species independently due to a combination of species-specific ecological preferences and resulting differential extents of the habitat change occurring at the barrier (Pyron & Burbrink, 2010). This is likely the case at the CFB, where populations have diverged at different times; future studies incorporating palaeo-niche modelling could address how these co-distributed taxa have shifted their distributions over time (e.g. Zink, 2014).

Many other taxonomic groups exhibit population genetic structure between the Sonoran and Chihuahuan Deserts, including vertebrates (Zink *et al.*, 2001; Pyron & Burbrink, 2010), invertebrates (Wilson & Pitts, 2010b) and plants (Rebernig *et al.*, 2010). Furthermore, research on sand dune (insects and *Uma* lizards) and aquatic habitat specialists (snails and pupfish) across arid North America suggest that no one single geological event is responsible for current distributions and biogeographical patterns (Van Dam & Matzke, 2016). Such a diverse array of taxonomic groups would likely show an even greater disparity in the number and timing of diversification events across this region. However, no current methods permit this level of community sampling of taxa for phylogeographical investigation. Further studies of widespread groups will likely reveal additional taxa that have diversified across the CFB. Given the results of previous studies as well as those presented here, it is clear that this region is responsible for repeatedly causing divergence between populations of co-distributed species, highlighting the importance of this region in generating the biodiversity of North America.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Specimen information, RDA, selection results.
Appendix S2 Variation in divergence time estimates.

DATA ACCESSIBILITY

All sequences have been submitted to GenBank, no. KX835536 - KX835995. Dryad Accession: doi:10.5061/dryad.74mn5—R scprits and .tre files from BEAST.

BIOSKETCHES

Edward A. Myers is broadly interested in population genomics, comparative phylogeography and systematics of herps.

Michael J. Hickerson is interested in comparative population genomics within the context of biogeography.

Frank T. Burbrink works on diversification of reptiles and amphibians.

Author contributions: E.A.M. and F.T.B. conceived the ideas; E.A.M. collected the data; E.A.M. and M.J.H. analysed the data, all authors contributed to writing the manuscript.

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