

MITOCHONDRIAL INTROGRESSION AND INCOMPLETE LINEAGE SORTING THROUGH SPACE AND TIME: PHYLOGENETICS OF CROTAPHYTID LIZARDS

Jimmy A. McGuire,^{1,2} Charles W. Linkem,³ Michelle S. Koo,⁴ Delbert W. Hutchison,⁵ A. Kristopher Lappin,⁶ David I. Orange,⁷ Julio Lemos-Espinal,⁸ Brett R. Riddle,⁹ and Jef R. Jaeger⁹

¹Museum of Vertebrate Zoology and Department of Integrative Biology, 3101 Valley Life Sciences Building, University of California, Berkeley, California 94720-3160

²E-mail: mcguirej@berkeley.edu

³Department of Ecology and Evolutionary Biology and Natural History Museum & Biodiversity Research Center, University of Kansas, Dyche Hall, 1345 Jayhawk Blvd., Lawrence, Kansas 66045-7561

⁴Museum of Vertebrate Zoology, 3101 Valley Life Sciences Building, University of California, Berkeley, California 94720-3160

⁵Biology Department, Whitman College, 345 Boyer Avenue, Walla Walla, Washington DC 99362

⁶Biological Sciences Department, California State Polytechnic University, 3801 West Temple Avenue, Pomona, California 91768-2553

⁷CTB McGraw-Hill, 10548 Armstrong Boulevard, Mather, California 95655

⁸Laboratorio de Ecología, UBIPRO, Facultad de Estudios Superiores, Iztacala UNAM, Av. de los Barrios No. 1, Los Reyes Iztacala, Tlalnepantla, edo. de Mexico, Mexico 54090

⁹School of Life Sciences, University of Nevada Las Vegas, 4505 Maryland Pkwy, Las Vegas, Nevada, 89154-4004

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We investigate the roles of mitochondrial introgression and incomplete lineage sorting during the phylogenetic history of crotaphytid lizards. Our Bayesian phylogenetic estimate for Crotaphytidae is based on analysis of mitochondrial DNA sequence data for 408 individuals representing the 12 extant species of *Crotaphytus* and *Gambelia*. The mitochondrial phylogeny disagrees in several respects with a previously published morphological tree, as well as with conventional species designations, and we conclude that some of this disagreement stems from hybridization-mediated mitochondrial introgression, as well as from incomplete lineage sorting. Unidirectional introgression of *Crotaphytus collaris* (western collared lizard) mitochondria into *C. reticulatus* (reticulate collared lizard) populations in the Rio Grande Valley of Texas has resulted in the replacement of ancestral *C. reticulatus* mitochondria over approximately two-thirds of the total range of the species, a linear distance of ~270 km. Introgression of *C. collaris* mitochondria into *C. bicinctores* (Great Basin collared lizard) populations in southwestern Arizona requires a more complex scenario because at least three temporally separated and superimposed introgression events appear to have occurred in this region. We propose an "introgression conveyor" model to explain this unique pattern of mitochondrial variation in this region. We show with ecological niche modeling that the predicted geographical ranges of *C. collaris*, *C. bicinctores*, and *C. reticulatus* during glacial maxima could have provided enhanced opportunities for past hybridization. Our analyses suggest that incomplete lineage sorting and/or introgression has further confounded the phylogenetic placements of additional species including *C. nebris*, *C. vestigium*, *C. insularis*, *C. grisei*, and perhaps *G. copei*. Despite many independent instances of interspecific hybridization among crotaphytid lizards, the species continue to maintain morphological and geographic cohesiveness throughout their ranges.

KEY WORDS: Bayesian phylogenetics, biogeography, crotaphytidae, ecological niche modeling, introgression conveyor.

For more than two decades, mitochondrial genes have served as the markers of choice for phylogeographic and species-level phylogenetic analyses of animals (Avice 2000). Mitochondrial data have assumed this position because they share a number of favorable properties such as matrilineal inheritance, a general lack of recombination, a high mutation rate, reduced effective population size, and availability of universal primers (Moore 1995; Avice et al. 1987; Moritz et al. 1987; Harrison 1989; Kocher et al. 1989). Analyses based on mitochondrial data will often provide robust phylogenetic and phylogeographic estimates, but it is widely recognized that under certain circumstances, a mitochondrial gene tree will reflect evolutionary processes other than phylogenetic descent (Pamilo and Nei 1988; Doyle 1997; Nichols 2001). Two such processes are introgressive hybridization and incomplete lineage sorting (Carr et al. 1986; Patton and Smith 1994; Maddison 1997; Ruedi et al. 1997; Funk and Omland 2003). Left undetected, introgression and incomplete lineage sorting can confound phylogenetic inference (Leaché and McGuire 2006). However, if the signatures of these processes are recognized, their occurrence can provide insights into the evolutionary process that would otherwise remain undetected if relying upon other data. For example, ancient mitochondrial introgression can provide the sole remaining signature of past hybridization (Weisrock et al. 2005).

While undertaking a molecular phylogenetic and phylogeographic study of collared and leopard lizards (Crotaphytidae), we discovered at least four instances of mitochondrial introgression and at least one apparent case of incomplete lineage sorting. Each case of introgression involves incorporation of the mitochondrial genome of *Crotaphytus collaris* into a neighboring species (one instance of introgression into *C. reticulatus*, three instances of introgression into *C. bicinctores*). The patterns that we have uncovered indicate a complex introgression scenario, involving unidirectional replacement of mitochondrial genomes with heterospecific copies across substantial geographical distances, with a remarkable spatial pattern that to our knowledge has not been observed previously in other systems. Here we describe the patterns of mitochondrial introgression in *Crotaphytus* using a phylogenetic approach, propose a model describing the historical development of these patterns for the more complex scenario involving *C. bicinctores*, and comment on the implications of our findings for the history of gene flow in these parapatrically distributed species. We furthermore apply ecological niche modeling to test the following three hypotheses: (1) that *C. bicinctores* has recently competitively replaced *C. collaris* in southwestern Arizona, (2) that the ranges of *C. bicinctores* and *C. collaris* are geographically separated during glacial maxima, and come into secondary contact during interglacials, thus promoting periodic hybridization, and (3) that the geographic distribution of *C. reticulatus* during glacial maxima extended westward out of the Rio Grande Valley of Texas and adjacent Mexico, bringing its range into substantial contact with that

of *C. collaris*, and thereby promoting introgressive hybridization. Finally, we attempt to disentangle the confounding signatures of introgression and gene flow from phylogenetic descent, to provide an improved phylogenetic hypothesis for Crotaphytidae.

THE STUDY ORGANISMS

Crotaphytid lizards are conspicuous predatory inhabitants of arid regions of Western North America (McGuire 1996). Crotaphytidae is not particularly species-rich, with two genera, *Crotaphytus* and *Gambelia*, comprises nine and three constituent species, respectively. However, Crotaphytidae appears to be a rather old clade as it represents one of eight major lineages that comprise the primarily New World pleurodont iguanians ("Iguanidae" sensu Macey et al. 1997b, but see Frost and Etheridge 1989). Within *Crotaphytus*, species delimitation is straightforward with all nine species geographically cohesive and diagnosable on the basis of morphology. Within *Gambelia*, the species status of *G. copei* is questionable, as this species is only weakly differentiated from *G. wislizenii*; McGuire (1996) argued that the species status of *G. copei* required confirmation, and this remains the case today.

Crotaphytus and *Gambelia* are broadly sympatric in western North America, but the species within each genus have parapatric distributions that are often separated by narrow bands of inappropriate habitat (McGuire 1996; see Fig. 1 for distributions of *Crotaphytus* species). For example, the ranges of *C. bicinctores* and *C. vestigium* approach one another within ~5 km in southern California; the ranges of *C. bicinctores* and *C. nebrius* approach within a few hundred meters of one another at two points in Arizona; *C. collaris* and *C. nebrius* are separated by the Tucson Valley (a linear distance of approximately 20 km); and *C. collaris* and *C. reticulatus* approach one another within 14 km in north-eastern Coahuila, México. Hybridization has been documented between two crotaphytid species pairs: *G. sila* and *G. wislizenii* (Montanucci 1970), and at two sites between *C. collaris* and *C. bicinctores* (Axtell 1972; Montanucci 1983). Although not verified definitively, Montanucci (1974) also inferred hybridization between *C. collaris* and *C. reticulatus*. Hybridization has not been inferred or suggested between *C. bicinctores* and *C. vestigium*, or between *C. bicinctores* and *C. nebrius* despite that the distributions of these species pairs are only narrowly geographically separated (McGuire 1996).

Crotaphytid lizards have become an important model system, especially for comparative ecology and biomechanics (e.g., Lappin and Husak 2005; Husak 2006; Husak and Fox 2006; Husak et al. 2006; Lappin et al. 2006), and it is therefore important to have a well-supported and well-resolved phylogenetic estimate for this group. Although the phylogenetics and taxonomy of Crotaphytidae have been studied extensively on the basis of morphology (McGuire 1996), a comprehensive molecular phylogenetic analysis has not been published to date.

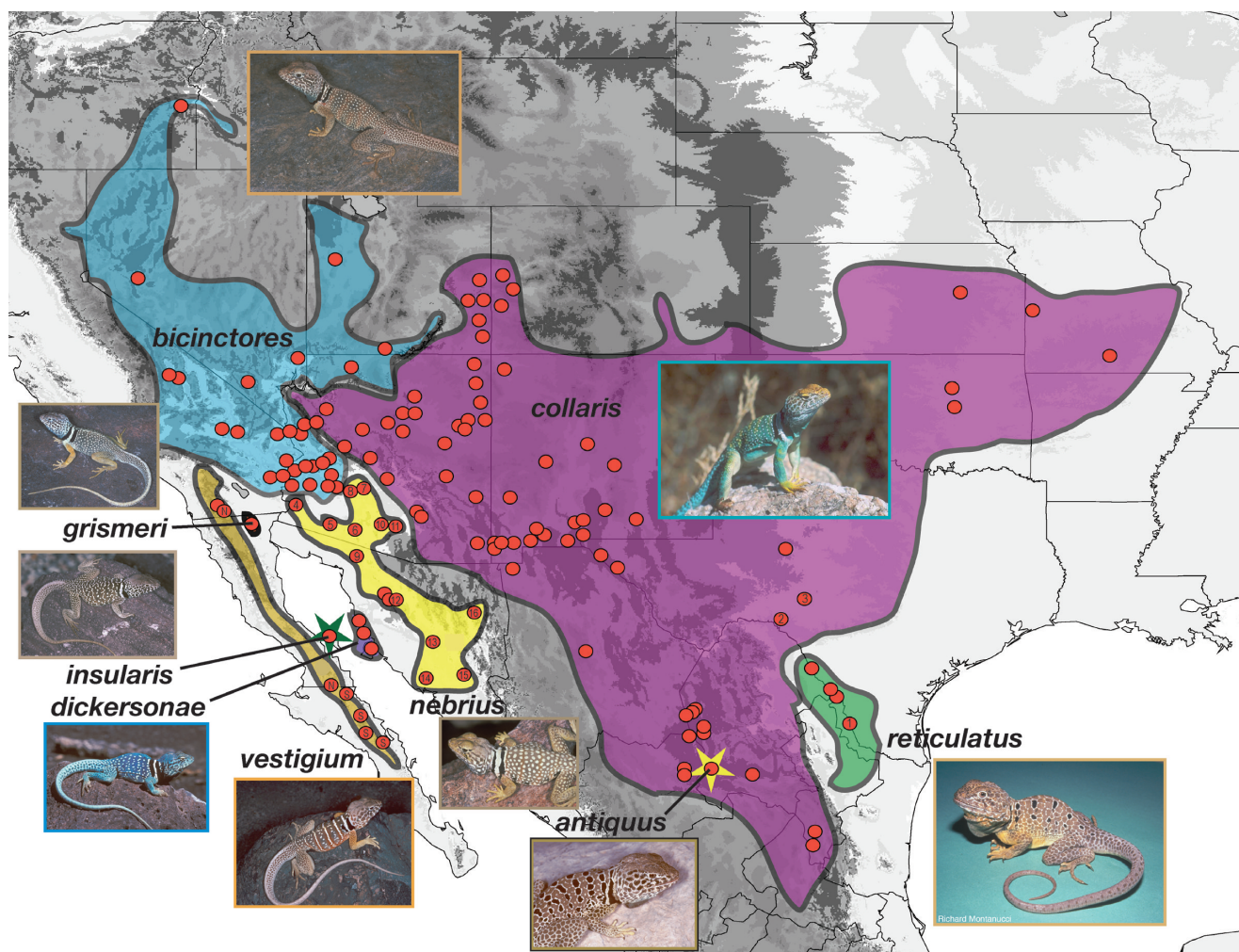


Figure 1. Distribution map for the nine species of *Crotaphytus*. Dots indicate sampling localities for the phylogenetic study. Dots with numbers or letters refer to localities mentioned specifically in the Results and Discussion. More detailed dot-distribution maps for each species are provided in McGuire (1996).

Materials and Methods

TAXONOMIC SAMPLING AND DNA SEQUENCE DATA

We obtained mitochondrial DNA sequence data for 408 exemplars representing the 12 extant species of crotaphytid lizards. This included relatively dense intraspecific sampling for select species (i.e., *C. bicinctores*, *C. collaris*, *C. nebris*, and *Gambelia wislizenii*), and sparse-to-moderate sampling for the remaining species. After initially detecting the signature of mitochondrial introgression, we increased our sampling of *C. collaris* and *C. bicinctores* in Arizona by collecting along three east–west transects that spanned the western margin of the range of *C. collaris*, continued westward through the range of *C. bicinctores* in southwestern Arizona, and ended in *C. bicinctores* populations in adjacent California (Fig. 1).

We isolated DNA using standard phenol-chloroform extraction as well as Qiagen DNeasy (Qiagen Inc., Valencia, CA)

extraction kits following standard protocols. We sequenced two mitochondrial fragments, corresponding to a 1246 aligned base pair (bp) segment comprising the complete NADH dehydrogenase subunit 2 (ND2) and partial or complete flanking tRNAs (methionine, tryptophan, alanine, and asparagine), and a 461 aligned bp fragment corresponding to the end of the glutamine tRNA and about one-third of the Cytochrome *b* (Cytb) gene. Amplification and sequencing of the ND2 (+tRNA) fragment required the use of external and internal primer sets. The external primers included METf.6 (L4437): 5′–AAGCTTTCGGGCCCCATACC–3′ and ASN.r2 (H5692): 5′–TTGGGTGTTTAGCTGTAA–3′. The internal primers used included ND2f.5 (L5002): 5′–AACCAAACCCAACTACGAAAAAT–3′ and ND2r.6 (H4980): 5′–ATTTTTCGTAGTTGGGTTTGRTT–3′ (Macey et al. 1997a). Amplification of the Cytb fragment required only the following external primer set: GLUDG-L: 5′–TGACT

TGAARAACCAAYCGTTG–3' and CB2-H: 5'–TCAGAATGATA TTTGTCCTCA–3'. We purified amplified PCR products on sephadex columns or using shrimp phosphatase and exonuclease (ExoSAPit), and sequenced purified PCR products directly using Big Dye Terminator sequencing reaction mix (Applied Biosystems, Foster City, CA) following manufacturer's protocols. Cycle-sequencing products were visualized on either an ABI 377, ABI 3700, or ABI 3730 automated sequencer. We were unable to obtain ND2 and Cytb sequences for four and three individuals, respectively. All DNA sequences generated for this study were deposited in Genbank (accession numbers EU037368–EU037771 and EU038399–EU038800). Our data matrix is available on the TreeBase website (project number: SN3480).

We aligned protein-coding mitochondrial sequences manually, with tRNA sequence alignments adjusted to account for secondary structure (Kumazawa and Nishida 1993; Macey and Verma 1997). MacClade 4.06 (Maddison and Maddison 2003) was used to verify that the protein-coding gene sequences remained in frame throughout their lengths. A three bp region between the tryptophan and alanine tRNAs was excluded due to alignment ambiguity.

PHYLOGENETIC ANALYSES

We performed Bayesian phylogenetic analyses using MrBayes (ver. 3.1.2; Huelsenbeck and Ronquist 2001). Model selection and tree visualization were undertaken using PAUP 4.0 (Swofford 2003). Because our combined dataset comprises two protein-coding mitochondrial genes, plus tRNA sequences, we performed partitioned analyses implementing separate nucleotide substitution models for subsets of the data more likely to have experienced similar evolutionary processes. We evaluated three partitioning regimes including unpartitioned, three partitions, and seven partitions. Three-partition analyses separated the data into Cytb, ND2, and tRNA subsets. Seven-partition analyses included separate substitution models for the tRNA sequences and each codon position within the ND2 and Cytb genes. We identified best-fitting models for each data partition (see below) using the Akaike Information Criterion (AIC; Akaike 1973; see Posada and Buckley 2004), as implemented in ModelTest 3.06 (Posada and Crandall 1998). The models implemented in our Bayesian phylogenetic analyses are listed in Table 1.

We attempted to select a best-fitting partitioning strategy for Bayesian analyses by (1) calculating standard Bayes factors (Nylander et al. 2004; Brandley et al. 2005), and (2) applying a Decision-Theoretic (DT) criterion (Minin et al. 2003; Abdo et al. 2005; Sullivan and Joyce 2005; McGuire et al. in press). Bayes factors were calculated using harmonic mean log likelihoods as provided by MrBayes. We took a fairly conservative approach by only accepting Bayes factors greater than 10 as evidence in support of a more partitioned model (Bayes factors > 10

Table 1. Alternative data partitions for phylogenetic analyses, and DNA substitution models applied to each. Abbreviations are defined as follows: Cytb, the entire Cytb gene fragment; ND2, the entire ND2 gene; Cytbpos1–3, first, second and third codon positions, respectively, for the Cytb gene fragment; ND2pos1–3, first, second and third codon positions for the ND2 gene; and tRNA, transfer RNA genes flanking the ND2 gene.

Unpartitioned: (All nucleotide positions: GTR+I+ Γ)
Three partitions: (Cytb, ND2, tRNA: GTR+I+ Γ)
Seven partitions: (Cytbpos3, ND2pos1, ND2pos3, tRNA: GTR+I+ Γ), (Cytbpos1, ND2pos2: HKY+I+ Γ), (Cytbpos2: HKY+ Γ)

are considered to be “very strong” according to the Kass and Raftery [1995] conventions). The DT approach was implemented using a modified version of the program DT-modelsel (script available at www.webpages.uidaho.edu/~jacks/DTModSel.html). See McGuire et al. (in press) for details. All partitioned analyses accommodated among-partition rate variation (APRV) through use of the “prset ratepr = variable” option in MrBayes.

We performed preliminary analyses under several branch length priors (mean = 10, 50, and 100; see Marshall et al. 2006) and found that the branch length exponential prior with a mean of 50 substantially outperformed the other prior values both in terms of a significantly higher harmonic mean log likelihoods and better convergence behavior and mixing. Consequently, all subsequent analyses employed a branch length prior of 50. We also performed preliminary analyses to find an optimal temperature regime for Metropolis-coupled Markov chain Monte Carlo analyses. The default temperature regime for heating ($T = 0.2$) resulted in no swapping of states between the heated and cold chains. We found that $T = 0.03$ resulted in state swap frequencies of 20–70% and consequently used this temperature regime for all subsequent analyses (see MrBayes 3.1.1 manual).

Each MrBayes analysis was run for 10 million generations, sampling from the chain every 1000 generations. We used two strategies to confirm that the chains had achieved stationarity. First, we evaluated “burn-in” plots by plotting log-likelihood scores, tree lengths, and all model parameter values against generation number using the program Tracer (ver. 1.3) or the statistical program Statview version 5.0. Second, we evaluated convergence by performing cumulative and sliding window analyses of posterior probability clade-support values to verify that these values were stable across all post-burn-in generations within each analysis using the online application Are We There Yet (AWTY) (Wilgenbuch et al. 2004). After determining chain convergence, which generally occurred within the first one million generations of each analysis (but required three million generations in one run), we discarded all samples obtained during the first four million generations as “burn-in.” We then generated consensus phylograms

with mean branch length estimates and posterior probability values for each node, credible sets of trees, and parameter estimates in the context of the final six million generations obtained during each analysis. We used the compare function in AWTY to compare recovered topologies and associated posterior probability values obtained across four independent analyses undertaken for each dataset, each of which was initiated from random starting points. Once we had discarded the pre-burn-in samples and verified that the four independent runs had converged on similar stationary distributions, we consolidated the data from the four analyses for final processing.

Because we obtained dense intraspecific sampling for several species, many individuals either shared identical mitochondrial haplotypes or were only weakly differentiated. To reduce the computational burden during Bayesian phylogenetic analyses, we removed individuals that exhibited less than 0.3% uncorrected pairwise sequence divergence from an included individual in the analysis. These individuals were then reincorporated onto the tree post-analysis, which simply involved adding these samples to strongly supported clades that lacked well-supported internal structure.

GIS MODELING

We used MaxEnt (ver. 2.3; Phillips et al., 2006) to model current and past geographical distributions of *C. bicinctores*, *C. collaris*, and *C. reticulatus* to identify likely points of contact between these species in the present, and how species contact zones might have been influenced by past glacial cycles. MaxEnt uses the principle of maximum entropy, in which the least biased estimation of an unknown probability distribution within a given space is the one that maximizes its entropy within the constraints of known data (e.g., sample points). The program produces spatially explicit models of continuous suitability distributions based on known occurrence samples and biologically meaningful climate variables. MaxEnt has demonstrated robust performance with small sample sizes of presence-only data (Elith et al. 2006). In addition, MaxEnt calculates receiver operating characteristic (ROC) for its area under the curve (AUC) value as a measure of model performance (Fielding and Bell 1997). We applied the following MaxEnt parameter values: (1) we randomly withheld 25% of available samples for testing, (2) we set the regularization multiplier to 1 (default), and (3) we allowed the algorithm to choose how the environmental layers were constrained (its “feature” type) based on training sample size (see Phillips et al., 2006).

We compiled georeferenced locality data for 894 *C. bicinctores*, 2110 *C. collaris*, and 93 *C. reticulatus* specimens from throughout their known ranges. From these, we sampled unique localities, resulting in 109 *C. bicinctores*, 376 *C. collaris*, and 24 *C. reticulatus* localities. The dataset of samples were modeled with 19 standard bioclimatic variables derived from mod-

ern temperature and precipitation (Worldclim 1.4; Hijmans et al. 2005) at a resolution of one square kilometer. To estimate distributions at the last glacial maximum, the general circulation model ECHAM 3 (DKRZ Modellbetreuungsgruppe 1992; Roeckner et al. 1992) was used as the simulated 21,000 YBP (years before present) paleoclimate on which we projected our model with the same variables and resolution as the modern climate dataset. Our area of analysis encompassed most of North America, including all of Mexico (lat 50° 5' N, 12° 52' S; long 125° 0' W, 53° 19' E). The area was purposefully larger than the extent of the species to overcome edge effects of the modeling routine. To allow direct comparisons of clade distributions, we converted the continuous models to presence/absence distributions by selecting a model-dependent threshold. We used the value where sensitivity (true positive) and specificity (true negative) are minimized as the threshold for presence/absence of species occurrence.

Results

PHYLOGENETIC ANALYSES

We performed Bayesian phylogenetic analyses on our combined mtDNA dataset comprising 1704 aligned nucleotide positions under three alternative partitioning regimes (unpartitioned, three partitions, and seven partitions). Analyses under each partitioning regime resulted in similar, well-supported and well-resolved topologies, especially with respect to interspecific relationships. However, the seven-partition analyses resulted in significantly greater Harmonic Mean Log Likelihoods than did three-partition and unpartitioned analyses (Table 2), and were preferred according to the DT criterion. Consequently, we base our interpretation of crotaphytid phylogeny and mitochondrial genome evolution on the seven-partition estimates (Fig. 2).

The phylogenetic estimate obtained here deviates in a number of respects from a previously published morphological assessment of species boundaries and phylogenetic relationships (McGuire 1996). For example, several morphologically distinct and diagnosable species are found to be either polyphyletic or paraphyletic, including *Gambelia wislizenii*, *C. bicinctores*, *C. collaris*,

Table 2. Data partition statistics including number of partitions, number of parameters per partitioning scheme, Harmonic Mean Log-Likelihoods (-HMLi), and 2 ln Bayes factors. Complete descriptions of the partitions are provided in Table 1.

No. of partitions	No. of parameters	-HML _i	2 ln Bayes factors
1	11	17,698.39	—
3	33	17,684.17	28.44
7	75	17,150.48	1067.38

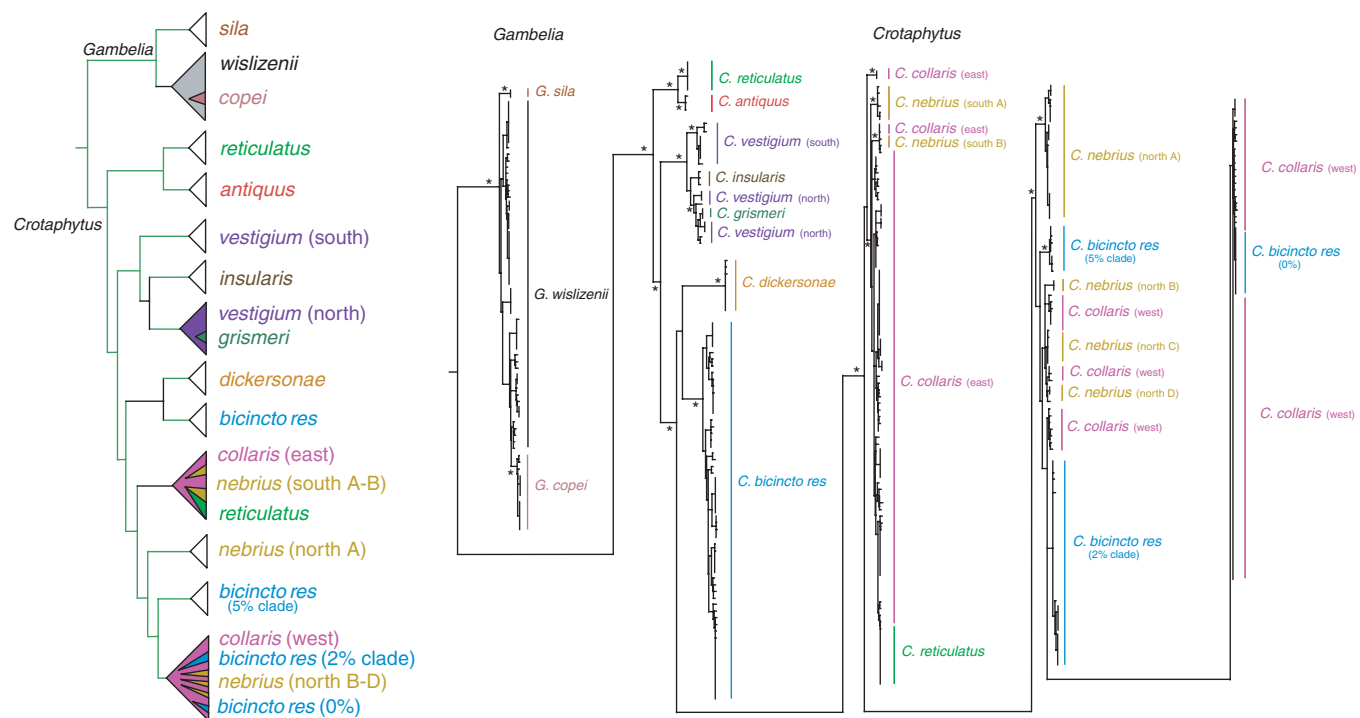


Figure 2. Phylogenetic estimate for Crotaphytidae based on seven-partition Bayesian analysis of mitochondrial DNA sequence data. Species are individually color coded. The figure on the left is a simplified version of the complete tree on the right. Asterisks indicate a subset of nodes that received $\geq 95\%$ posterior probability values.

C. nebris, *C. reticulatus*, and *C. vestigium*. Furthermore, interspecific relationships based on the mitochondrial topology disagree in several respects with the morphological topology (Fig. 3). This disagreement appears to result from a combination of (1) a more accurate phylogenetic estimate for some nodes based on mtDNA data than was obtained on the basis of morphology, (2) sections of the mitochondrial gene tree that disagree with the morphology tree because of incomplete lineage sorting, and (3) sections of the mitochondrial gene tree that disagree with the morphology tree because of mitochondrial introgression following hybridization at secondary contact zones. Below, we explore these issues in detail.

Crotaphytus reticulatus and *C. collaris*

We include mitochondrial sequence data for 15 *C. reticulatus* individuals collected from several localities in the Rio Grande Valley of Texas. Our sampling included the northwestern-most point in the species' distribution (near Eagle Pass), and a series of localities extending to the southeast terminating at Falcon Reservoir—a linear distance of approximately 270 km (Fig. 1). Our *C. collaris* sample from adjacent Texas and Mexico is sparse, only approaching within 140 km of the known range of *C. reticulatus*. Nevertheless, we found that mtDNA haplotypes obtained from all of our *C. reticulatus* samples except those from Falcon Reservoir (Fig. 1, locality 1) are identical or nearly so to *C. collaris* haplotypes

from our most proximate sampling localities in Sutton and Val Verde Counties, Texas (Fig. 1, localities 2–3). Furthermore, six of 11 individuals from Falcon Reservoir also are identical ($n = 8$) or nearly so ($n = 2$) to Texas *C. collaris* haplotypes (Fig. 2). The remaining five *C. reticulatus* samples from Falcon Reservoir together represent the sister clade of the geographically disjunct, but morphologically similar species, *C. antiquus* (McGuire 1996).

Crotaphytus bicinctores and *C. collaris*

Phylogenetic relationships among *C. bicinctores* and *C. collaris* mtDNA sequences were unexpected in that neither species was found to be monophyletic (Fig. 2). In particular, *C. bicinctores* haplotypes were placed in four distinct sections of the *Crotaphytus* topology, with three of these groupings nested within *C. collaris*. One highly divergent *C. bicinctores* clade (10–11% uncorrected patristic distances from *C. collaris*) was placed outside of *C. collaris* as the sister taxon of *C. dickersonae*. This clade comprises samples collected throughout most of the range of the species, including all but southwestern Arizona. We refer to these haplotypes as representing the suite of ancestral (or authentic) mitochondrial genomes for *C. bicinctores*. Two additional strongly supported *C. bicinctores* clades (which we refer to as the “5%” and “2%” clades) are nested within a primarily western *C. collaris* clade (Fig. 2). The 5% *C. bicinctores* clade comprises individuals collected in the Dome Rock, Kofa, and Laguna Mountains in extreme western

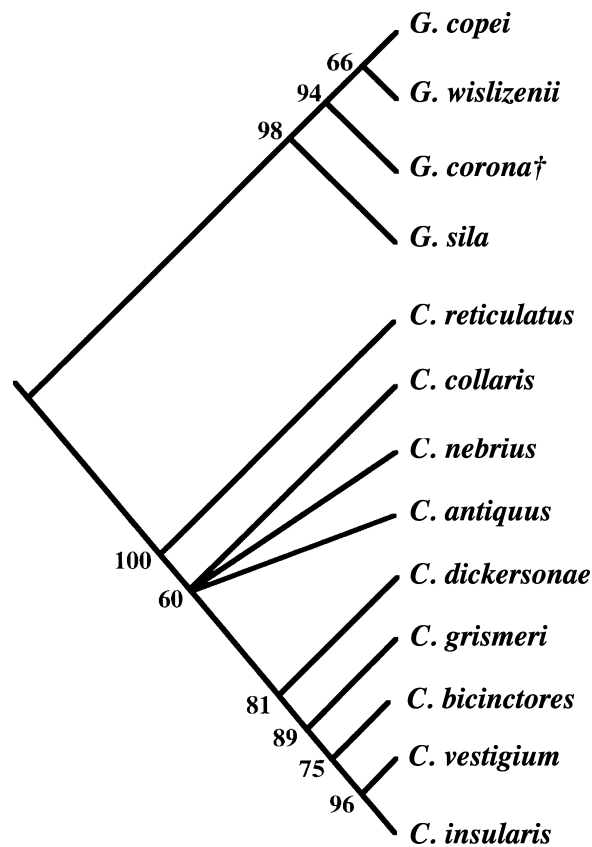


Figure 3. Phylogenetic estimate for crotophytid lizards based on morphological and allozyme data (adapted from McGuire 1996).

Arizona (Fig. 5; localities 1–3), with the Colorado River serving as a discrete boundary between these haplotypes and ancestral *C. bicinctores* haplotypes in adjacent California (Fig. 5; orange dots). We have not yet identified the exact boundary between the ancestral and 5% haplotype clades further north in Arizona. The haplotypes forming the 5% clade are ~5% divergent (uncorrected patristic distances) from the most similar *C. collaris* haplotypes. The 2% *C. bicinctores* clade comprises individuals collected between the Kofa Mountains (Fig. 5, locality 2) in the west, and the Harquahala, Belmont, and Gila Bend Mountains to the east (Fig. 5, localities 4–6). The haplotypes forming this clade are ~2% divergent from the most similar *C. collaris* haplotypes (which are found in adjacent western Arizona). Finally, haplotypes representing additional *C. bicinctores* localities to the east, including from the Belmont, Vulture, and White Tank Mountains (Fig. 5, localities 5, 7, 8), do not form an exclusive clade and instead group phylogenetically with a set of *C. collaris* samples from the adjacent extreme western margin of the range of *C. collaris*, primarily samples from the vicinity of Wickenburg (Fig. 5, locality 9). Although not all of these *C. bicinctores* and *C. collaris* samples share identical haplotypes, several *C. bicinctores* from the Vulture Mountains share an identical haplotype with a *C. collaris* from the Constel-

lation Road immediately ENE of Wickenburg. Consequently, we refer to this set of *C. bicinctores* haplotypes as “0% divergent.” The remainder of these “0% divergent” haplotypes differ by one or a few substitutions from adjacent *C. collaris* samples, exhibiting < 0.5% patristic distances from the most similar *C. collaris* haplotypes. Although we have not detected geographical overlap between the divergent ancestral *C. bicinctores* haplotypes found primarily outside of Arizona and the 5%, 2%, and 0% divergent haplotypes that are nested within the (primarily) *C. collaris* clade, there is overlap in the distributions of the 5% and 2% haplotypes (in the Kofa Mountains; Fig. 5, locality 2), and between the 2% and 0% haplotypes (in the Belmont Mountains; Fig. 5, locality 5).

Crotaphytus nebrius and *C. collaris*

The phylogenetic results for *C. nebrius* mtDNA are even more complex than are those for *C. bicinctores*. *Crotaphytus nebrius* was found to be polyphyletic, appearing in several widely separated positions within the *Crotaphytus* tree. A well-supported *C. nebrius* subclade (*C. nebrius* north A, Fig. 2) is placed as the sister taxon of a large clade comprising western *C. collaris* and introgressed *C. bicinctores* (as well as several additional *C. nebrius* samples discussed below). This large subclade includes samples obtained throughout much of the northern part of the species’ range (i.e., Gila, Mohawk, Little Ajo, and Estrella Mountains, and Buckeye Hills of Arizona, and one locality in northwestern Sonora, Mexico; Fig. 1, localities 4–9). However, several additional samples from the northeastern portion of *C. nebrius*’ range in Arizona (Silverbell and Tucson Mountains; Fig. 1, localities 10–11), as well as three samples from north-central Sonora (vicinity of Caborca; Fig. 1, the three localities labeled as 12) are further nested within the primarily western *C. collaris* clade in which they are placed in a mixed clade with several *C. collaris* samples from southeastern Arizona (*C. nebrius* north B-D; Fig. 2). A second major subclade of *C. nebrius* (*C. nebrius* south A, Fig. 2) is placed as the sister taxon to a large clade comprising eastern *C. collaris* and introgressed *C. reticulatus* samples, as well as two *C. nebrius* samples discussed below. The *C. nebrius* south A subclade is comprised primarily of samples from the southern portion of the species’ range including localities south of Hermosillo, Mexico (Fig. 2, localities 13–15). However, one of two samples from Guisamopa (Fig. 2, locality 15) and our sample from Villa Hidalgo (Fig. 2, locality 16) are placed in a more nested clade with *C. collaris* samples from extreme southeast Arizona.

Crotaphytus vestigium, *C. insularis*, and *C. grisei*

Our sampling for *C. vestigium*, *C. insularis*, and *C. grisei* is fairly sparse with just 19 exemplars representing this clade. The phylogenetic estimate provides strong support for the monophyly

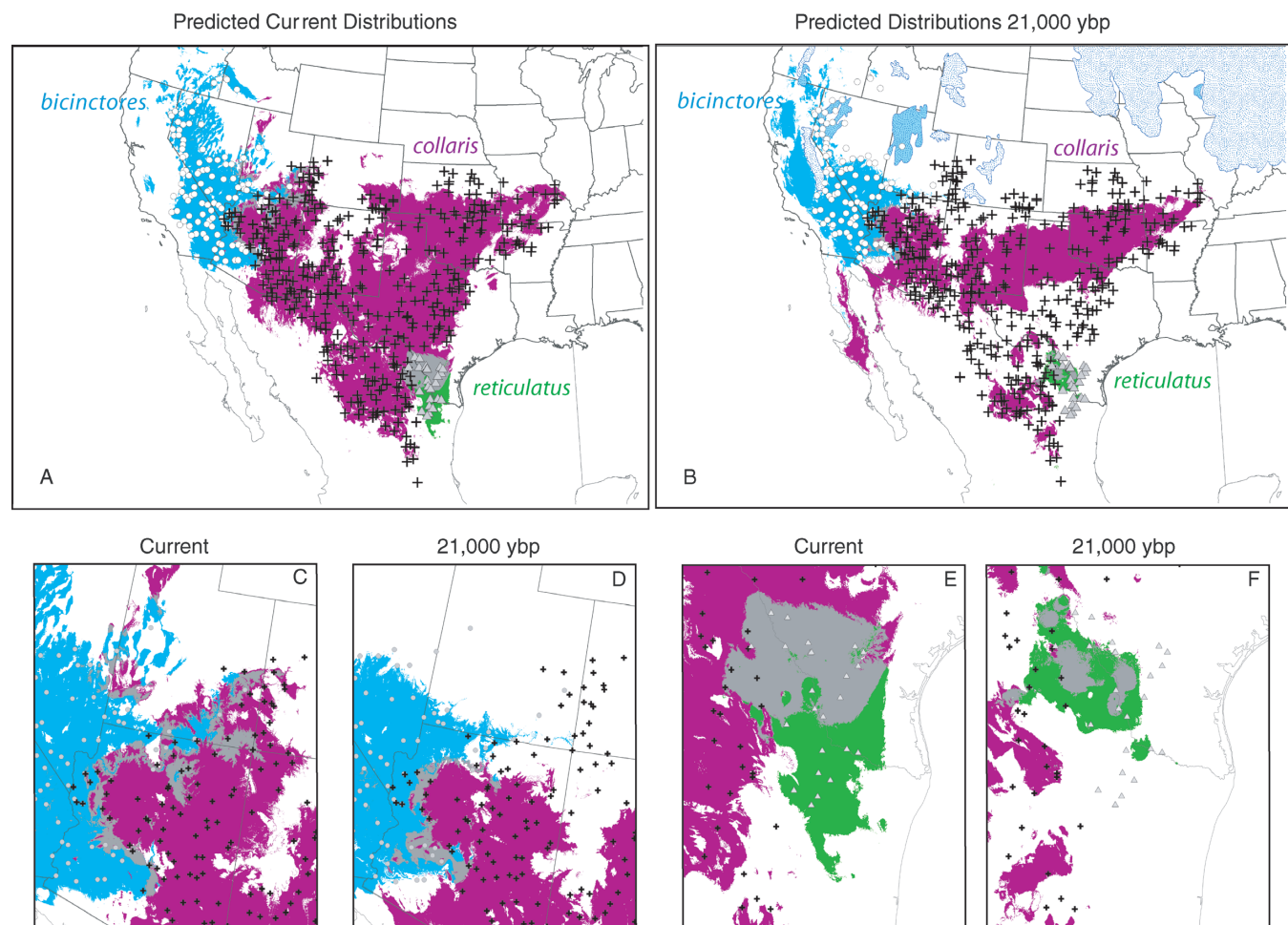


Figure 4. GIS ecological niche models for *Crotaphytus*. The upper panel illustrates the current scenario (A) and the 21,000 YBP paleo scenario (B) for *C. bicinctores*, *C. collaris*, and *C. reticulatus*. The lower panel presents close-up views of the species contact zones given the current and paleo reconstructions for *C. bicinctores* and *C. collaris* (C,D), and for *C. collaris* and *C. reticulatus* (E,F). Pale blue indicates the predicted range of *C. bicinctores*, purple indicates *C. collaris*, green indicates *C. reticulatus*, and gray reflects range overlap between *C. bicinctores* and *C. collaris*, and between *C. collaris* and *C. reticulatus*. Circles, crosses, and triangles represent georeferenced localities for *C. bicinctores*, *C. collaris*, and *C. reticulatus*, respectively.

of these three taxa as a group, but both *C. insularis* and *C. grismeri* are nested within *C. vestigium*, rendering the latter species paraphyletic. This clade appears to be divided into three strongly supported subclades: the first comprises southern populations of *C. vestigium* (Santa Agueda and southward; Fig. 2, localities denoted by “S”), the second corresponds to *C. insularis*, and the third to northern *C. vestigium* (El Arco northward; Fig. 2, localities denoted by “N”), plus *C. grismeri*.

Gambelia sila, *G. copei*, and *G. wislizenii*

Gambelia sila is weakly placed as the sister taxon of all other *Gambelia* samples (Fig. 2), which is consistent with its morphological differentiation and current taxonomy (McGuire 1996). *Gambelia copei* forms a well-supported clade that is deeply nested in the *G. wislizenii* clade.

GIS MODELING

Our GIS modeling analyses resulted in five primary findings, all of which are evident in Figure 4. First, the predicted contemporary distribution of *C. collaris* in southwestern Arizona does not extend much beyond the current geographical range limits of the species. This finding is inconsistent with our hypothesis that *C. collaris* recently occupied the southwestern portion of the state now occupied by introgressed *C. bicinctores* (Hypothesis 1, see Introduction). However, the distributions of *C. bicinctores* and *C. collaris* in southwestern Arizona are predicted to have overlapped more extensively during the last glacial maximum (21,000 YBP) than is the case today, with *C. collaris* extending further westward and *C. bicinctores* extending further eastward. This finding is inconsistent with the hypothesis that glacial cycling has mediated bouts of periodic secondary contact between these species, but does render it plausible that *C. bicinctores* replaced *C. collaris*

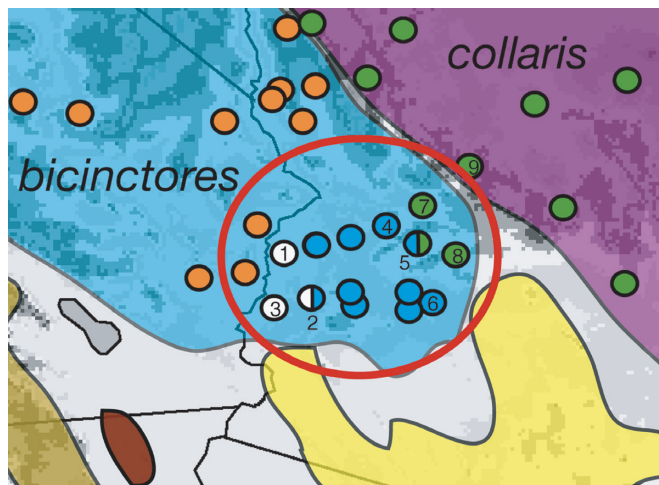


Figure 5. Map illustrating the interface between the ranges of *Crotaphytus bicinctores* and *C. collaris* in southwestern Arizona. Orange dots indicate ancestral *C. bicinctores* haplotypes. Green dots within the range of *C. collaris* indicate contemporary *C. collaris* haplotypes. Green dots within the range of *C. bicinctores* denote introgressed "0% divergent" *C. collaris* haplotypes. Blue and white dots within the distribution of *C. bicinctores* denote the "2%" and "5%" divergent introgressed *C. collaris* haplotypes. Dots with numbers refer to localities mentioned specifically in the Results.

in southwestern Arizona during a glacial maximum (Hypothesis 2). A third finding is that the predicted contemporary ecological niche of *C. collaris* in the Rio Grande Valley would allow its range to overlap extensively with that of *C. reticulatus* in areas where *C. reticulatus* are carrying introgressed *C. collaris* haplotypes. Fourth, the predicted distribution of *C. reticulatus* 21,000 YBP does not extend substantially westward, and its range is not predicted to overlap more substantially with that of *C. collaris* than is predicted for interglacials (Hypothesis 3). Finally, although not directly related to our hypotheses regarding the influence of glacial cycling on introgressive hybridization, our GIS modeling indicates that *C. bicinctores*, *C. collaris*, and *C. reticulatus* may have experienced substantial range contractions during glacial maxima. Our paleo reconstructions suggest that northern populations of *C. bicinctores* were either extirpated or present in a narrow northwestern refugium. The predicted paleo distribution of *C. collaris* is perhaps most surprising in that the species range is not predicted to have simply contracted southward, but may have, instead, become concentrated in an east/west-oriented band in the northern portion of the species' current distribution. Much of the central and southern portion of the species' range is predicted to have been unoccupied 21,000 YBP.

Discussion

The mitochondrial gene tree recovered here conflicts substantially with a previously published morphological phylogenetic estimate

for Crotaphytidae (McGuire 1996). We suspect that this incongruence has been driven to a large extent by introgressive hybridization and incomplete lineage sorting, processes that complicate the use of mitochondrial data for phylogenetic reconstruction. Although mitochondrial introgression and incomplete lineage sorting can confound phylogenetic inference, they are important components of lineage history and can elucidate key features of evolutionary processes. Here, we present evidence that introgression and incomplete lineage sorting have profoundly influenced the distribution of mitochondrial haplotypes among crotaphytid lizards. Our data clearly indicate that hybridization has played a significant and temporally extended role during crotaphytid evolution, and suggest that species boundaries within this group have been maintained in the face of repeated opportunities for lineage merging. Below, we first comment on the manner in which we can distinguish between mitochondrial introgression and incomplete lineage sorting in select cases. We then discuss introgressive hybridization and its role in determining the observed patterns of mitochondrial variation in *Crotaphytus* lizards. Finally, we discuss the phylogenetic implications of our mtDNA analysis, and attempt to glean meaningful phylogenetic information despite the confounding signatures of hybridization and incomplete lineage sorting.

MITOCHONDRIAL INTROGRESSION VERSUS INCOMPLETE LINEAGE SORTING

In some circumstances, mitochondrial introgression and incomplete lineage sorting can result in similar phylogenetic patterns (Avice and Ball 1990; Morando et al. 2004). However, there are two expectations given incomplete lineage sorting that can be used to differentiate these processes under some circumstances. First, incomplete lineage sorting requires that the true species divergence event postdates the corresponding bifurcation in the gene tree. When two species share an identical haplotype, incomplete lineage sorting can only explain this finding if speciation occurred so recently that there has not been time for genetic divergence. For example, some *C. reticulatus* and *C. collaris* share an identical mitochondrial haplotype despite that these taxa are morphologically and ecologically distinct. If this results from incomplete sorting of haplotypes present in their common ancestor, then the speciation events that gave rise to their very distinct morphologies and ecologies must have occurred recently enough that the mitochondrial genomes retained in the descendent species have yet to accumulate independent mutations. This scenario seems unlikely for *C. reticulatus*, and for *C. bicinctores*, especially in light of the fact that other populations representing these species are characterized by mitochondrial haplotypes that are very divergent from those of *C. collaris*. Second, if multiple alleles present in a common ancestor have been retained in descendent species, the alleles are expected to be randomly distributed in the descendant populations,

and not concentrated geographically near species boundaries or known hybrid zones (Barbujani et al. 1994; Hare and Avise 1998; Masta et al. 2002). In the present examples, the populations of *C. reticulatus* and *C. bicinctores* that carry mitochondrial haplotypes identical or only weakly divergent from those of adjacent *C. collaris* are not randomly distributed throughout the ranges of these species, but rather are concentrated near their geographic points of contact with *C. collaris*. Thus, we conclude that introgressive hybridization rather than incomplete lineage sorting has resulted in the unusual complement of mitochondrial haplotypes present in *C. reticulatus* and *C. bicinctores*.

RECENT MITOCHONDRIAL INTROGRESSION BETWEEN *C. COLLARIS* AND *C. RETICULATUS*

Crotaphytus reticulatus and *C. collaris* are distinct species that are well differentiated morphologically and ecologically (Montanucci 1971, 1974; McGuire 1996). Thus, there is no reason to believe that polyphyly of *C. reticulatus* in our mitochondrial tree is the result of poorly understood species boundaries. Rather, our data indicate that *C. reticulatus* mitochondrial genomes have been replaced by those of *C. collaris* over a substantial portion of the range of *C. reticulatus* in the Rio Grande Valley of Texas (Fig. 1). In our phylogenetic analysis of mtDNA data, *C. reticulatus* haplotypes fell into two widely separated sections of the *Crotaphytus* tree, with one set of haplotypes placed as the sister taxon of *C. antiquus*, and a second set deeply nested within eastern *C. collaris* (Fig. 2). We interpret the haplotypes that are placed phylogenetically as the sister of *C. antiquus* to be derived from ancestral *C. reticulatus* mitochondrial genomes, and the remainder to represent recent introgression from *C. collaris*. Although the morphological study of McGuire (1996) did not find support for *C. reticulatus* and *C. antiquus* as sister taxa, these species share a number of morphological characters that are consistent with a sister taxon relationship including their dorsal color pattern composed of an extensive white reticulum with some of the cells filled with black pigments, and jet-black femoral pore glands and secretions (apparently unique among lizards). We therefore conclude that the *C. reticulatus* mitochondrial data are informative both from the standpoint of the phylogeny (in that they provide strong support for a sister taxon relationship between this species and *C. antiquus*), and in supporting recent introgressive hybridization between *C. reticulatus* and *C. collaris*.

Introgressed *C. collaris* haplotypes are not limited to the immediate vicinity of the *C. reticulatus*/*C. collaris* species boundary, and the pattern instead appears to reflect one of two plausible processes. One possibility is that *C. reticulatus* has recently replaced *C. collaris* in the northern half of the Rio Grande Valley, and during this process captured the mitochondrial genome of *C. collaris* throughout the region. This hypothesis is consistent with our ecological niche model for *C. collaris*, which suggests that this area

should provide appropriate habitat for this species. However, this scenario seems unlikely to us because the habitat requirements of *C. reticulatus* and *C. collaris* are quite distinct, with *C. collaris* requiring rocky substrates and *C. reticulatus* occurring in open mesquite flats. Although the climate in the northern portion of the range of *C. reticulatus* might also be appropriate for *C. collaris*, the topography and substrate is not. A second hypothesis—and the one that we favor—is that there is an ongoing sweep (Avise 2000) of the *C. collaris* mitochondrial genome through the range of *C. reticulatus*, with the sweep having already covered approximately two-thirds of the range of *C. reticulatus*. If this interpretation is correct, the leading edge of the “wave front” is in the neighborhood of Falcon Reservoir (Fig. 1, locality 1; where roughly half of the lizards collected along the same limestone bluff carried *C. collaris* haplotypes and the other half carried ancestral *C. reticulatus* haplotypes). Thus, our analysis possibly provides a snapshot of an ongoing mitochondrial replacement event that could eventually result in all *C. reticulatus* carrying *C. collaris* mitochondria.

No hybrid zones or areas of range overlap have been documented for *C. reticulatus* and *C. collaris* (Montanucci 1974), although it is known that their ranges approach within 14 km of one another in northeastern Coahuila, Mexico (McGuire 1996). Montanucci (1974) proposed that the range of *C. reticulatus* once expanded further westward, overlapping that of *C. collaris*, and thereby mediating introgressive hybridization. Our mitochondrial data support Montanucci's (1974) hypothesis of recent introgressive hybridization, but the process that brought these species into secondary contact remains open to conjecture. Our contemporary ecological niche model for *C. reticulatus* suggests that its range may extend further westward in northern Nuevo Leon than is currently documented, which would provide broad geographical overlap with *C. collaris* (Fig. 4). Thus, the ranges of the two species could overlap in this area today or perhaps overlapped in historical times but fail to do so now because of habitat degradation. It is also possible that the range of *C. reticulatus* extended further westward during the last glacial maximum, thereby providing enhanced opportunities for hybridization. Indeed, our paleoecological niche model for *C. reticulatus* predicts a westward expansion out of the Rio Grande Valley, and more extensive overlap with *C. collaris*. Regardless of whether they were in contact during glacial maxima or minima, we suggest that it is much more likely that range shifts in *C. reticulatus* have brought this species into contact with *C. collaris* than vice versa because *C. reticulatus* is not limited to rocky habitats that do not shift spatially with changes in climate as does *C. collaris*. We further note that the sister taxon relationship between *C. reticulatus* and *C. antiquus* provides additional evidence that the range of *C. reticulatus* (or the common ancestor of *C. reticulatus* and *C. antiquus*) once extended much further westward than it does today. Indeed, *C. antiquus* is restricted to the rocky slopes of three contiguous but isolated

mountains in southwestern Coahuila, Mexico, approximately 300 km west of the current range of *C. reticulatus* (Axtell and Webb 1995; McGuire 1996), and the intervening region is now occupied exclusively by *C. collaris*.

TEMPORALLY SEPARATED AND REPEATED INTROGRESSION BETWEEN *C. COLLARIS* AND *C. BICINCTORES*: THE "INTROGRESSION CONVEYOR"

The pattern of mitochondrial introgression between *C. collaris* and *C. bicinctores* is unusual in that introgression appears to have occurred on at least three separate occasions at the same geographical boundary during the past few million years. *Crotaphytus bicinctores* is a well-differentiated, morphologically distinct species, easily distinguishable from *C. collaris* on the basis of numerous morphological differences (McGuire 1996). Despite the morphological and geographic cohesiveness of this species, *C. bicinctores* haplotypes are placed phylogenetically in four separate sections of the *Crotaphytus* mitochondrial tree. Throughout most of its geographical range, *C. bicinctores* is characterized by a monophyletic suite of mitochondrial haplotypes that are approximately 10–11% genetically divergent (uncorrected) from haplotypes typical of *C. collaris*. In contrast, in southwestern Arizona, all *C. bicinctores* carry mitochondrial haplotypes that appear to be derived from western *C. collaris* populations. These introgressed haplotypes are not uniform. Rather, the haplotypes fall into three discrete groups (i.e., 0%, 2%, and 5% divergent haplotypes, see Results), each of which is geographically localized to a particular section of southwestern Arizona (Fig. 5). The 0% haplotypes are found nearest to the zone of potential contact with western *C. collaris* (an actual hybrid zone has yet to be identified in this area), the 2% divergent haplotypes are in an intermediate geographic position west of the 0% haplotypes, and the 5% divergent haplotypes are found even further west relative to the *C. collaris* interface (and bounded on their west by the Colorado River). This pattern does not simply reflect isolation by distance because parastrophic genetic distances do not increase continuously from east to west. We posit that the 0%, 2%, and 5% divergent haplotypes are the result of temporally isolated introgression events that occurred via hybridization along the *C. bicinctores*/*C. collaris* geographical interface.

Two alternative hypotheses could explain the observed pattern. One possibility is that *C. collaris* occupied southwestern Arizona and was recently replaced by *C. bicinctores*. This hypothesis requires a number of complicating assumptions that limit its plausibility. First, it requires that *C. collaris* in this region was genetically structured (5% and 2% divergent haplotypes), despite that our contemporary and 21,000 YBP ecological niche models indicate that the region has only represented appropriate habitat for *C. collaris* during glacial maxima (suggesting the need for recolonization, which generally results in absence of genetic

structuring; Fig. 4). Second, the hypothesis requires that mitochondrial gene capture occurred many times independently as *C. bicinctores* swept through from west to east, such that the underlying genetic structure present in *C. collaris* continues to be reflected in *C. bicinctores*. We propose an alternative scenario in the form of the "introgression conveyor" model (Fig. 6), which is based on repeated hybridization-mediated introgression events at or near the contemporary *C. collaris*/*C. bicinctores* geographical interface, followed by slow westward migration of introgressed mitochondrial genomes through *C. bicinctores* populations. Although we cannot place precise dates on the three hypothesized introgression events, a very coarse calibration of 2% per million years for these mitochondrial markers (Brown et al. 1979; Wilson et al. 1985) implies that the 5% and 2% haplotype clades originated via introgressive hybridization approximately 2.5 million and one million YBP, whereas the 0% haplotypes result from relatively recent hybridization. In the absence of a time-calibrated (relaxed) molecular clock specific to these lineages, these proposed introgression dates are speculative, but we tentatively conclude that introgressive hybridization between *C. collaris* and *C. bicinctores* has occurred periodically throughout at least the latter half of the Pleistocene, if not longer.

The pattern of mitochondrial introgression observed in southwestern Arizona is unusual in several respects. First, we argue that the earliest introgression event occurred on the order of two to three million YBP. Mitochondrial introgression typically is only proposed when the observed mitochondrial haplotypes in the parental species are identical or only weakly divergent because, with increasing haplotype divergence, it becomes progressively more difficult to discriminate between introgression and incomplete lineage sorting (Avice and Ball 1990; Funk and Omland 2003). However, in the present case, the continued occurrence of substantially more divergent (i.e., ancestral) mitochondrial genomes in more geographically distant populations of *C. bicinctores* provides compelling evidence for ancient introgression followed by substantial divergence.

Second, the pattern of repeated, temporally separated, unidirectional introgression also is unusual. These features of *C. collaris*–*C. bicinctores* introgression are difficult to explain on the basis of conventional mitochondrial genome capture theory, which usually involves one of two alternative explanations for mitochondrial capture and replacement across an extended geographical area: (1) neutral genetic drift associated with frequent hybridization, or (2) selective sweeps associated with rare hybridization events (Ballard 2000; Ballard and Whitlock 2004). A neutral genetic drift hypothesis cannot be rejected based on available evidence, but is nevertheless difficult to reconcile with the observed data given the extended temporal period during which these haplotypes appear to have been present in the region (i.e., millions of years). Selective sweep hypotheses are often based on

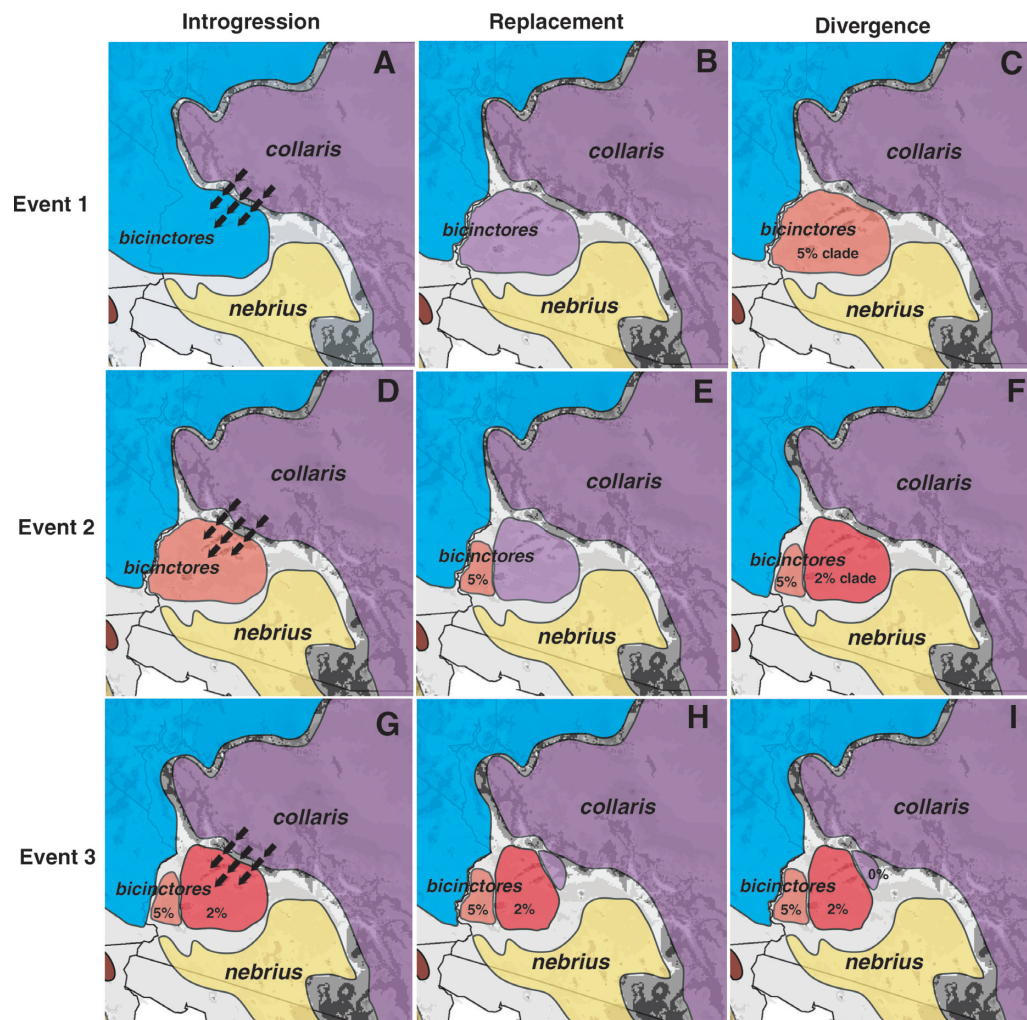


Figure 6. Model describing the “introgression conveyor” by which *Crotaphytus bicinctores* populations in southwest Arizona have acquired mitochondrial haplotypes from adjacent populations of *C. collaris*. A crude molecular clock calibration of 2%/million years suggests that introgression event 1 occurred approximately two to three million years ago (MYA), event 2 occurred approximately 1 MYA, and event 3 has taken place relatively recently.

the plausible argument that introgressed mitochondrial genomes are better adapted to the thermal environment of the recipient species than was the species’ naturally occurring mitochondrial genome (Doi et al. 1999; Somero 2002; Mishmar et al. 2003; Ballard and Whitlock 2004). A selective sweep scenario based on thermal adaptation seems unlikely in the present case because *C. bicinctores* is widespread in the most extreme desert regions of western North America, including the colder Great Basin Desert to the most thermally extreme environments of the Mojave Desert (i.e., Saline and Death Valleys). Thus, it is difficult to accept the premise that the mitochondria of *C. collaris*, which occurs in less extreme thermal environments, is better adapted to conditions in southwestern Arizona. The pattern of repeated mitochondrial introgression events in southwestern Arizona is, in fact, difficult to reconcile with *any* selective sweep scenario, regardless of whether it is connected to thermal adaptation. Although the 5% divergent

C. collaris mitochondrion might plausibly have provided a selective advantage relative to the ancestral mitochondrion of *C. bicinctores*, the likelihood of selectively advantageous mutations first driving replacement of the 5% divergent mitochondria with the 2% mitochondria, followed by a similar selection-driven replacement of 2% mitochondria with the more recently introgressed 0% version seems remote. At present, evolutionary processes mediating introgression in this system remain unknown.

Crotaphytus bicinctores and *C. collaris* are known to hybridize in nature, with two well-documented hybrid zones having been described in the literature (Axtell 1972; Montanucci 1983). Interestingly, both of the documented hybrid zones are outside of the region of mitochondrial introgression in southwestern Arizona. Montanucci (1983) described a narrow hybrid zone in the Little Colorado River drainage area of northern Arizona, and showed unequivocal examples of hybridization based on

allozymic data. Axtell's (1972) hybrid zone occurred along the north slope of the Cerbat Mountains near Dolan Springs in northwestern Arizona. JAM and CWL revisited this site in 2005 and concluded that the hybrid zone was no longer active, with *C. collaris* still present on the north slope of the Cerbat Mountains, and *C. bicinctores* restricted to the south slope of the White Hills approximately 2 km to the north (separated by the narrow valley within which resides the town of Dolan Springs). Surprisingly, we did not detect any evidence of mitochondrial introgression in this region, suggesting that the hybridization events responsible for introgression must have occurred further south along the geographical interface between *C. bicinctores* and *C. collaris*. This finding suggests that mitochondrial introgression is not an inevitable result of hybridization between these species, and might in fact be an extremely rare event relative to the frequency of hybridization.

Finally, we note that the morphological phylogenetic analysis of McGuire (1996) found strong support for a monophyletic assemblage composed of *C. bicinctores*, *C. vestigium*, *C. grisei*, and *C. insularis*. Indeed, these taxa (excluding the more recently described *C. grisei*) were considered to be conspecific in earlier taxonomies (Axtell 1972; Stebbins 1985). In contrast, the mitochondrial phylogenetic estimate suggests that *C. bicinctores* are more closely related to *C. collaris* (assuming that the ~10% haplotypes indeed represent the nonintrogressed *C. bicinctores* mitochondrial genome) than to *C. vestigium*, *C. grisei*, and *C. insularis*, albeit as the weakly supported sister taxon of *C. dickersonae*. There are several possible explanations for this phylogenetic incongruence including error in the morphology tree, incomplete sorting of mitochondrial haplotypes, and ancient mitochondrial introgression. We speculate that given the apparent occurrence of repeated introgression between these species, that the ancestral *C. bicinctores* mitochondria might actually be derived from an even earlier introgression event with *C. collaris* than the three events discussed above.

CROTAPHYTUS NEBRIUS AND C. COLLARIS

Mitochondrial introgression or incomplete lineage sorting could also explain other unexpected phylogenetic relationships recovered in our analysis of mtDNA data (i.e., *C. grisei*, *C. insularis*, *C. nebris*, and *G. copei*). The mitochondrial phylogenetic results for *C. nebris* and *C. collaris* are complex, with *C. nebris* haplotypes appearing in at least six different places in the *Crotaphytus* topology. Given several well-documented cases of mitochondrial introgression between *C. collaris* and other *Crotaphytus* species, it is tempting to suggest that this simply represents another series of introgression events. Although this may be true, there are other plausible explanations that cannot be discounted at this time. One possibility is that *C. nebris* is not a valid species distinct from *C. collaris*. *Crotaphytus nebris* is easily differentiated from *C. collaris* on the basis of morphology, but the morphological phylo-

genetic analysis of McGuire (1996) suggested that they are closely related (Fig. 3). Another possibility is incomplete lineage sorting. Most *C. nebris* from northern populations are placed in a well-supported clade that is sister to the large western *C. collaris* (plus introgressed *C. bicinctores*) clade. Similarly, most of the southern *C. nebris* populations are placed in a well-supported clade near the base of the large eastern *C. collaris* clade. If all northern and southern *C. nebris* were placed in these basal *C. nebris* clades, a natural explanation would be incomplete lineage sorting between recently diverged sister species. However, the somewhat random placements of additional *C. nebris* within the western and eastern *C. collaris* clades suggest that perhaps a combination of incomplete lineage sorting and recent introgressive hybridization best explains the observed distribution of *C. nebris* mitochondrial haplotypes. This complex pattern requires further evaluation with multilocus genetic data.

CROTAPHYTUS GRISMERI, C. INSULARIS, AND C. VESTIGIUM

The mitochondrial phylogenetic analysis provides strong support (100% posterior probability) for the monophyly of *C. grisei*, *C. insularis*, and *C. vestigium*. This result contradicts the morphological phylogenetic study of McGuire (1996), who found strong support for the monophyly of these species only if *C. bicinctores* was also included. Although we have reservations about the mitochondrial phylogenetic position of *C. bicinctores* (see above), the monophyly of *C. grisei*, *C. insularis*, and *C. vestigium* is sensible from a biogeographical standpoint as each of these species is associated with the Baja California Peninsula. *Crotaphytus vestigium* occurs on mainland Baja California and in the contiguous Peninsular ranges of southern California; *C. insularis* is endemic to Isla Ángel de La Guarda, a continental island in the Sea of Cortez that separated from the Baja California Peninsula approximately two to three million YBP when a leaky transform fault shifted from the east side to the west side of the island (Lonsdale 1989; Carreño and Helenes 2002); and *C. grisei* is endemic to the Sierra de Los Cucapás and Sierra El Mayor in northeastern Baja California (McGuire 1994), which separated from the main block of the Peninsular ranges in the Pliocene and are separated from them by the Laguna Salada floodplain (Gastil et al. 1983).

The phylogenetic relationships of *C. grisei*, *C. insularis*, and *C. vestigium* again suggest a combination of incomplete lineage sorting and recent mitochondrial introgression. *Crotaphytus vestigium* is divided into relatively deeply divergent southern and northern clades (~5.5% uncorrected patristic distance). This pattern is consistent with many other Baja California taxa that exhibit north-south genetic splits (Upton and Murphy 1997; Riddle et al. 2000; Lindell et al. 2005; Riginos 2005; Crews and Hedin 2006; Lindell et al. 2006). Although the timing of this putative mid-peninsular divergence is unknown in *C. vestigium*, it appears to

predate the separation of Isla Ángel de La Guarda (two to three million YBP) because *C. insularis* is weakly placed as the sister taxon of the northern *C. vestigium* clade rather than that of all *C. vestigium*. Given the deep mitochondrial divergence between *C. insularis* and both the northern and southern *C. vestigium* clades, and the fact that *C. insularis* is restricted to Isla Ángel de La Guarda (rendering gene flow less likely), we conclude that this is an example of incomplete lineage sorting and not ancient introgression. *Crotaphytus grisei*, on the other hand, is virtually undifferentiated from *C. vestigium* according to the mitochondrial data. This finding suggests either that this species only recently became isolated and morphologically divergent from *C. vestigium* (with insufficient time for lineage sorting), or has experienced recent mitochondrial introgression as seen in some other *Crotaphytus* species. As is the case for *C. nebris*, the independent lineage (species) status of the morphologically differentiated *C. grisei* should be further evaluated using multilocus genetic data.

GAMBELIA COPEI AND G. WISLIZENII

Our mitochondrial data suggest that *G. copei*, which occurs primarily on the Baja California Peninsula, is deeply nested within the wide-ranging species, *G. wislizenii*. This suggests that *G. copei* is a recent peripheral isolate from the morphologically similar *G. wislizenii*, and perhaps that the species status tentatively assigned to it by McGuire (1996) was unjustified. McGuire (1996) indicted that the species status of *G. copei* required confirmation by evaluating the presence or absence of gene flow at their putative contact zone in northeastern Baja California. Our results here indicate that such a study would still be desirable.

PHYLOGENETIC ASSESSMENT

We have shown that the mitochondrial gene tree estimated here reflects a number of processes beyond phylogenetic descent, including several clear cases of mitochondrial introgression and incomplete lineage sorting. We have also identified less substantiated cases that might also reflect these processes. Because introgression and incomplete lineage sorting will result in misleading phylogenetic estimates if the gene tree is taken at face value, care must be taken to glean phylogenetic signal from these data. Nevertheless, several aspects of the mitochondrial gene tree are consistent either with the morphological phylogenetic estimate of McGuire (1996) or suites of morphological character states described in that study. For example, the mitochondrial data are consistent with the monophyly of *Crotaphytus* and *Gambelia*. Within *Gambelia*, the mitochondrial data are consistent with a sister taxon relationship between *G. sila* and other *Gambelia* (albeit with weak support). Within *Crotaphytus*, *C. antiquus* and *C. reticulatus* are placed as sister taxa, and together represent the sister taxon of all other *Crotaphytus*. Monophyly of *C. grisei*, *C. insularis*, and *C. vestigium* slightly contradicts McGuire (1996), but is more consistent

with biogeographical expectations given that all three species are associated with the Baja California Peninsula. The placement of *C. insularis* as the sister taxon of the northern clade of *C. vestigium* also makes biogeographical sense because the separation of *C. vestigium* into northern and southern clades prior to the separation of Isla Ángel de La Guarda from the northern Baja California Peninsula (and concomitant isolation of *C. insularis*) is consistent with many other Baja California taxa. Finally, if *C. nebris* proves to be a valid species, it very likely represents a relatively recent derivative of *C. collaris*, which is consistent with the mitochondrial tree. Other aspects of the mtDNA tree are more questionable given their disagreement with well-supported nodes in the morphological study (McGuire 1996), including the phylogenetic placement of *C. bicinctores* and *C. dickersonae* as sister taxa, and their placement together as the sister taxon of *C. collaris* plus *C. nebris*. The phylogenetic relationships of *crotaphytid* lizards clearly require further study with multilocus genetic data, preferably in concert with the previously published morphological data. Nevertheless, despite evidence for mixed signals in the mitochondrial data, we conclude that we are now closer to having a comprehensive phylogenetic estimate for *Crotaphytidae*.

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Appendix

Collection and voucher data for genetic samples. Museum codes follow Leviton et al. (1985). Nonstandard abbreviations are as follows: ADL, Adam D. Leaché; AKL, A. Kristopher Lappin; BKS, Brian K. Sullivan; DGM, Daniel G. Mulcahy; DWH, Delbert W. Hutchison; JAS, James A. Schulte; JFH, Jerry F. Husak; JLE, Julio Lemos-Espinal; JQR, Jonathan Q. Richmond; LEB, Lars E. Bell; LSUHC, La Sierra University Herpetological Collection; MCB, Matthew C. Brandley; TJL, Travis J. LaDuc; TWR, Tod W. Reeder.

Crotaphytus antiquus

MEXICO: Coahuila: JLE 12615 (Sierra de San Lorenzo), TNHC 53153, 53158 (Sierra de San Lorenzo at Santa Eulalia).

Crotaphytus collaris

MEXICO: Chihuahua: JLE 12300 (halfway between Sierra de En Medio and Rancho Nogales), JLE 12313 (Rancho Penoles), JLE 12314 (Sierra de En Medio, pradera de Janos), LSUMZ H6957 (W of Asencion); Coahuila: JLE 12241 (La Virgen, 25 km S Quimicas del Rey), JLE 12242, 12500-502 (10 km S Quimicas del Rey), JLE 12243 (9.5 km S Quimicas del Rey), JLE 12244, 12503 (Estacion El Oro), JLE 12311 (near La Esmeralda), JLE 12312, 12504 (S La Esmeralda), JLE 12513 (km 166 on rd to Quimicas del Rey), JLE 14058-60 (Adelante de Parras de las Fuentes, Coahuila. 3.5 km E Ejido Abrevaderos); ROM GAA 83-101 (Mohovano); Durango: LSUMZ H6961 (no specific locality), ROM GAA 83-9, 83-11, 83-23 (Mapimi); Nuevo Leon: ROM GAA 83-96, 83-105 (Dr. Arroyo), ROM GAA 83-60, 83-89, 83-90 (Matehuala vicinity); USA: Arizona: Apache County: DWH 4 (Little White House Canyon), DWH 5 (Ganado), DWH 6 (Petrified National Forest), DWH 7 (St. Johns area); Cochise County: AKL 227-228 (Foothills Rd. N of Portal [within 10 km]), JAS 299 (~4 miles North of Intersection of State Route 186 and Apache Pass Road on Apache Pass Road), JAS 300 (1 mile North of Dos Cabezas off State Route 186), JAS 301 (on Rucker Canyon Road, ~4 miles from intersection of Hwy 191), LSUMZ 18418-419 (Coronado National Forest, AMNH Southwest Research Station, Portal); Coconino County: ADL 208 (Angel Rd., exit 211 off I-40), AKL 193, 195, 316 (Buffalo [Range] Rd. [exit 225] off I-40 E Flagstaff), TWR 2031-32, 2034-35 (9.7 mi S of Gray Mountain, 0.9 mi W of Hwy 89), TWR 2040-42 (along Meteor Crater Rd., 4 mi N of Meteor Crater), TWR 2058 (along Hwy 99, 1.7 mi NW of jct of Hwy 99 and I-40); Gila County: DWH 8 (Cassadore Spring); Graham County: DWH 9 (Old Safford Road); Maricopa County: AKL 224 (0.8 mi E on Flat Top Mesa Rd. off of I-17 N of Phoenix), LVT 2241-43 (first dirt rd E Hwy 87 just past 4 peaks scenic vista, 3.2 mi down rd. c. 10 mi NE of Scottsdale off Hwy 87 [near FR 142/Cline Cabin Road to 4 Peaks Wilderness and Roosevelt Lake]); Mohave County: LVT 2250 (25.4 mi S. Pierce Ferry Rd. on Stockton Rd. 14.6 mi N. Kingman), MVZ 244226 (W slope of Cerbat Mts. on Mineral Park Rd.), MVZ 244227 (N face of Cerbat Mts. at Dolan Springs), TWR 2014 (6.1 mi NNE of Antares Rd., along "precursor" to Indian Rd. 2); Navajo County: ADL 219 (Woodruff Rd.), ADL 221-222 (Old Woodruff Rd., between Woodruff and Snowflake), LEB 022 (Off of Old Woodruff Rd.), LEB 152 (On Concho-Snowflake Rd., east of Snowflake), LEB 153 (On Rt. 77, 2.5 mi south of Taylor), LVT 2302 (Holbrook, 8th & Greer, north of town), TWR 661 (27.7 mi S of Hwy 377 and 77 jct, along Hwy 377 [= Heber Rd.]); Pima County: DWH 10 (Tucson area), LSUMZ H5538-39 (Santa Catalina Mountains on Burlington Road); Pinal County: BKS 1125 (Santan Mountains); Yavapai County: AKL F1, M1, M2 (W of Sedona off of FR 152 going N off of HW 89), AKL 189, 252, 254-55 (Bloody Basin Rd. off of I-17), AKL 317, 320, 322 (Chino Valley, just down

road S of Seligman [exit 123 off of I-40]), LVT 2277-79 (NE of Wickenburg on Constellation Rd. 12.2 mi from junction w/ Jack Burden Rd.), LVT 2280 (Bagdad, on Lindahl Rd. 1 mi. N. of junction w/ Copper Rd. on Ledge east of Rd.), MVZ 244225 (ENE of Wickenburg on Constellation Rd.), MVZ 244228-30 (vicinity of Wickenburg on Rincon Rd. and Scenic Loop); Colorado: Garfield County: TWR 1802-03 (1.9 mi N of the jct of CO 13 and CO 325); Montrose County: ADL 278-279 (4.8 mi. N of Naturita along SR 141); Otero County: CAS 223559 (Vogel Canyon Picnic Area, 1.27 mi S of CR 802 [Davidson Cyn. Rd.], 2.2 mi W of Hwy 109); Kansas: Geary County: LVT 2233, 2235 (Fort Riley Camp); Missouri: Ozark County: DWH 20 (Long Bald [Caney Mountain Wildlife Refuge]); Vernon County: DWH 19 (Eve Quarry); New Mexico: Doña Ana County: AKL 122, 268, 355 (Baylor Canyon Road off of HW 70), LVT 2220-21 (2.45 mi on Aguirre Springs Loop, Organ Mtns. 3.9 mi S. of 70/82), LVT 2323 (4.2 mi. W. of Co. Rd. A-3 and 0.7 mi. S Co. Rd. A-008), Eddy County: DWH 15 (El Paso Gap); Grant County: LVT 2325 (6.2 mi. S mile 45 on NM 9, 2.2 mi. W of Hachitas); Hidalgo County: DWH 13 (Animas area), TWR 210 (2.2mi W jct. NM 9 & NM 338, just off N side NM 9); Lincoln County: LVT 2286 (Valley of Fires BLM area, Malapais Lava Flows); Luna County: DWH 12 (Rockhound State Park), LVT 2259, TJL 319 (Victorio Mtns, C020 rd, 2.7 mi S Interstate 10, 0.4 mi S C 020 on quarry rd.), TWR 227 (just outside entrance to Spring Canyon State Park), LVT 2285 (Hermanas-Deming Rd. 2.6 mi N. of Hwy 9 on boulder by rd.); Otero County: DWH 14 (Otero Mesa); San Juan County: LVT 2306 (18.1 mi. S. of Shiprock (Town), E. of Hwy 666); Sierra County: DWH 11 (City of Rocks State Park); Socorro County: JQR 163 (Goat Draw Canyon just north of Hwy 60E); Oklahoma: Kiowa County: JAS 195-97 (intersection of E9490 and N2160 Roads); Oklahoma County: LVT 2298-2301 vicinity of Arcadia); Texas: Coke County: DWH 18 (Lake Spence); El Paso County: AKL 353-354 (Cottonwood Springs turnout off State/County Rd. 375 [junction with I-10 ca. 7 mi S of NM/TX border] in the Franklin Mtns.); Hudspeth County: LVT 2331-33 (Indio Mtns., Indio Research Station 0.5 km N of rd. tank on road to Squaw Peak), LVT 2334 (10.2 rd. mi. S I-10 on rd. to Hot Wells, 5.8 rd. mi. S Hot Wells on rd. to Eagle Mtns); Sutton County: DWH 17 (Texas A&M Research Station); Travis County: 30 mi. SW Austin at Gill Ranch; Val Verde County: DWH 16 (Seminole Canyon St. Park); Utah: Grand County: AKL 301 ("Hittle Bottom" off HWY 128 NE of junction with US 191), AKL 302 (Mile Marker 24 on HW 128 NE of junction with US 191), AKL 305 (NE of Moab), AKL 307, 311-12 (near junction between La Salle Mtn. Loop Rd. and Geyser Pass Rd., E Moab), DWH 1 (Rock Corral Road), LVT 2251 (2.0 mi. N Thompson on Sego Petroglyphs Rd.), TWR 1789 (13.7 mi NE of jct. UT 128 and US 191, 0.2 mi E UT 128); San Juan County: DWH 2 (Davis Canyon Rd.), DWH 3 (Holly Trail), LVT 2254-55 (T27S R23E S20).

Crotaphytus bicinctores

USA: Arizona: La Paz County: LVT 2270 (1.8 mi S SR 72 on Yellow bird Rd. NW of Bouse at Plomosa Mtns.), MVZ 244170 (W edge Harquahala Mts., ca. 4 km SE of Salome off Centennial Park Rd.), MVZ 244171-74 (Plomosa Mtns. via Bouse-Quartzsite Rd. [= Plomosa Rd.]), MVZ 244175-77 (Dome Rock Mts. N of I-10); Maricopa County: AKL M1-M5 (Gila Mtns. near Gillespie Dam [within 5 km along pipeline road]), LVT 2239-40 (3.1 mi S Hwy 60 East of Eagle Eye Rd., east edge Harquahala Mtns.), LVT 2275 (Right side of Gillespie bridge just W of bridge), MVZ 244178 (Sentinel, ca. 6 mi N I-8), MVZ 244279-81 (road to Painted Rock Dam), MVZ 244182-85 (White Tank Mtns.), MVZ 244186-89 (Vulture Mtns.), MVZ 244190-92 (Harquahala Mts., ca. 5 km S of Aguila via Eagle Eye Rd.), MVZ 244193-95 (Belmont Mts., 4.2 mi S of intersection of Wickenburg Rd. and Vulture Mine Rd.); Mohave County: AKL 181, 183 (25 mi. S state line on Mt. Trumbull Loop), LVT 2244 (17.1 mi E Oatman Rd. CG (by I-40), 4.9 mi. W Oatman), MVZ 244196-97 (E slope of Black Mts., ca. 5 km S of Yucca via I-40), MVZ 244198-205 (W slope of Black Mts. on Old Hwy. 66), MVZ 244206-09 (S slope of White Hills at Dolan Springs); Yuma County: LSUMZ H5523, 5525, 5527, 5529 (Kofa Mtns., Palm Canyon), MVZ 244210-12 (Agua Caliente, off Sentinel-Hyder Rd.), MVZ 244213-14 (Laguna Mountains, via road to N. R. Adair Park and shooting range), TWR 2146 (Kofa NWR, ~8.3 mi E Hwy 95, near beginning of Kofa Queen Canyon); California: Imperial County: AKL 124 (5 mi up Black Mountain Rd. off of HW 78), AKL 125 (5.2 mi up Black Mountain Rd. off of HW 78); Inyo County: AKL 139 (1 mi. up dirt road N of HW 190 3.2 mi W of HW 190/Olancho Darwin Rd. intersection), AKL 140 (Saline Valley Rd., 17.6 mi N of junction with HW 190), MVZ 137454 (7 mi. E Hwy. 190, Saline Valley Rd.), MVZ 137645 (0.5 mi W of Inyo National Forest boundary, Hwy. 168), MVZ 137679 (5 mi E [by road] Panamint Springs, Hwy. 190, Death Valley National Monument); San Bernardino County: AKL 11, 48A (Newberry Mtns. where they reach the National Trails HW and I-40 just W of Newberry Springs), AKL 103, 105 (Pisgah Crater), LVT 716 (NE of Old Dad Mtn., just E of N Big Game Guzzler 0.25 mi E of NE corner S11), LVT 717 (1.4 Mi. E of Kelbaker Rd., N Foothills of Marble Mtns., NE corner of S30), LVT 718 (Old Woman Mtns., Rattlesnake Canyon), MVZ 244219-24 (E slope of Sacramento Mts. ca. 5 km S of I-40), MVZ 244215-18 (Big Maria Mts., N of Blythe, ca. 15 km N (via Hwy. 9) of I-10/Hwy. 95 intersection), ROM 14584 (9 mi. E Ludlow); Nevada: Churchill County: AKL 165 (HW 50, 14.9 mi W of HW 361/50 junction), LVT 676 (Eetza Mtn., Lahontan Mtns, 1.9 mi N. Grimes Pt [I-50], 11.8 Mi E of Fallon [I-50/I-95]); Clark County: LVT 2218 (1.3 mi N. of 160 on 159), LVT 2249 (Cabin Creek Canyon, Virgin Mtns.), LVT 2257 (Blue Diamond Rd., 5 mi. past red rock turnoff); Esmeralda County: LVT 651 (3.3 mi S. Goldfield Summit, E of I-95); Pershing County: CAS 227918 (ca

100 m N of dirt rd., SW side Hwy [SSR34], 14 mi N of Gerlach [Jct 447]); Washoe County: CAS 227925 (Empire Ranch Rd., SW of SSR447); White Pine County: CAS 223401 (3 mi SW [by rd] of 487, along Snake Creek Rd., Snake Range); Oregon: Harney County: LVT 2226 (31.3 mi. S of Hwy 78, 6 mi. E. of Cattle-guard turnoff); Utah: Juab County: LVT 5183-85 (W side Topaz Mountain); Kane County: LVT 2252 (47 mi. E of Kanab & 15 mi W. Glen Canyon off Hwy 89, 0.8 mi. up Cottonwood Canyon); Millard County: CAS 223399 (Knoll Sprg. Rd., ca. 1 mi. [by air] NE Eskdale, SE Conger Range).

Crotaphytus dickersonae

MEXICO: Sonora: LSUMZ H5487 (Bahía Kino), LSUMZ H5500 (3 mi. E Bahía Kino), LSUMZ H5570 (24.5 mi. N Bahía Kino), MVZ 241181 (), MVZ 241186 (), MVZ 241192-93 (35.0 road mi N of Bahía de Kino Nuevo via the road to Punta Chueca and El Desemboque, 2.0 road mi E of Punta Chueca and El Desemboque road), ROM 15051 (Bahía Kino).

Crotaphytus grisei

MEXICO: Baja California: UNAM MZFC-Herp 6646-1, 6647-1 (Sierra de Los Cucapas).

Crotaphytus insularis

MEXICO: Baja California: LSUHC 421, ROM 34013, 34870 (Isla Ángel de La Guarda),

Crotaphytus nebrius

MEXICO: Sonora: JLE 11835-36 (Guisamopa); LSUMZ H5509 (60 km S Hermosillo), LSUMZ H5512 (75 mi. NW Caborca), LSUMZ H5577 (Punta San Carlos), MVZ 24119-96 (2.9 mi S Xolotl rd., Hermosillo), MVZ 241197-98 (6.5 mi E Heroica Caborca via Mexico Hwy. 46), MVZ 241199 (7.8 mi NNW Heroica Caborca via Mexico Hwy. 2), TWR 1250 (Villa Hidalgo), TWR 1393 (San Carlos); USA: Arizona: Maricopa County: AKL CnF1 (near Robbins Butte in Buckeye Hills, about 15 km NE Gillespie Dam), AKL CnM1 (at base of Powers Butte in Buckeye Hills, about 10 km N of Gillespie Dam), AKL 225, 256 (Buckeye Hills near Gillespie Dam), LVT 2312-13 (Sierra Estrella Mtns., near Phoenix), MVZ 244231-36 (Estrella Mts., immediately S of Phoenix International Raceway), MVZ 244237-38 (Little Ajo Mtns.), MVZ 244239-43 (Tucson Mountain Park), MVZ 244244-45 (Silverbell Mts., 19.9 mi W of intersection of Avra Valley Rd. and Hwy. 10 via Avra Valley Rd.), MVZ 244246-47 (Mohawk, N side I-8); Yuma County: DGM 471 (southern end of Gila Mountains), LSUMZ H5518-20 (N edge of Gila Mountains)

Crotaphytus reticulatus

USA: Maverick County: LSUMZ H5574-75 (1 mi. E Eagle Pass); Starr County: JAM 3983-85, JFH J1, J3, J4, J6, J7, K2, K3, P1 (Falcon State Park); Webb County: JAM 3986 (24.1 mi. NW intersection FM 1472 and FM 255), JAM 3987 (16.2 mi. NW intersection FM 1472 and FM 255).

Crotaphytus vestigium

MEXICO: Baja California: ROM JRO 330-331 (2 mi. N El Arco); Baja California Sur: MVZ 236264 (7.5 mi E San Isidro via Hwy. 1), ROM 13557-58 (Santa Agueda, 2.5 mi. W Hwy 1), ROM 13573-74 (Santa Agueda, 4.9 mi. W Hwy 1), ROM RWM 546 (29 mi. S Mulege), SDSNH F350 (San Jose de Magdalena); USA: Imperial County: TWR 1913 (Dos Cabezas); Riverside County: MVZ 249150 (San Jacinto Mts., Palm Oasis); San Diego County: CAS 223631 (McCain Valley, near mining Pit #667 [McCain Pit #667], 15.6 mi S of Hwy 78 along Hwy S2), LSUMZ H5534 (Mountain Springs Road), LSUMZ H5536 (NE Vallecito).

Gambelia copei

MEXICO: Baja California: MVZ 161173 (11.4 mi S by Mexico Hwy. of junction with Rd. 10 Guerrero Negro), MVZ 161174 (7 km W (by road) Bahía de Los Angeles), ROM JRO 363, 365, 368, 370, 373 (Isla Cedros, Arroyo Cardón); Baja California Sur: ROM RWM 1031, 1056 (1.3 mi. NE Punta Abreojos), ROM RWM 1066 (19 mi. NE Punta Abreojos), ROM RWM 1067 (9 mi. NE Punta Abreojos), SDSNH F393 (Santa Rosalia).

Gambelia sila

USA: California: Madera County: LSUMZ H5586, H5592 (5.9 mi. E Firebaugh).

Gambelia wislizenii

MEXICO: Coahuila: ROM GAA 83-102 (Mohovano); Durango: ROM 15102, ROM GAA 83-1, ROM GAA 83-8, ROM GAA 83-18 (Mapimi); Sonora: LSUHC 3215 (Isla Tiburón), LSUMZ H5501 (4.1 mi. E Bahía Kino), LSUMZ H5502 (25 mi. E Hermosillo), LSUMZ H5503 (Punta Tepoca), LSUMZ H5504 (Sano Flats, 2 mi. E Punto Libertad), LSUMZ H5505 (32.8 mi. N Bahía Kino), LSUMZ H5573 (17.4 mi. N Bahía Kino), ROM 14165 (41 km SW Sonoita); USA: Arizona: Coconino County: TWR 2048 (Along Hwy 99, 16.4 mi NW of jct. of Hwy 99 an I-40), TWR 2054 (5 mi NW of jct of Hwy 99 an I-40, along Hwy 99);

Yavapai County: MVZ 244251 (vicinity of Wickenburg on Rincon Rd. and Scenic Loop); Yuma County: AKL 225 (near intersection Ave. 3E and County 19th Str., just W Barry M. Goldwater Air Force Range); California: Imperial County: ROM 13775, 13852 (Ocotillo, Shell Canyon Rd.), TWR 540-41 (Dos Cabezas Road); Inyo County: AKL 30 (6 mi up Homewood Canyon Road from Trona Road, 7 mi. N Trona), AKL 151 (Eureka Valley Rd. 34.1 mi S of junction with Oasis Rd. [near HW 266/168 junction]); Kern County: AKL 104 (4.5 mi N of California City near Phillips Rd. [dirt], 2 mi. from Desert Tortoise Preserve), MCB 225 (Near El Mirage Dry Lake and Adelanto, ~1.5 mi. W Koala Road on Chamberlaine), MCB 227 (Near El Mirage Dry Lake and Adelanto, ~1.0-1.5 mi. W Koala Road on Chamberlaine); Los Angeles County: ROM 13985-87 (Longview and Palmdale), ROM 14589, 14608-09 (50th and Palmdale Blvd.); Riverside County: SDSNH 68904 (Hwy 10, 10 mi. N Corn Springs exit); San Bernardino County: AKL 19 (base of S slope Kelso Dunes), AKL 77 (up dirt road ca. 3 mi. N on Lucerne Valley Cutoff off of HW 247), AKL 137 (Pisgah Crater), AKL 188 (Camp Rock Rd. 18 mi SE of exit off I-40 [junction near Daggett] at NW margin of Frye Mtns.), JQR 127 (Mojave National Preserve, ca. 3 miles NE of junction Kellbaker Rd/Brant Cima Rd. on Brant Cima Rd.), ROM 13750 (N of Kelso), ROM 14646 (10 mi. W Newberry Springs), TWR 1965 (0.4 mi S of Hwy 62 on Iron Mt. Pumping Plant Rd., 7.2 mi NE of jct of Hwy 177 and Hwy 62); San Diego County: LSUMZ H5507 (Anza Borrego Park, N of Mount Palm), ROM 14021-22, 16107 (7.6 mi. NW Ocotillo); Nevada: Mineral County: MVZ 150063-64 (3 mi. S of Rattlesnake Wells, 0.3 mi. E of junction of Rattlesnake Wells Rd. and Hwy. 31), MVZ 150065 (0.1 mi. S Rattlesnake Wells, 0.3 mi E of junction of Rattlesnake Wells Rd. and Hwy. 31); Nye County: AKL 154, 156 (Gabbs, near mine shaft and junk pile); Washoe County: AKL 166 (off of Fish Springs Rd. 10.9 mi. W of Fish Springs Rd./HW445 junction), AKL 167 (off of Fish Springs Rd. 15.7 mi W of Fish Springs Rd./HW445 junction); Utah: Grand County: AKL 299-300 ("Hittle Bottom" off of HW 128 NE of junction with US 191).