

# Novel patterns of historical isolation, dispersal, and secondary contact across Baja California in the Rosy Boa (*Lichanura trivirgata*)

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## Abstract

Mitochondrial DNA (mtDNA) sequence variation was examined in 131 individuals of the Rosy Boa (*Lichanura trivirgata*) from across the species range in southwestern North America. Bayesian inference and nested clade phylogeographic analyses (NCPA) were used to estimate relationships and infer evolutionary processes. These patterns were evaluated as they relate to previously hypothesized vicariant events and new insights are provided into the biogeographic and evolutionary processes important in Baja California and surrounding North American deserts. Three major lineages (Lineages A, B, and C) are revealed with very little overlap. Lineage A and B are predominately separated along the Colorado River and are found primarily within California and Arizona (respectively), while Lineage C consists of disjunct groups distributed along the Baja California peninsula as well as south-central Arizona, southward along the coastal regions of Sonora, Mexico. Estimated divergence time points (using a Bayesian relaxed molecular clock) and geographic congruence with postulated vicariant events suggest early extensions of the Gulf of California and subsequent development of the Colorado River during the Late Miocene–Pliocene led to the formation of these mtDNA lineages. Our results also suggest that vicariance hypotheses alone do not fully explain patterns of genetic variation. Therefore, we highlight the importance of dispersal to explain these patterns and current distribution of populations. We also compare the mtDNA lineages with those based on morphological variation and evaluate their implications for taxonomy.

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## 1. Introduction

The extreme southwestern region of North America, including Baja California, has experienced some of the most dramatic geological events and ecological transformations as a result of tectonic interactions (Stock and Hodges, 1989). Most notable are the interactions responsible for the origin of the Baja California peninsula. Over the last 4 to 12 million years, expansion between the Pacific and North American plates has rifted the Baja California peninsula approximately 300 km to the northwest along the San Andreas Fault zone, leading to the formation of the Gulf

of California and resulting in one of the longest peninsulas in the world (Savage, 1960; Stock and Hodges, 1989; Holt et al., 2000; Oskin and Stock, 2003). More recently (15–30ka), an ecological transformation has changed the region from relatively mesic conditions toward increasing aridity and spreading deserts (Axelrod, 1979; Van Devender and Spaulding, 1979; Van Devender et al., 1987). This complex interplay of geological and ecological events, operating at different timescales, provides an opportunity for examining the evolution among the arid-adapted fauna inhabiting this region.

Using a synthesis of plate-driven rifting models, paleogeography, and paleoecology, Murphy (1983) and Grismer (1994a) presented general models for the evolution of the Baja California peninsula that promoted the importance of pre-Pleistocene vicariant events to explain current

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distribution patterns and/or areas of endemism for different squamate reptile taxa. Two primary vicariant hypotheses were proposed, with emphasis placed on the biological consequences of these historical events. Notably, species that were centered along more southerly portions of the expansion zone were separated first during the earliest inception of the Gulf of California around the late Miocene (between 11 and 6 Ma), designated the ‘southern gulf vicariance’ hypothesis. Sometime later (between 8 and 4 Ma), species distributed further north were separated when northern extensions of the Gulf of California reached their maximal limits and inundated lower elevations of the continental deserts of southern California and Arizona (‘northern gulf vicariance’ hypothesis; Murphy, 1983; Grismer, 1994a).

These vicariant hypotheses and biogeographical scenarios countered previous ideas that emphasized waves of dispersal mediated by Pleistocene climatic changes as the dominant force in the evolution of arid-adapted herpetofauna of Baja California (e.g., Savage, 1960). However, some case examples proposed by Murphy (1983) and Grismer (1994a) suffered definitive support for vicariant explanations because no well-corroborated phylogenies existed at the time. Recently, a number of phylogenetic studies of vertebrate species in southwestern North America have demonstrated the importance of pre-Pleistocene processes in shaping the distribution of lineages, providing support for these vicariant hypotheses (Riddle et al., 2000a,b; Jaeger et al., 2005; Devitt, 2006). These studies (as do others [e.g., Upton and Murphy, 1997; Orange et al., 1999; Nason et al., 2002; Crews and Hedin, 2006; Recuero et al., 2006]) highlight the importance of using independent lines of evidence (e.g., phylogeny) to validate such biogeographic hypotheses. Thus, vicariance is now considered by many to have been the dominant force underlying biogeographic patterns of the vertebrate fauna in the desert southwest. Yet, it has been recently demonstrated that both vicariance and dispersal are natural processes affecting current distributions of taxa, so dispersal should not be rejected a priori (Austin et al., 2003; Jaeger et al., 2005; Noonan and Chipindale, 2006).

Molecular systematics, coupled with recently developed phylogeographic approaches, can now be incorporated allowing the potential to discriminate between hypotheses such as whether historical or more current evolutionary processes have shaped genetic variability (Avise, 1998; Templeton, 1998; Knowles and Maddison, 2002). In addition, this allows the ability to compare the depths and positions of intraspecific phylogeographic breaks with the magnitude and locations of extrinsic factors such as geology and climatic changes. Combined, these approaches enable determination of the roles both dispersal and/or vicariance have played in structuring the spatial distributions of lineages within a species (or group of species). This is especially useful for species with complex geographic ranges that do not fit into straightforward, vicariance patterns (see Grismer, 1994a).

In this study, we examine patterns of mtDNA sequence variation in the Rosy Boa (*Lichanura trivirgata*) to provide new insights into the biogeographic and evolutionary processes important in Baja California and the southwestern region of North America. *L. trivirgata* is well suited for testing vicariant hypotheses that may have affected the phylogeography of other Baja California taxa for several reasons. First, *L. trivirgata* has an extensive geographic distribution, ranging from the Death Valley region of southeastern California southward to the tip of Baja California, and eastward to south-central Arizona and the west coast of Sonora, Mexico. This extensive extra-peninsular distribution makes it difficult to assign to any one proposed vicariant group, but the unique geographic range allows an assessment of population differentiation across significant biogeographic boundaries (sensu Grismer, 1994b; Riddle et al., 2000a). Second, two ecological characteristics of *L. trivirgata* may influence genetic variation. This species usually inhabits the lower terraces of boulder-strewn canyons and desert foothills, much of which occurs in disjunct arrays (particularly in desert regions). Concomitantly, the sedentary nature of *L. trivirgata* suggests minimal dispersal abilities (Diffendorfer et al., 2005). Given these constraints, interpopulation gene flow may be limited in *L. trivirgata*, thus increasing the likelihood of exhibiting high levels of geographically structured genetic divergence between populations.

The primary objective of this study is to use mitochondrial gene sequences to infer the phylogeographic relationships within *L. trivirgata*. We evaluate these patterns as they relate to previously hypothesized vicariant events associated with the formation of Baja California and surrounding North American deserts. We focus on the genetic structure of populations using a geospatial framework and where possible rely upon nested clade phylogeographic analysis (NCPA) to guide inferences regarding biogeographic and evolutionary processes that may have structured the genetic variation (Templeton, 1998; Templeton, 2004). Second, we use the mtDNA phylogeny to define lineage relationships among populations in order to define evolutionary units and potential species boundaries. We compare the evolutionary lineages delineated in our study with morphological variation and evaluate support for taxonomic implications using the evolutionary species concept.

## 2. Materials and methods

### 2.1. Sampling and tissue acquisition

Tissue samples were obtained from a total of 131 specimens of *L. trivirgata* from 76 localities throughout California, Arizona, and Mexico (Fig. 1; Appendix). These localities generally represent the distributional range of the species. Non-destructive sampling techniques (i.e., drawn blood, ventral scale clips, salvaged specimens, shed skin) were used to obtain tissue for DNA extraction in

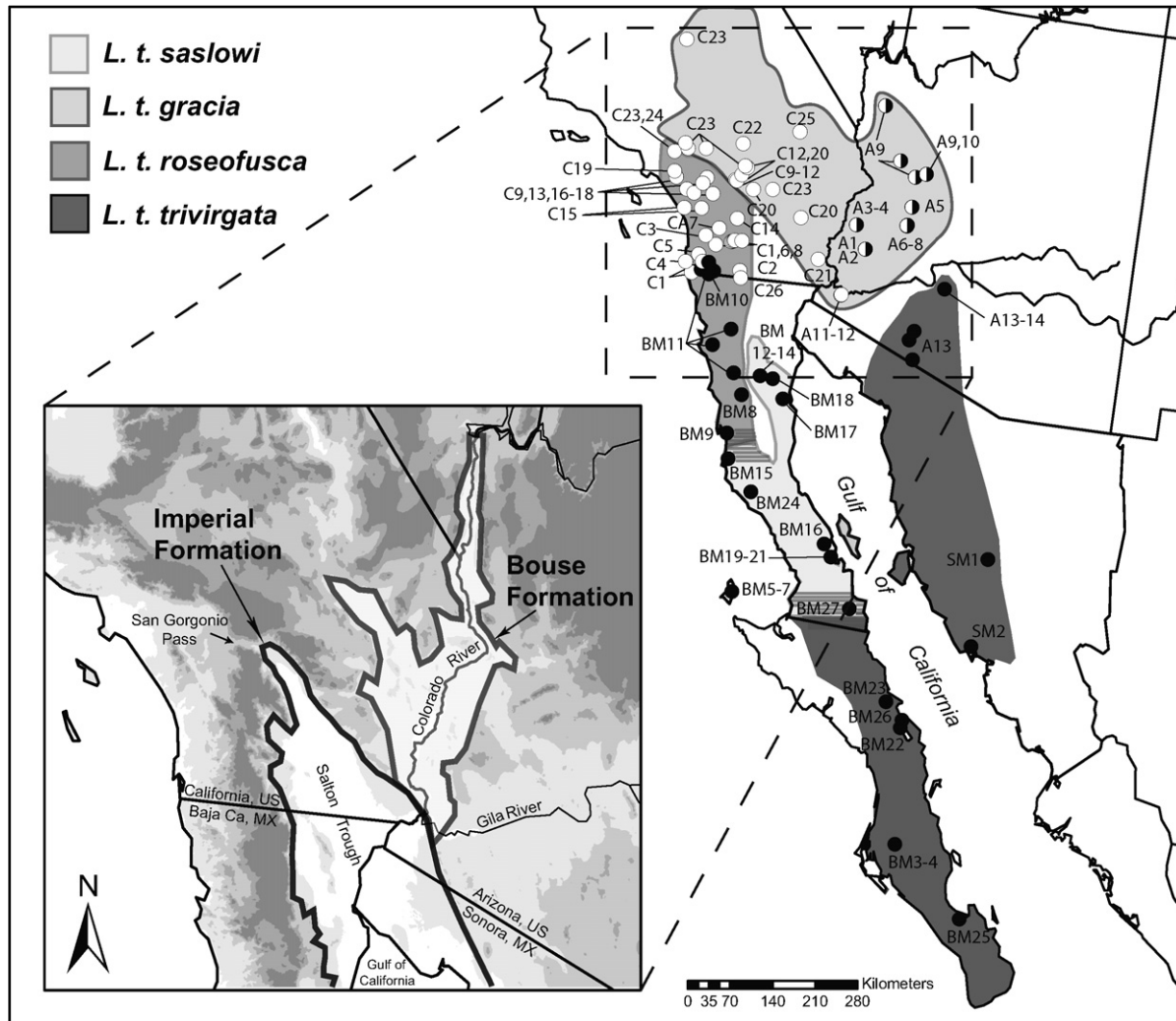


Fig. 1. Geographic distribution and subspecies delimitation of *Lichanura trivirgata* with collection localities included in this study. Haplotypes present in each lineage are indicated at the collection locations (numbers follow those in the Appendix and Fig. 2); open circles indicate haplotypes aligned to Lineage A, half filled circles indicate haplotypes aligned to Lineage B, and closed circles indicate haplotypes to Lineage C. The inset shows the inferred historical events responsible for the formation and diversification of lineages within *Lichanura*. The location and extent of the Imperial Formation within the Salton Trough is outlined by the thick black line. The extent of the Bouse Formation along the Colorado River is outlined by the thick dark grey line. Geographic areas mentioned in the text are labeled accordingly. Shading indicates elevation with lightest grey between 0 and 400 m, light grey between 480 and 800 m, grey 820 to 1100, and dark grey above 1200 m.

most cases. To prevent multiple tissue sampling from the same individual within a population, wild-caught snakes (that were not vouchered) were marked with passive-integrated-transponder tags (PIT tags) before release.

## 2.2. Molecular methods

Portions of the mitochondrial ND1 (945 bases) and 16S (160 bases) genes were used to estimate population structure and infer phylogenetic relationships within *L. trivirgata*. Standard techniques were used to extract DNA from tissues and amplify the targeted gene regions using the polymerase chain reaction (PCR). The primers used were 16dR (5'-CTACGTGATCTGAGTTCAGACCGGAG-3'; Leaché and Reeder, 2002) and t1le-R (5'-TCTCRGGCAC AYTTCATTGTGGT-3'; this study). PCR products were

purified and directly sequenced on an ABI 377 automated sequencer. Sequences were edited using Sequencher™ 4.5 and aligned using CLUSTAL W (Thompson et al., 1994). Alignments were not problematic due to conserved amino acid codon positions and relatively low levels of genetic divergence.

## 2.3. Phylogenetic analysis

Partitioned Bayesian phylogenetic analyses were conducted using MRBAYES version 3.1.2 (Ronquist and Huelsenbeck, 2003). Given the heterogeneity of DNA sequence models of evolution within and/or across genes (e.g., Nylander et al., 2004; Brandley et al., 2005), the mtDNA data were divided into four data partitions: three for each codon position of the protein coding ND1 gene

and one for the 16S region. Only a single partition (vs. separate stem and non-stem partitions) was used for the 16S data because it consisted of only 160 bp and ~85% of those represent non-stem forming positions. Appropriate models of sequence evolution for each partition were determined using the likelihood ratio test (LRT) implemented with MRMODELTEST version 2.1 (Nylander, 2002). Two simultaneous, completely independent partitioned analyses were run to help determine when convergence had been achieved (i.e., stationarity). Each analysis consisted of  $1.0 \times 10^7$  generations with a random starting tree and four incrementally heated Markov chains (using default heating values), sampling the Markov chains at intervals of 1000 generations (40,000 generations discarded as burn-in). Clades with posterior probabilities of  $\geq 0.95$  were considered strongly supported (Wilcox et al., 2002; Alfaro et al., 2003).

#### 2.4. Root estimation and divergence time estimates

We explored rooting the *Lichanura* phylogeny using *Charina bottae* (extant sister species of *L. trivirgata*; Noonan and Chippindale, 2006) as the outgroup (all *Lichanura* mtDNA sequences were re-aligned). A Bayesian approach (similar to that described by Brandley et al. (2005)) was employed to test alternative rooted hypotheses. The 95% credible set of unique trees was created using the *sumt* command in MRBAYES. This methodology was used to test whether alternative root placements not supported with high posterior probabilities (i.e.,  $Pp < 0.95$ ) could be statistically rejected by the data (i.e., alternative root placements absent from the 95% credible set; Buckley et al., 2002; Brandley et al., 2005).

While we acknowledge the difficulty in applying molecular clock estimates (Hillis et al., 1996; Cranston and Rannala, 2005; Rutschmann, 2006) and agree they should be used with caution, approximate times of lineage divergence were estimated using a Bayesian relaxed clock method, implemented in MULTIDISTRIBUTE (Thorne et al., 1998; Thorne and Kishino, 2002). This method uses the Markov chain Monte Carlo (MCMC) procedure to derive a posterior distribution of rates and times, and provides credibility intervals for estimated divergence times (Kishino et al., 2001). We followed the most commonly used procedures detailed in the step-by-step manual by Rutschmann (2005). Model parameters were estimated using the F84 +  $\Gamma$  model using BASEML, a program that is part of the PAML package (Yang, 1997). Second, maximum-likelihood branch lengths, along with a variance-covariance matrix of branch lengths, were estimated using the F84 +  $\Gamma$  model parameters and a pre-specified tree topology (our Bayesian inference), implemented in ESTBRANCHES. The last step uses the program MULTIDIVTIME to approximate the posterior distribution of substitution rates and divergence times using a MCMC procedure. Before the analysis can be implemented, MULTIDIVTIME requires the user to specify several priors. The age of the root node was given as a mean value of eight million years

ago (Ma) with a standard deviation set equal to the mean. This value was based on the earliest known fossils identified as *Lichanura* sp. from Clarendonian (Late Miocene; 10.5 to 6 Ma) deposits (Whistler and Burbank, 1992), but we acknowledge that the oldest known fossil does not necessarily reflect the earliest occurrence of this species. Default values were used for all other priors. Each analysis consisted of  $1.0 \times 10^6$  generations with sampling of Markov chains at intervals of 100 generations (100,000 generations discarded as burn-in).

#### 2.5. Nested clade phylogeographic analysis

NCPA was used to statistically assess the association of geographic and phylogenetic position of haplotypes and guide inferences regarding population level processes using GEODIS version 2.4 (Posada et al., 2000). Intraspecific haplotype networks were inferred using statistical parsimony (SP; Templeton et al., 1992), as implemented in TCS version 1.21 (Clement et al., 2000). Multiple networks were obtained that were comparable to the clades inferred from Bayesian analyses; however, the networks could not be joined because divergences between them exceeded the limits of SP (>14 steps). Since determination of interior/tip status between these clades was problematic we restricted NCPA to a subset of our data. Population structure inferences were made following the most recent inference key of Templeton (2004). Ambiguities in the recovered network was resolved using published guidelines (Templeton and Sing, 1993; Crandall and Templeton, 1993; Castelleo and Templeton, 1994).

### 3. Results

#### 3.1. Molecular variation

A total of 1105 base pairs (bp) of mtDNA (160 bp 16S rRNA and 945 bp ND1) were aligned and used for subsequent analyses. Within *L. trivirgata*, 171 are variable and 132 are parsimony informative. In all, 67 unique haplotypes are identified (Appendix). The average sequence divergence (uncorrected) between all *L. trivirgata* haplotypes is 3.3%, (ranging from 0.09% to 6.3%). Sequence divergence estimates between *L. trivirgata* and its nearest relative (*C. bottae*) range from 14.4% to 16.7%.

#### 3.2. Phylogenetic analyses

Hierarchical model testing indicated that the best-fit model of sequence evolution for the 16S, 1st and 3rd codon position partitions was HKY +  $\Gamma$ , with the HKY model being a better fit for the 2nd codon positions. The two independent partitioned analyses converged on similar average log-likelihood values ( $-\ln L = 3306.23$  and  $3305.83$ ). A 50% majority rule consensus tree of the combined pool of trees (120,002 trees) reveals three major lineages (Fig. 2). These are referred to as lineages A, B, and C, and haplotypes are designated as A, C, BM, and SM to correspond



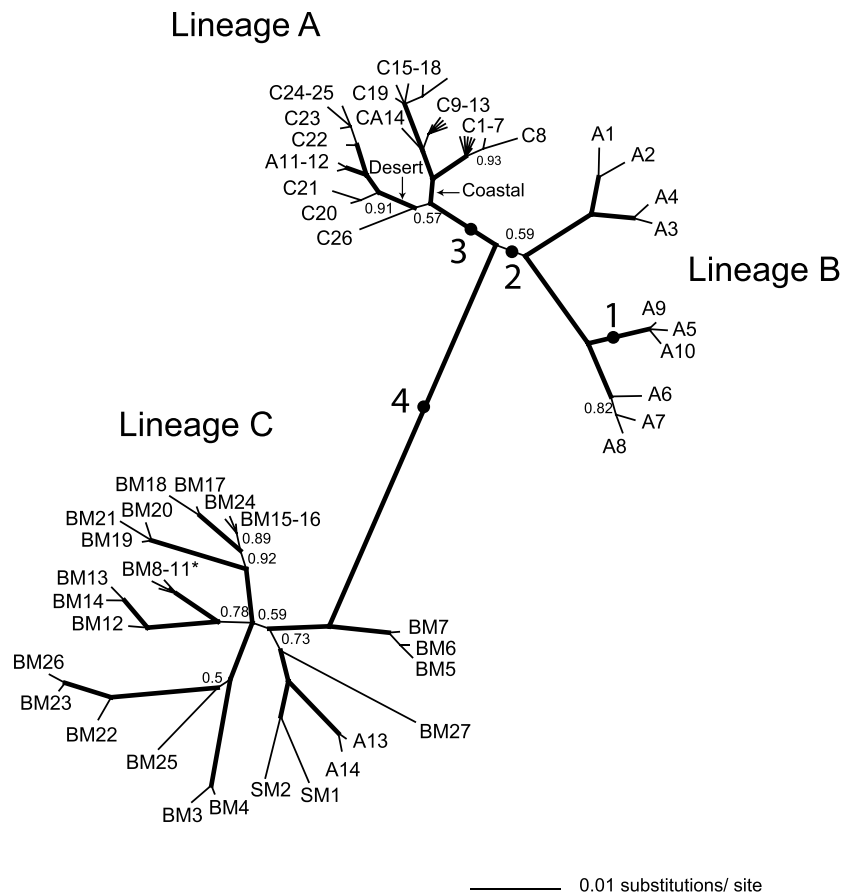


Fig. 2. Unrooted Bayesian inference phylogram (50% majority rule) of *Lichanura trivirgata* derived from 67 unique haplotypes. The three major lineages are discussed in the text and referenced in Figs. 4 and 5. Branches supported by Bayesian posterior probabilities  $\geq 0.95\%$  are thickened, those values below 0.95% are indicated by a number, and those less than 0.50 are not shown. Haplotype names follow those in Fig. 1 and the Appendix. The Bayesian inferred rooting from the 'outgroup method' is indicated by the black dot numbered 1. Alternative root placements explored are indicated by numbers 2–4 as in Table 2.

to their geographical location (i.e., A = Arizona, C = California, BM = Baja California, Mexico, and SM = Sonora, Mexico). Lineage C is appreciably more divergent from the other two lineages (A and B) than the latter are from each other (Fig. 2; Table 1). Posterior probabilities ( $Pp$ ) reveal strong support for Lineages A and C ( $Pp \geq 0.95$  for both; Fig. 2), while lineage B is only weakly supported ( $Pp = 0.59$ ; Fig. 2).

The geographical distributions of haplotypes within the major lineages exhibited very little overlap. Lineages A and B are predominately separated along the Colorado River bordering Arizona and California. With the exception of haplotypes from the Gila Mountains, Arizona (A11–12),

haplotypes in Lineage A occur primarily west of the Colorado River. Two well supported clades are found within Lineage A (Fig. 2). A 'desert' clade is found primarily within the western Mojave and Sonoran Deserts of California, and haplotypes along the northern extensions of the Peninsular Ranges within southern California form a 'coastal' clade. Haplotypes of Lineage B occur east of the Colorado River and north of the Gila River throughout the Sonoran and Mojave Deserts of Arizona (Fig. 1). Lineage C consists of two geographically disjunct groups of populations: (1) populations distributed along the Baja California peninsula (including extreme southern San Diego County); and (2) populations found in south-central desert ranges in Arizona, southward along the coastal regions of Sonora, Mexico. An area of contact between Lineages A and C occurs along the border region of Baja California and California (Fig. 1). Haplotypes of Lineage C (BM10–11) are found throughout the Tijuana/Otay watershed (sites 41–47, 53–54; Appendix) in California, and haplotypes from both lineages (CA1, BM11) were only recovered at one of our collection sites (site 53, Hollenbeck Canyon, California).

Table 1  
Average uncorrected genetic divergence calculated for intra-lineage comparisons, and minimum and maximum genetic divergences for all inter-lineage comparisons

Clade	Lineage A	Lineage B	Lineage C
Lineage A	0.85%	—	—
Lineage B	1.86–2.80%	1.40%	—
Lineage C	4.18–5.93%	4.65–6.33%	1.93%

3.3. Root estimation and divergence time estimation

The phylogeny inferred from the Bayesian analyses including *C. bottae* (model parameters re-estimated with addition of the outgroup) differs slightly from the unrooted analysis because of the root placement within Lineage B (rooting number # 1; Fig. 2). The rooted phylogeny does not support the regionally proximate populations in Lineage B as an exclusive group, with the northwestern Arizona haplotypes (A5, A9–10) now being the sister lineage to all remaining *L. trivirgata*. Also, Lineages A and C are rendered sister lineages. However, the posterior probabilities indicate weak support for both relationships ( $Pp = 0.74$  and  $Pp = 0.58$ , respectively). Alternative root hypotheses (2–4; see Fig. 2) could not be rejected since they are present in the 95% credible set of trees (Table 2).

Bayesian posterior estimates of divergence times were congruent between separate runs, indicating convergence. The posterior estimates and 95% credibility intervals are listed in Table 3, and estimates implicate late Miocene to early Pliocene time frame for the earliest divergence events between the major mtDNA lineages of *L. trivirgata*.

3.4. Nested clade phylogeographic analysis

We restricted NCPA to a subset of the data for which we had relatively dense geographical sampling, corresponding to 39 collection sites from Lineage A. Haplotypes were connected by SP using a 95% parsimony limit that imposed

Table 2  
Results of alternative rooting hypotheses using the partitioned Bayesian analysis including outgroups

Alternative Root Hypothesis	Number of congruent trees
Total trees in 95% CI	6683
1. Bayesian analysis root	~6200
2. Lineage B (monophyletic) (Lineage A + C)	20
3. Lineage A (Lineage B + C)	73
4. Lineage C (Lineage A + B)	144

The presence of any trees with alternative rootings in the 95% credible set of trees indicates that the hypothesis cannot be rejected by the data.

Table 3  
Bayesian estimates of divergence times in millions of years ago (Ma) with 95% credibility intervals estimated under the F84 +  $\Gamma$  model of sequence evolution

Lineage divergence	Divergence date with 95% CI	Historical event
(AB) from C	7.4 (4.1, 9.7)	Imperial formation \ Gulf of California
A from B	4.8 (2.1, 7.8)	Bouse formation \ Colorado River

The node connecting *Charina bottae* to all other *Lichanura* sequences (position 4, Fig. 2) was constrained to between 6 and 10.5 based on fossils of *Lichanura* sp. taken from Late Miocene deposits.

a maximum of 14 mutational steps between connections (Fig. 3). Of the nine “nesting clades” that included both geographical and genetic variation (clades 1-2, 2-2, 2-4, 2-6, 3-1, 3-4, 4-1, 4-2, 5-1), we could reject the null hypothesis of no statistical association between haplotype distributions and geography in six cases (clades 1-2, 3-1, 3-4, 4-1, 4-2, 5-1). Table 4 summarizes the results and inferences about population structure and history, and the geographic associations of haplotype clades at the 3 and 4 step levels are plotted on Fig. 4. Restricted gene flow with isolation by distance was inferred at lower clade levels, particularly within the coastal clades of Lineage A (clades 1-2 and 3-1), with range expansion inferred at the higher clade level (4-1). Range expansion is also a potential driving force in haplotype distribution along the desert regions within Lineage A. Nesting clades found primarily within the northern Mojave Desert (clade 3-4) and within clade 4-2 (the clade containing all desert haplotypes) both had patterns of range expansion (Table 4). However, no definitive conclusions (i.e., restricted gene flow or contiguous range expansion) could be drawn at the highest clade level (clade 5-1) because of problems determining interior/tip status.

4. Discussion

We were able to detect three geographically subdivided lineages within *L. trivirgata* (Fig. 2). Despite the distinctiveness of the three major lineages, our rooted phylogenetic analysis was unable to order the divergence events among the lineages with great confidence (Table 2). Intra-specific phylogeny root estimations have been shown to be especially problematic (Templeton, 1993; Castelleo and Templeton, 1994), particularly when outgroup sequences are divergent with respect to the ingroup sequences (as is

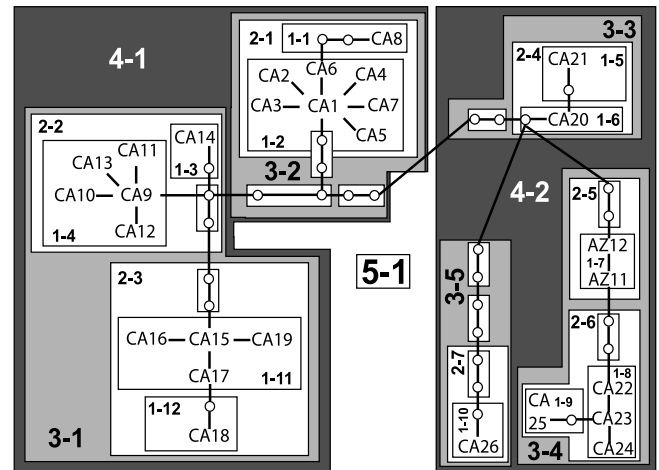


Fig. 3. Nested clade diagram for mtDNA haplotypes of Lineage A based on the statistical parsimony network. Each line in the network represents a single mutational change between the haplotypes. The small open circles between branches represent unobserved interior haplotypes that were not sampled. Haplotype names and their geographical distributions are listed in the Appendix.

Table 4  
Inferences from NCPA for clades with significant associations with geography

Clade	Chi-square <sup>a</sup>	Clades	D <sub>c</sub> <sup>b</sup>	D <sub>N</sub> <sup>b</sup>	Chain of inference	Inference <sup>c</sup>
<i>Lineage A (coastal)</i>						
1–2	0.004	C1(Int)	27	29 <sup>S</sup>		
		C2	0	52		
		C3	0 <sup>S</sup>	34		
		C4	0	47		
		C5	0	24		
		C6	7 <sup>S</sup>	49 <sup>L</sup>		
		C7	0	29		
3–1	0.001	I–T	25	–11 <sup>S</sup>	1–2–3–5–6–7–Yes	Restricted gene flow
		2–2(Int)	48 <sup>L</sup>	51 <sup>L</sup>		
		2–3	24 <sup>S</sup>	30 <sup>S</sup>		
4–1	0.000	I–T	25 <sup>L</sup>	21 <sup>L</sup>	1–2–3–4–No	Restricted gene flow with IBD
		3–1	41 <sup>S</sup>	67		
		3–2(Int)	37 <sup>S</sup>	65	1–2–11–12–No	Contiguous range expansion
		I–T	–9	–8		
<i>Lineage A (desert)</i>						
3–4	0.222	2–5(Int)	0 <sup>S</sup>	290 <sup>L</sup>	1–19–20–2–11	Range expansion
		2–6	84 <sup>S</sup>	86 <sup>S</sup>		
		I–T	–84	204 <sup>L</sup>		
4–2	0.008	3–3(Int)	71	81	1–2–11–12	Range expansion
		3–4(Int)	107	118		
		3–5	0	156		
		I–T	97 <sup>S</sup>	–48		
<i>Lineage A (coastal vs. desert)</i>						
5–1	0.000	4–1	66 <sup>S</sup>	74 <sup>S</sup>	1–2–? Tip/Interior status	Inconclusive outcome
		4–2	110 <sup>L</sup>	130 <sup>L</sup>		
		I–T	45 <sup>L</sup>	56 <sup>L</sup>		

<sup>a</sup> Chi-square results based on exact permutational (10,000 resamples) contingency analysis of categorical variation (clade × geographic).

<sup>b</sup> Statistical significance of DC and DN determined by 10,000 random permutations of clades against sampling location. Superscript S/L.

<sup>c</sup> Inferences were made following the updated version (11-Nov-2005) of the inference key provided in Templeton (2004).

the case in our study). Given these limitations, we primarily rely on the overall shape of the unrooted Bayesian tree and levels of sequence divergence to lend support for the timing of lineage diversification within *Lichamurra*.

#### 4.1. Pre-Pleistocene patterns

Phylogenetic analyses reveal support for the isolation of Lineages A + B from Lineage C as the deepest divergence event in the *L. trivirgata* mtDNA phylogeny (Fig. 2), although rooted trees render populations in Lineage B paraphyletic with respect to all other haplotypes. Comparing the magnitude of divergences between the lineages reveals that Lineages A and B are appreciably more divergent from Lineage C (uncorrected *p*-distances = 4.2–6.3%) than the former are from each other (uncorrected *p*-distances = 1.9–2.8%; Fig. 2). Thus, patterns of relationships suggest lineage diversification within *L. trivirgata* has developed over differing time frames: (1) a basal divergence indicating long temporal separation between Lineage C and Lineage A + B, and (2) a more recent divergence between Lineages A and B. Late Miocene to early Pliocene events (7–4 Ma; Table 3) provide the best supported explanation for the divergence events in *Lichamurra*. Under this

scenario, the earliest phylogenetic division was likely initiated by development of the Baja California peninsula.

Two primary vicariant hypotheses responsible for the development of the Baja California peninsula (Murphy, 1983; Grismer, 1994a; Murphy and Aguirre-Léon, 2002) could be attributed to a pre-Pleistocene divergence of the *L. trivirgata* lineages: the southern gulf vicariance (early Miocene) and the northern gulf vicariance (Late Miocene–Early Pliocene). A past hypothesis, consistent with the southern gulf vicariance, suggests populations of *Lichamurra* originated in the Cape region of Baja California as it broke away from mainland Mexico about 5–12 million years ago, leaving populations isolated from each other on both sides of the developing Gulf of California (Welsh, 1988). The fact that the same subspecies (*L. t. trivirgata*) can be found on both sides of the gulf was taken as evidence supporting this hypothesis. Our estimated timing of the earliest divergence between populations of *L. trivirgata* is certainly within a late Miocene time frame (7 Ma, constrained to between 10 and 4 Ma; Table 3) Nonetheless, we find little phylogenetic support for the above scenario. Under a southern gulf vicariance, we would expect to find a genetic divergence between populations from Baja California and mainland Mexico similar in magnitude (or greater) as that observed between Lineage C and Lineage

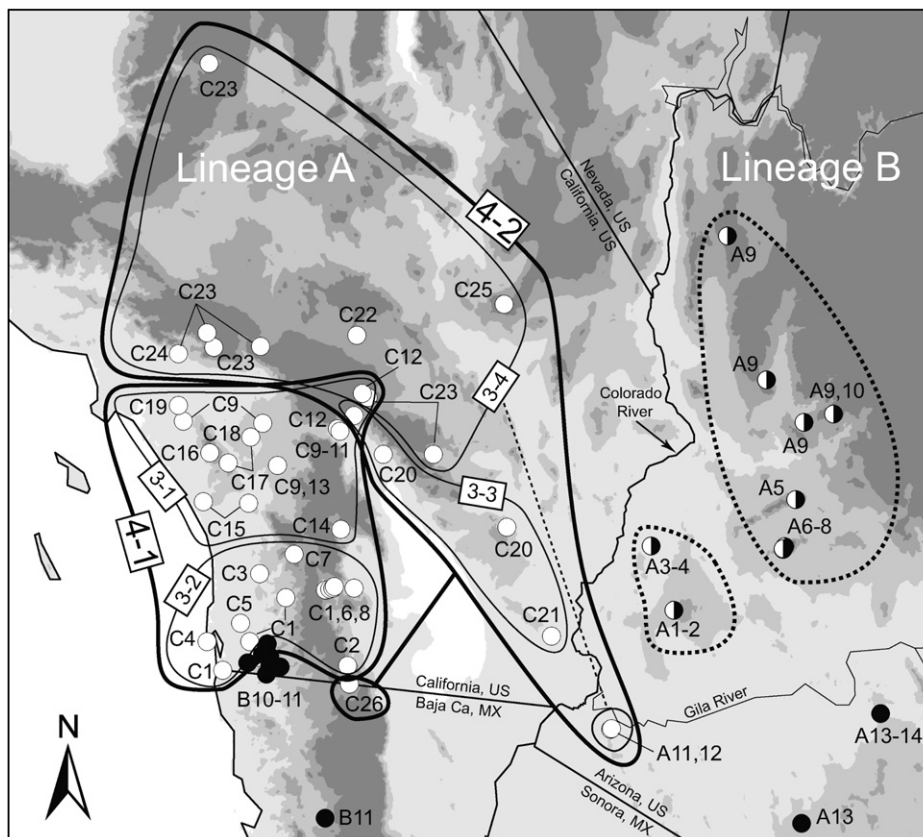


Fig. 4. An overlay of nested clades belonging to Lineage A from Fig. 3 on geography. For clarity purposes only those haplotype clades that exhibited statistically significant associations with geography at the 3 to 4 step nesting levels are mapped (Table 4). Haplotypes present in each lineage follow the color coding as in Fig. 1. For Lineage B, the dotted lines roughly outline haplotype clade boundaries with high posterior probabilities ( $Pp < 0.95$ ; Fig. 2) throughout Arizona and do not relay complete distributional information for *Lichanura trivirgata*. Shading indicates elevation with lightest grey between 0 and 400 m, light grey between 480 and 800 m, grey 820 to 1100, and dark grey above 1200 m.

A + B. Instead, our analyses reveal haplotypes from Sonora, Mexico (SM) and Baja California (BM) are nested within Lineage C (Fig. 2). Thus, divergence between these populations seems to have occurred sometime after the development of the Gulf of California. Furthermore, southern gulf vicariance events have been hypothesized to show patterns where peninsular populations are derived from tropical ancestral populations from mainland Mexico (e.g., *Sceloporus angustus* group and *S. utiformis*, Flores-Villela et al., 2000; *Bipes biporus*, Papenfuss, 1982; for others see Murphy and Aguirre-Léon, 2002). Our data suggest ancestral populations of *L. trivirgata* were likely more widely distributed to the north prior to development of the Gulf of California, extending from southwestern Arizona into the developing Baja California peninsula, a hypothesis that is plausible given our root placement (yet we acknowledge the weak statistical support of the rooting). The sister relationship with *C. bottae*, a Pacific Northwest boa, and the extra-peninsular distribution of *Lichanura* (primarily to the north) provides further support for more northern warm desert ancestral populations (Grismer, 1994a).

Thus, northern gulf vicariance events provide the best supported explanation for the deep divergence in *Lichan-*

*ura*. For warm desert species with population distributions around the Gulf of California prior to this marine transgression, a sister lineage relationship should be observed on opposite sides of the northern gulf region (Grismer, 1994a). The geographical distribution of Lineage A + B and Lineage C, coupled with the relatively high sequence divergence between these lineages, is consistent with the expected pattern of vicariance between northern and peninsular biotas (Figs. 4 and 5). Under this scenario, isolation, estimated to have occurred 7 Ma (Table 3), can be attributed to early marine extensions of the Gulf of California as a result of Miocene tectonic activity (Lucchitta, 1972; Holt et al., 2000; Stock and Hodges, 1989; Dorsey et al., 2007). These marine transgressions, recognized as the Imperial Formation, inundated the Salton Trough and as far north as the San Gorgonio Pass (Winker and Kidwell, 1986; Fig. 1). Age assignments for the Imperial Formation have been estimated to between 10 and 6 Ma (Peterson, 1975; Schrepf, 1981). Although more recent restricted age estimates (7.4 to  $\geq 6.0$  Ma), consistent with our molecular clock estimates, have been reported based on radiometric, microfossils, and paleoclimatic considerations (Rymer et al., 1994; Matti and Morton, 1993; McDougall et al., 1999).



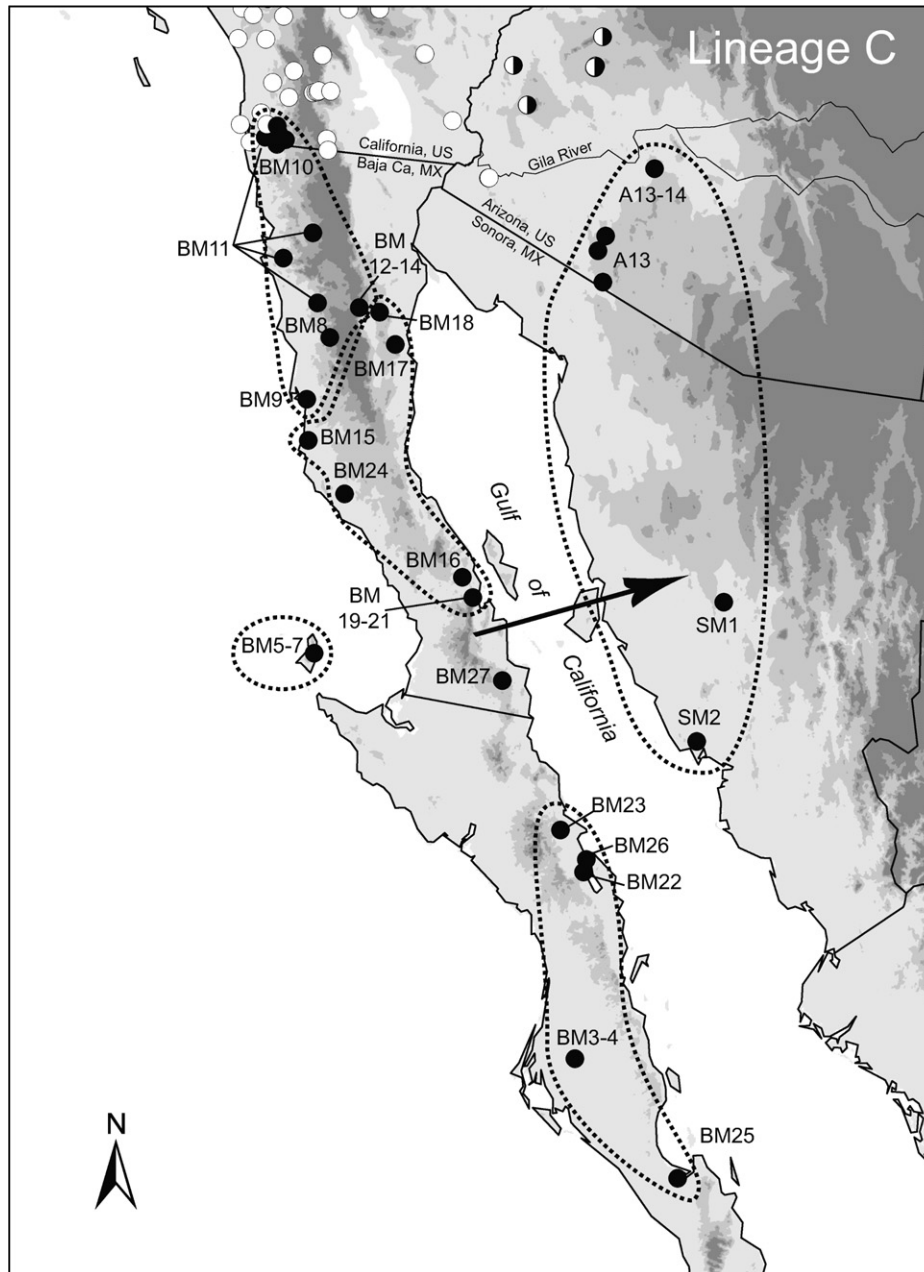


Fig. 5. An overlay of major haplotype clades with high posterior probabilities ( $P_p < 0.95$ ; Fig. 2) belonging to Lineage C on geography and do not relay complete distributional information for *Lichanura trivirgata*. The arrow indicates the rough location of the midriff island chain that extends across the Gulf of California forming a partial bridge between the peninsula and the Sonoran coast, and the likely means of trans-gulf dispersal for *Lichanura* to the mainland. Schematics are the same as in Fig. 4.

The isolation of Lineage A from Lineage B (Fig. 2) is the next deepest divergence in the phylogeny, estimated to have occurred 5 Ma (constrained to between 2 and 8 Ma; Table 3). The distribution of each lineage primarily on west and east sides of the Colorado River suggest that the formation of the lower Colorado River played an active role in limiting dispersal among populations on either side of the river (Fig. 4). A second geologic event, known as the Bouse Formation, involved extensive inundations from the Yuma area as far north as Nevada (Fig. 1; Metzger, 1968). Whether this inundation was the result of further estuarine

incursions from the Gulf of California or the result of freshwater deposition from rapid lake-spillover events fed by an early Colorado River remains at the center of debate (Spencer and Patchett, 1997; Poulson and John, 2003; Dorsey et al., 2007). Nonetheless, the shallow water embayment, responsible for the Bouse Formation and early Colorado River system, encompassed a geographic region that likely served as a substantial barrier to dispersal. In addition, ages assignments from early deposits of the Colorado River along Lake Mead south to the Salton Trough (dated between 6.0 and 5.3 Ma; Spencer et al., 2001; House

et al., 2005; Dorsey et al., 2007) closely approximate our estimated age of divergence between Lineages A and B (5 Ma; Table 3).

Thus, early extensions of the Gulf of California during the Late Miocene–Pliocene led to the long term isolation between Lineage A + B and Lineage C, while subsequent development of the Colorado River played an important role in limiting (and possibly preventing) dispersal between Lineage A and B. However, placement of Gila Mountain, Arizona haplotypes (AZ11–12) within the desert clades of Lineage A (west side of the Colorado River) suggests that the lower portions of the Colorado River may have allowed periodic dispersal of individuals. Similar patterns have been observed in other reptiles, where genetically differentiated groups of populations meet along the Colorado River and yet at certain areas, populations on opposite sides are genetically similar (desert tortoise, McLuckie et al., 1999; sand lizards, Trépanier and Murphy, 2001; Murphy et al., 2006; horned lizards; Mulchay et al., 2006). McLuckie et al. (1999) suggested the possibility of periodic river meandering to account for the transfer of floristic and faunistic components on opposite sides of the river. Interestingly, periodic avulsions during the Pleistocene are known to have shifted the flow of the Colorado River northwestward, forming Lake Cahuilla (Stokes et al., 1997). Thus, the meandering river hypothesis seems plausible and may explain the establishment of haplotypes from Lineage A across the river in the Gila Mountains, Arizona.

Northern gulf vicariant events, coupled with patterns of phylogenetic divergence, have been documented in mammals, birds, reptiles, amphibians, plants, and arthropods (Petren and Case, 1997; Riddle et al., 2000a,b; Zink et al., 2001; Nason et al., 2002; Hurtado et al., 2004; Crews and Hedin, 2006; Devitt, 2006); however, most attribute patterns of divergence to a single vicariant event as described by Murphy (1983) and Grismer (1994b). To our knowledge, our study is the first to document and resolve multiple vicariant events at differing timescales within this region. While some taxa exhibit clear phylogenetic evidence predicted by the northern gulf vicariance (e.g., mammals, Riddle et al., 2000a; amphibians, Jaeger et al., 2005; cactus, Nason et al., 2002), others seem less clear. For example, Riddle et al. (2000b) regarded the northern gulf vicariance as the general isolation mechanism for peninsular and continental forms of *Chaetodipus*, but also noted the importance of the present day Colorado River as a continuing barrier to gene flow between lineages. In addition, an east/west genetic barrier has been observed across the Colorado River in the desert tortoise (Lamb et al., 1989), fringe-toed lizards (Trépanier and Murphy, 2001), and the flat-tailed horned lizard (Mulchay et al., 2006), yet these species do not occur south along the peninsula of Baja California. Furthermore, geological evidence supports multiple marine and lacustrine incursions in this region at different time periods (Lucchitta, 1979; McDougall et al., 1999; Dorsey et al., 2007). Therefore, instead

of one single vicariance event (sensu Murphy, 1983; Grismer, 1994a), multiple events as posited by Crews and Hedin (2006), may be responsible to explain the patterns of divergence across such a diverse array of taxa. For instance, patterns of divergence in some studies might correspond to early phase opening of the Gulf of California and subsequent filling of the Salton Trough, known as the Imperial Formation (Lucchitta, 1979; McDougall et al., 1999); whereas, patterns of east–west divergence across the Colorado River might reflect the progressive and more extensive inundation of the Gulf of California (i.e., the Bouse Formation) that ultimately lead to the arrival of the Colorado River (Metzger, 1968; Boehm, 1984; Spencer et al., 1998; House et al., 2005). With recent advances in the emergent field of comparative phylogeography, it is now possible to statistically evaluate simultaneous vicariance hypotheses of co-distributed taxa (Edwards and Beerli, 2000; Knowles, 2004; Hickerson et al., 2006). For example, Leaché et al. (2007) used a Bayesian method (approximate Bayesian computation; Beaumont et al., 2002; Hickerson et al., 2007) to demonstrate that shared mtDNA genealogical patterns across the mid-peninsular region of Baja California from six small mammals and six reptiles support two vicariant episodes, rather than one (as traditionally purported). As phylogeographic data of co-distributed taxa accumulate in the desert southwest, particularly at the population level, evaluating whether one or more episodes of diversification best explain the northern gulf vicariance will be possible and should increase our understanding regarding the historical and modern biogeographic influence of the Colorado River.

#### 4.2. Phylogeographic structure within clades

Vicariance hypotheses alone do not fully explain patterns of genetic variation and population distributions of *Lichanura*. Dispersal must be used to explain some aspects of current distributions. Two patterns of subsequent dispersal merit discussion: the west-to-east dispersal of Baja California peninsular populations into mainland Sonoran Desert regions (Lineage C), and the apparent secondary contact between peninsular populations of Lineage A and Lineage C in California.

Phylogenetic analysis of the disjunct Sonoran Desert populations found within south-central Arizona (A13–14) and Sonora, Mexico (SM1–2) suggest a rather novel dispersal hypothesis. Analyses reveal haplotypes from these mainland populations nested within Lineage C. Since the northern gulf vicariance was implicated between Lineage C and all other populations, we consider subsequent dispersal of peninsular populations into the Sonoran Desert of mainland Mexico the best explanation for the phylogenetic patterns and current distribution. A dispersal hypothesis (presumably circum-gulf) was proposed earlier by Murphy (1983), since no allozyme genetic divergences were observed between *L. trivirgata* populations on

either side of the Gulf of California. However, a circum-gulf route seems unlikely given the phylogenetic relationship between mid-peninsular haplotype BM27 (El Progreso, Baja California) and all other south-central mainland Sonoran haplotypes (Fig. 5). Additionally, the northern gulf region was likely dominated by unsuitable habitat, such as broad flat plains and estuaries, very similar to the Colorado River valley habitats found in the region today (Van Devender and Spaulding, 1979). Thus, a trans-gulf dispersal hypothesis seems more likely. Proximate to the El Progreso collection site (haplotype BM27) is a midriff island chain (Ángel de la Guarda, San Lorenzo, San Esteban, and Tiburón) that extends across the Gulf of California forming a partial bridge between the peninsula and the Sonoran coast, providing a likely means of over-water dispersal for *Lichanura* to the mainland. Interestingly, the gulf seaway is at its narrowest in this region, and the largest present-day water gap between this island chain is 15 km between San Lorenzo and San Esteban. However, most of these islands were likely connected by land bridges to either the peninsula (e.g., Ángel de la Guarda, San Lorenzo) or the mainland (e.g., Tiburón) prior to the rise in sea level during the current interglacial period (Soulé and Sloan, 1966; Carreño and Helenes, 2002). Although *L. trivirgata* are not known to occur on San Lorenzo and San Esteban islands, they have been found on Tiburón and just north of San Lorenzo on Ángel de la Guarda (Grismer, 1999; Grismer, 2002).

Trans-gulf dispersal across the midriff islands is known or presumed to occur in some plants and reptiles due to the relatively close geographic proximity of these islands to each other (Grismer, 1994b; Petren and Case, 1997; Cody et al., 2002; Murphy and Aguirre-Léon, 2002). A sizable group of plants with peninsular origins are thought to have reached the Sonoran Desert in mainland Mexico by the midriff island chain following the eastward spread of arid conditions since the last pluvial period (Cody et al., 2002). For reptiles, the only obvious cases of dispersal from west-to-east involves the leaf-toed gecko (*Phyllodactylus xanti*). This species is widespread and endemic to Baja California, but can be found on many islands in the Gulf of California including San Lorenzo, San Esteban, and Isla Tiburón. Generally, species found on the midriff islands are presumed to be of mixed origin (i.e., colonized by conspecifics from both mainland and peninsula). However, many studies regarding the evolution of insular fauna in the Gulf of California thus far have not been based on phylogenetic investigation, especially at the population level (Grismer, 1994b; Murphy and Aguirre-Léon, 2002). While our mtDNA data supports a relatively novel phylogeographic pattern of trans-gulf dispersal from west-to-east (including a successful mainland invasion), we recognize further work is needed to evaluate this dispersal hypothesis.

Since northern extensions of the Gulf of California reached as far north as the San Gorgonio Pass, Murphy

(1983) and Murphy and Aguirre-Léon (2002) suggested that there was a water connection between the inundated Los Angeles Basin and lower elevation basins in the Salton Trough, which would have completely isolated the peninsular land mass from North America. Although no firm geological evidence exists for the San Gorgonio seaway, the hypothesis has largely been supported by marginally congruent phylogenetic patterns from mesic adapted vertebrate species where peninsular populations exhibited genetic discontinuity between conspecific populations further north and east of San Gorgonio Pass (Upton and Murphy, 1997; Rodríguez-Robles et al., 1999). However, the largest genetic signature in *Lichanura* (i.e., Lineage A + B and C) is found much further south of the San Gorgonio Seaway Barrier along the US–Mexico border in southern San Diego Co., California (Figs. 4 and 5). We suggest two possible scenarios (although not necessarily mutually exclusive of one another) that may explain the apparent incongruence. The first possibility involves active dispersal, where populations from each lineage dispersed into newly formed habitat along the Peninsular Ranges once the Gulf of California waters had receded to their present conditions. Dispersal from the northern deserts of California (Lineage A) southward into the peninsular ranges may have shifted the genetic signature of vicariance (from the San Gorgonio Pass) southward. The presence of both ‘desert’ and ‘coastal’ clade haplotypes (C9–12, 20, 23; Fig. 4) of Lineage A within the San Gorgonio Pass region (Sites 62, 69–70; Appendix) and the relatively shallow divergence between haplotypes of the ‘coastal clade’ provide support for this hypothesis.

Alternatively, a second hypothesis involves the possibility of a novel geographic and/or physiographic barrier along the US–Mexico border region. Interestingly, this is an area where several other taxa (especially reptiles) endemic to the Baja California peninsula reach their northern limit (e.g., plants: *Agave shawii*, *Ornithostaphylos oppositifolia*, *Fraxinus parryi*, and *Ambrosia chenopodiifolia*; reptiles: *Coleonyx switaki*, *Urosaurus nigricaudus*, *Bogertophis rosaliae*; and *Masticophis fuliginosus*). However, in the absence of any known geographic barrier along the US–Mexico border region, dispersal abilities for these species may be restricted in relation to structural and environmental variables that are known to change in this region (e.g., temperature, moisture availability, soil characteristics; Westman, 1981; Cody et al., 2002). Although we prefer a dispersal (range expansion) hypothesis with secondary contact between lineages to explain the more southern genetic break between lineages, the accumulation of phylogeographic data on taxa inhabiting this border region will help to elucidate the processes involved that have potentially driven differential responses to the northern vicariance events. Our data suggest that different locations of contact between peninsular and continental lineages may simply reflect differing species-specific attributes of dispersal and habitat affinities once the barrier to dispersal is removed.



### 4.3. Taxonomic implications of mtDNA variation

Initial studies examining morphological geographic variation recognized two species (Cope, 1861, 1868; Klauber, 1931), but more recent studies have suggested the recognition of a single, polytypic species composed of at least four subspecies (see Fig. 1.; Ottley, 1978; Yingling, 1982; Gorman, 1985; Spiteri, 1988). Discordance between subspecies (based primarily on color patterns) and mtDNA lineages is seemingly a consistent pattern in snakes (e.g., Rodriguez-Robles et al., 1999; Burbrink et al., 2000; Burbrink, 2002; Janzen et al., 2002). The phylogeny recovered in this study partitions the haplotype clades geographically in ways that are not entirely concordant with the divisions suggested by the currently recognized subspecies. In particular, the molecular data indicate two cases in which recovered clades are inconsistent with a priori predictions based on subspecies taxonomy. These cases involve the subspecies *L. t. gracia* and *L. t. roseofusca*. There are at least three alternative explanations for this discordance, including inadequate morphological taxonomy (i.e., members of a single subspecies are actually non-synonymous), incomplete lineage sorting of mtDNA variation, and/or ongoing gene flow across subspecies boundaries (see Funk and Omland, 2003).

In the case of *L. t. gracia*, inadequate taxonomy seems the most plausible explanation (although incomplete lineage sorting is also possible). In describing this new form, Klauber (1931) noted that *L. t. gracia* and *L. t. roseofusca* “are virtually indistinguishable in scale counts”, but based the diagnosis on the color and pattern of the dorsal and lateral stripes. Phylogenetic analyses indicate that *L. t. gracia* populations in California (within Lineage A) are actually more closely related to *L. t. roseofusca* than they are to their subspecific counterparts in Arizona (=Lineage B), which makes this subspecies paraphyletic (non-exclusive) as currently described. Another discrepancy between mtDNA and currently recognized subspecies involves populations found throughout the coastal peninsular ranges (particularly along the US–Mexico border region). The boas in this region are relatively variable in color and pattern (Klauber, 1931), but have been historically assigned to *L. t. roseofusca* (see Fig. 1.). However, the mtDNA haplotype tree reveals a cryptic lineage divergence (actually the highest divergence observed) in this geographic region. Thus, one explanation for this apparent disparity is that introgression is occurring between these two distinct lineages and the ‘roseofusca’ background is displacing the previous subspecies color pattern (possibly ‘saslowi’). Alternatively, it may be that the currently recognized subspecies designation actually masks the apparent phylogenetic diversity in this region. Snakes along these coastal peninsular populations of California and Baja California are noticeably darker and tend to lose the lighter dorsal striping pattern found in their arid dwelling conspecific populations, suggesting that perhaps this “coastal” color pattern is a selectional response for enhanced thermoregulatory capabilities in cooler mesic environments (Lawson

and King, 1996; Burbrink et al., 2000). Nonetheless, both alternatives suggest sharing of this particular coastal color pattern (i.e., “roseofusca”) does not appear to be an indication of close phylogenetic relationship.

Elucidating patterns of genetic structure within a species can often provide new insight on previously overlooked and/or unrecognized patterns of morphological variation among populations, which may have important taxonomic implications (e.g., Zamudio and Greene, 1997; Parkinson et al., 2000; Shaffer et al., 2004; Vredenburg et al., 2007). Studies of morphological variation within *Lichanura* have generally provided evidence of character overlap (Ottley et al., 1980; Spiteri, 1988). However, re-examination of unpublished morphological data (Spiteri, 1986, 1988) and additional museum specimens across the zone of contact between Lineages A + B and Lineage C provide morphological support that the two lineages are evolving independently. Mean scale counts of both ventral and subocular scales in Lineage A + B are higher (ventral scales  $\cong$  235 and subocular scales  $\cong$  3–7) than Lineage C (ventral scales  $\cong$  228 and subocular scales  $\cong$  0–2). While these data are tentative, they do suggest independent nuclear evidence (i.e., morphological traits presumably encoded by the nuclear genome) against which the mtDNA gene tree may be evaluated. If this character concordance holds up after further and more extensive geographic morphological analyses (especially across the contact zone), it will further corroborate the existence of separate species.

While species concepts in principal and practice remain rather contentious, de Queiroz (1998) suggested that there is a commonality present across these “concepts” (i.e., species are independent lineages [=the general lineage concept of species]). Given that speciation is a time-extended process, most species definitions (*sensu de Queiroz, 1998*) (i.e., biological [Mayr, 1942], evolutionary [Simpson, 1961; Frost and Hillis, 1990], phylogenetic [Cracraft, 1983], and cohesion [Templeton, 1989]) differ primarily in the description of the general lineage concept of species and/or the preferred criterion used for the delimitation of separate lineages. Based on this knowledge, utilizing the evolutionary species definition (Frost and Hillis, 1990) and DNA tree-based delimitation (Wiens and Penkrot, 2002), the currently available evidence (our mtDNA and preliminary morphological characters) suggests that a minimum of two evolutionary species exist within *Lichanura* that are likely narrowly sympatric along the US–Mexico border region of California and Baja California, Mexico. Populations within Lineages A and B (including the Peninsular Range populations of Baja California) were formerly considered a distinct species, *Lichanura roseofusca*, based on morphological differences (Cope, 1861, 1868; Klauber, 1931). However, *L. roseofusca* was later relegated to subspecific status based on presumed intergradation with *L. trivirgata* populations along the mid-peninsular region of Baja California (Miller and Stebbins, 1964; Ottley et al., 1980). The inference of historical fragmentation of Lineage C from Lineage A + B provided evidence of genetic isola-



tion between these divergent lineages (~5–6% uncorrected sequence divergence), and considerable sampling ( $n = 16$  individuals) within the geographic region of apparent contact failed to provide any clear evidence of introgression (i.e., no broad mixing or overlap of haplotypes between these lineages).

We can appreciate the arguments that have been made against the delimitation of species based primarily on mtDNA (e.g., Moritz et al., 1992; Sites and Crandall, 1997); however, equally convincing arguments have been put forth advocating the strengths of mtDNA for inferring species phylogenies and defining species limits (e.g., Moore, 1995, 1997; Wiens and Penkrot, 2002). Also, while preliminary in nature, the morphological data provides additional independent evidence on the distinctiveness of these mtDNA lineages. In the absence of strong genetic evidence of gene flow between these major mtDNA lineages within *Lichanura* and the lack of information on mating systems (see Hoelzer, 1997 for examples of how some mating systems could cause discordance between mtDNA trees and species trees), we prefer to recognize the historical diversification within *Lichanura* by recognizing the two evolutionary species, one represented by Lineage A + B (= *L. orcutti*, Stejneger, 1889a,b), and the other represented by Lineage C (= *L. trivirgata*, Cope, 1861).

#### 4.4. *Lichanura orcutti* (Stejneger, 1889a)

*Lichanura orcutti* Stejneger (1889a) The West American Scientist, 6, 83. Type locality “Colorado Desert, San Diego County, California.”

*Lichanura simplex* Stejneger (1889b) Proceedings of the United States National Museum, 12, 95–99. Type locality: “San Diego, Cal.”

**Distribution.** North of the US–Mexico border within San Diego, County in California along the coastal Peninsular Ranges, northward into the Mojave Desert and eastward in the Sonoran Desert of California and Arizona. In Arizona this species inhabits areas north of the Gila River, except for individuals inhabiting the Gila Mountains.

**Remarks.** The name *Lichanura orcutti* was given priority since the type specimens of *L. roseofusca* and *L. myriolepis* were collected in the same geographic region (i.e., northern part of Lower California) where Lineage C can be found. Lineage C extends north of the US–Mexico border within the Tijuana/Otay watersheds (see below). Therefore these names are not available. *Lichanura orcutti* was given precedence over *L. simplex* since Stejneger’s description of *L. orcutti* was published prior to *L. simplex* in The West American Scientist (Stejneger, 1889a). This predates his description in the Proceedings of the United States National Museum (Stejneger, 1889b), where *L. orcutti* is described again along with the first description of *L. simplex*. Additionally, the geographic description for the type locality of *L. simplex* is too vague (i.e., San Diego Co., Cal.) since *L. trivirgata* extends into San Diego County.

#### 4.5. *Lichanura trivirgata* (Cope, 1861)

*Lichanura trivirgata* Cope (1861) Proceedings of the Academy of Natural Science Philadelphia, 13, 292–306. Type locality “the southern region of Lower California.”

*Lichanura roseofusca* Cope (1868) Proceedings of the Academy of Natural Science Philadelphia, 20, 2. Type locality “northern part of Lower California [Mexico].”

*Lichanura myriolepis* Cope (1868) Proceedings of the Academy of Natural Science Philadelphia, 20, 2. Type locality “northern part of Lower California [Mexico].”

**Distribution.** Extreme southern San Diego County, California within the Tijuana River and Otay watersheds, southward throughout the Baja California peninsula, and northwestern Mexico in the state of Sonora. In Arizona it can be found throughout isolated mountain ranges south of the Gila River in Maricopa, Pima, and Pinal Counties.

We acknowledge that these species delimitations are hypotheses that can (and should) be tested with additional data from nuclear molecular markers and/or morphology to reinforce or falsify the species limits suggested by our mtDNA data, but until then we believe this new classification is a better reflection of the species diversity within *Lichanura*. Additionally, this recognition exemplifies priority that might be most relevant to habitat conservation plans in San Diego County, California and Maricopa, Pinal, and Pima Counties in Arizona.

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## Appendix

Site number, locality, haplotype number, and tissue sample and/or voucher numbers. Numbers in parentheses following haplotypes indicate the occurrence of that haplotype represented in the sample

Site	Locality <sup>a</sup>	Haplotypes	Sample No.	GenBank
<i>Lichanura trivirgata</i>				
1	USA: Arizona, Yuma Co., Kofa Mountains	A1, 2	DAW079, 80	EU280479-480
2	USA: Arizona, Yuma Co., Gila Mountains	All, 12	ASU 33393, 35561	EU280476-477
3	USA: Arizona, La Paz Co., Dome Rock Mountains	A3(2), 4	DAW111, 211, 216	EU280482-483; EU280481
4	USA: Arizona, Maricopa Co., Harquahala Mountains	A6(2), 7, 8	DAW078, 93, SDSU 4262, 178	EU280495-496; EU280493-494
5	USA: Arizona, La Paz Co., Harcuvar Mountains	A5(3)	ASU 34525, DAW120, 177	EU280490-492
6	USA: Arizona, Yavapai Co., Nothing	A9	DAW110	EU280487
7	USA: Arizona, Yavapai Co., Bagdad	A9, 10(2)	DAW147, 148, 149	EU280485; EU280488-489
8	USA: Arizona, Mohave Co., Hualapai Mountains	A9	DAW193	EU280486
9	USA: Arizona, Mohave Co., Cerbat Mountains	A9	ASU 35064	EU280484
10	USA: Arizona, Pima Co., Puerto Blanco Mountains	A13(3)	DAW074, 135, 163	EU280522-524
11	USA: Arizona, Maricopa Co., North Maricopa Mountains	A13(2), 14	SDSNH 68894, DAW189, 190	EU280525-526; EU280529
12	USA: Arizona, Pima Co., Little Ajo Mountains	A13	DAW191	EU280527
13	USA: Arizona, Pima Co., Scarface Hills	A13	DAW192	EU280528
14	Mexico: Baja California, San Felipe	BM17	DAW181	EU280513
15	Mexico: San Felipe Valley	BM18	MVZ 233285	EU280512
16	Mexico: Baja California, El Rosario	BM15	DAW159	EU280515
17	Mexico: Baja California, La Calentura	BM11	UABC H069	EU280499
18	Mexico: Baja California, Santo Tomas	BM11	UABC 1330	EU280500
19	Mexico: Baja California, Sierra Juarez	BM11	UABC 1329	EU280501
20	Mexico: Baja California, Sierra San Pedro Martir	BM8	UABC 1264	EU280497
21	Mexico: Baja California, San Matias	BM12, 13, 14	DAW160-162	EU280534-536
22	Mexico: Baja California, Bahia de los Angeles	BM16, 19(3), 20-21	DAW134, 154, 155, 156, 210, 214	EU280516-521
23	Mexico: Baja California, San Quintin	BM9	DAW165	EU280498
24	Mexico: Baja California, Santa Catarina	BM24	SDSNH 68903	EU280514
25	Mexico: Baja California Sur, Ciudad Constitucion	BM3-4	DAW101, 146	EU280533; EU280532
26	Mexico: Baja California Sur, El Progreso	BM27	DAW224	EU280544

(continued on next page)

## Appendix (continued)

Site	Locality <sup>a</sup>	Haplotypes	Sample No.	GenBank
27	Mexico: Baja California Sur, San Jose de Magdalena	BM23	DAW220	EU280541
28	Mexico: Baja California Sur, Mulege	BM22, M26	DAW219, 222	EU280540; EU280543
29	Mexico: Baja California Sur, La Paz	BM25	DAW221	EU280542
30	Mexico: Baja California Sur, Isla Cedros	BM5-7	DAW085, 86, 179	EU280537-539
31	Mexico: Sonora: Hermosillo	SMI	DAW212	EU280530
32	Mexico: Sonora: Guaymas	SM2	DAW070	EU280531
33	USA: California, San Diego Co., Cigarette Hills	Cl(2), 6	SDSNH 72825, DAW038, SDSU 4255	EU280416-417; EU280429
34	USA: California, San Diego Co., Sentenac Canyon	C1, 6, 8	DAW025, 36, 51	EU280418; EU280430; EU280432 EU280431
35	USA: California, San Diego Co., The Narrows	C6	DAW045	EU280433-434
36	USA: California, San Diego Co., Alder Creek	C14(2)	SDSNHM 68994, DAW143	EU280478
37	USA: California, San Diego Co., Jacumba	C26	DAW117	EU280427
38	USA: California, San Diego Co., Inkopah Mountains	C2	SDSNH 72826	EU280414-415
39	USA: California, San Diego Co., Wild Animal Park	C3(2)	SDSNH 72824, DAW130	EU280419
40	USA: California, San Diego Co., Ramona	C1	DAW119	EU280420
41	USA: California, San Diego Co., Cedar Canyon	C1	SDSNH 72822	EU280502-503
42	USA: California, San Diego Co., Barrett Junction	BM11(2)	SDSNH 72823, DAW057	EU280504
43	USA: California, San Diego Co., Honey Springs	BM11	DAW052	EU280505
44	USA: California, San Diego Co., Skyline Truck Trail	BM11	SDSNH 68975	EU280421-422
45	USA: California, San Diego Co., Sweetwater	C1(2)	RNF2264, DAW060	EU280506-508
46	USA: California, San Diego Co., Marron Valley	BM11(3)	RNF2265, DAW061, SDSNH 72827	EU280423
47	USA: California, San Diego Co., Goat Canyon	C1	DAW035	EU280426
48	USA: California, San Diego Co., Point Loma Reserve	C4	DAW029	EU280428
49	USA: California, San Diego Co., Mission Trails	C5	DAW043	EU280425
50	USA: California, San Diego Co., Palomar Mountain	C7	DAW072	EU280452
51	USA: California, San Diego Co., Camp Pendelton	C15	DAW073	EU280453-454
52	USA: California, San Diego Co., Santa Margarita Reserve	C15(2)	DAW027, 28	EU280509; EU280424
53	USA: California, San Diego Co., Hollenbeck Canyon	BM11, C1	RNF6009, RAJSP14	

## Appendix (continued)

Site	Locality <sup>a</sup>	Haplotypes	Sample No.	GenBank
54	USA: California, San Diego Co., Otay Valley	BM10-11	DAW040, RNF6008	EU280511; EU280510
55	USA: California, Orange Co., Wier Canyon	C9, C17	DAW065, WEIR340	EU280432; EU280447
56	USA: California, Orange Co., Trabuco Canyon	C16	DAW068	EU280456
57	USA: California, Orange Co., Yorba Linda	C19	DAW209	EU280455
58	USA: California, Riverside Co., North Hills	C9(2), 13	DAW022, 23, 24	EU280436-437; EU280440
59	USA: California, Riverside Co., Motte Reserve	C17, 18	DAW053, DAW077	EU280448; EU280451
60	USA: California, Riverside Co., Lake Elsinore	C17(2)	DAW039, RNF 2263	EU280449-450
61	USA: California, Riverside Co., Moreno Valley	C9	DAW102	EU280438
62	USA: California, Riverside Co., Whitewater Canyon	C9, 10(2), 11, 12	DAW076, 84, 100, 108, 139	EU280439; EU280441-444
63	USA: California, Riverside Co., Chuckwalla Mountains	C20(2)	DAW075, 124	EU280457-458
64	USA: California, Riverside Co., Thousand Palms	C20	SDSNH 72828	EU280459
65	USA: California, San Bernardino Co., Morongo Valley	C20	DAW109	EU280460
66	USA: California, San Bernardino Co., Silverwood Lake	C23	DAW064	EU280464
67	USA: California, San Bernardino Co., Johnson Valley	C22	DAW132	EU280475
68	USA: California, San Bernardino Co., Stoddard Ridge	C23	MVZ 137796	EU280463
69	USA: California, San Bernardino Co., Pioneertown	C23(2), CA12	DAW174, RNF2462, BUR109	EU280465-466; EU280445
70	USA: California, San Bernardino Co., Pipes Canyon	C12	RNF2464	EU280446
71	USA: California, San Bernardino Co., Granite Mountains	C25	HBS 26953	EU280474
72	USA: California, Kern Co., Onyx	C23	DAW083	EU280467
73	USA: California, Los Angeles Co., Azusa Canyon	C23(3), 24	DAW150-152, 173	EU280468-470; EU280473
74	USA: California, Los Angeles Co., Mount Baldy	C23	DAW175	EU280471
75	USA: California, Los Angeles Co., Fish Fork Creek	C23	DAW223	EU280472
76	USA: California, Imperial Co., Black Mountain	C21(2)	DAW123, 185	EU280461-462
	<i>Charina bottae</i>		DAW081, CAS 209228	EU280412-413

<sup>a</sup> Specific locality and voucher information can be obtained from the first author.

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