Phylogeography of the Western Lyresnake (*Trimorphodon biscutatus*): testing aridland biogeographical hypotheses across the Nearctic–Neotropical transition

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Abstract

The Western Lyresnake (*Trimorphodon biscutatus*) is a widespread, polytypic taxon inhabiting arid regions from the warm deserts of the southwestern United States southward along the Pacific versant of Mexico to the tropical deciduous forests of Mesoamerica. This broadly distributed species provides a unique opportunity to evaluate a priori biogeographical hypotheses spanning two major distinct biogeographical realms (the Nearctic and Neotropical) that are usually treated separately in phylogeographical analyses. I investigated the phylogeography of *T. biscutatus* using maximum likelihood and Bayesian phylogenetic analysis of mitochondrial DNA (mtDNA) from across this species' range. Phylogenetic analyses recovered five well-supported clades whose boundaries are concordant with existing geographical barriers, a pattern consistent with a model of vicariant allopatric divergence. Assuming a vicariance model, divergence times between mitochondrial lineages were estimated using Bayesian relaxed molecular clock methods calibrated using geological information from putative vicariant events. Divergence time point estimates were bounded by broad confidence intervals, and thus these highly conservative estimates should be considered tentative hypotheses at best. Comparison of mtDNA lineages and taxa traditionally recognized as subspecies based on morphology suggest this taxon is comprised of multiple independent lineages at various stages of divergence, ranging from putative secondary contact and hybridization to sympatry of 'subspecies'.

Keywords: Mexico, mitochondrial DNA, North America, phylogeography, *Trimorphodon*, vicariance

Received 17 March 2006; revision accepted 2 May 2006

Mexico has drawn special attention from biogeographers because of its exceptional biodiversity that lies at the interface between two distinct biogeographical regions, the Nearctic and the Neotropical. Following the seminal works of Sclater (1858) and Wallace (1876) defining the world's biogeographical regions, a number of authors have noted the difficulty in delimiting the Nearctic–Neotropical boundary because of the composite character of Mexico's fauna (Wallace 1876; Halffter 1976; Lomolino *et al*. 2005). This confluence of distinct regional faunas has led some authors to suggest that Mexico and the southwestern United States constitute a broad transition zone between distinct northern and southern biotas (Halffter 1976; Heilprin 1887). Recent efforts using biogeographical mapping approaches and various taxonomic groups have further demonstrated that no single line may be drawn that is optimal for all taxa, highlighting the transitional nature of the Nearctic–Neotropical boundary (e.g. Ortega & Arita 1998; Marshall & Liebherr 2000; Morrone & Márquez 2001; Escalante *et al*. 2004; Huidobro *et al*. 2006; Morrone 2006).

In addition to recent biogeographical mapping efforts, an increasing number of phylogeographical analyses have provided key insights into the history and formation of species across western North America. These investigations have consistently revealed species boundaries, hybrid zones, and genetic discontinuities in diverse taxa that are highly concordant with geographical barriers, a pattern consistent with a model of vicariant allopatric

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divergence and speciation. Middle Miocene to Pleistocene vicariant events and more recent Quaternary climatic shifts have been invoked to explain this pattern, and provide testable hypotheses that may be evaluated through phylogeographical investigations of codistributed taxa. The majority of previous studies have focused on species inhabiting the Nearctic deserts of northern Mexico and the southwestern United States (e.g. Riddle & Honeycutt 1990; Lee *et al*. 1996; Upton & Murphy 1997; Zink & Blackwell 1998; Orange *et al*. 1999; Riddle *et al*. 2000a, b, c; Zink & Blackwell-Rago 2000; Zink *et al*. 2000; Zink *et al*. 2001; Murphy & Aguirre-Leon 2002; Nason *et al*. 2002; Zink 2002; Hurtado *et al*. 2004; Hafner & Riddle 2005; Jaeger *et al*. 2005; Riddle & Hafner 2006), with fewer works examining species found in the seasonally dry tropical deciduous forests of Mesoamerica (e.g. Parkinson *et al*. 2000; Sasa Marin 2000; Becerra 2005; Hasbún *et al*. 2005). Although no single line can be drawn to mark precisely where the Nearctic meets the Neotropics, many tropical species reach their northernmost limit in southern Mexico and are usually treated separately in phylogeographical analyses. A unique opportunity to link the evolutionary histories of arid-adapted temperate and tropical biota in western North America is provided by species distributed across the biologically diverse Nearctic–Neotropical transition zone. Genetically dissecting such widespread species may contribute to a better understanding of how historical forces have shaped genetic, species, and community diversity in the aridlands of North and Middle America.

Here, I extend previous efforts investigating the evolutionary history and biogeography of arid western North American biota through a phylogeographical investigation of the Western Lyresnake, *Trimorphodon biscutatus*. This widespread taxon is well suited for an investigation of the role of historical geographical barriers in shaping contemporary patterns of population genetic differentiation because its distribution spans several major biogeographical breaks previously hypothesized to have played a role in the origin and evolution of other codistributed organisms, such as the Gulf of California (Upton & Murphy 1997; Riddle *et al*. 2000a, b, c; Murphy & Aguirre-Leon 2002; Nason *et al*. 2002; Hurtado *et al*. 2004), Sierra Madre Occidental (Riddle & Honeycutt 1990; Lee *et al*. 1996; Zink & Blackwell 1998; Orange *et al*. 1999; Riddle *et al*. 2000b; Zink & Blackwell-Rago 2000; Zink *et al*. 2000, 2001; Zink 2002; Becerra 2005; Jaeger *et al*. 2005), trans-Mexican volcanic belt (TMVB; Mulcahy & Mendelson 2000; Mateos *et al*. 2002; Amman & Bradley 2004; Hulsey *et al*. 2004; Pauly *et al*. 2004; Zaldívar-Riverón *et al*. 2004; Becerra 2005; Mateos 2005), and Isthmus of Tehuantepec (Peterson *et al*. 1999; Sullivan *et al*. 2000; Fig. 1).

Accurate estimation of absolute divergence dates among lineages is a powerful tool for inferring the contribution of geological and climatic events in speciation (Yoder & Yang 2004). Although the molecular clock hypothesis (Zuckerkandl & Pauling 1965) may be useful for estimating divergence times when the data conform to the clock assumption, the clock is usually violated, even among closely related species (Huelsenbeck *et al*. 2000; Welch & Bromham 2005). Because rate and time are confounded in phylogenetic analysis, divergence time estimation becomes problematic when the assumption of the clock is relaxed (Huelsenbeck *et al*. 2000; Yang & Yoder 2003). Fortunately, methods for estimating absolute divergence times among lineages using DNA sequences while allowing for variation in the rate of molecular evolution have recently been developed (Sanderson 1997, 2002; Thorne *et al*. 1998; Huelsenbeck *et al*. 2000; Yoder & Yang 2000; Kishino *et al*. 2001; Thorne & Kishino 2002; Yang & Yoder 2003; Seo *et al*. 2004). Bayesian relaxed-clock methods are available that permit the use of multiple calibration points in the form of upper and lower constraints on node times (Thorne *et al*. 1998; Thorne & Kishino 2002), allowing for biogeographical reconstruction in lineages with incomplete or absent fossil records (Welch & Bromham 2005).

Methods

Molecular methods

New mitochondrial DNA (mtDNA) sequences were obtained from 91 individuals of *Trimorphodon biscutatus* from across the species' range, including samples of all currently or formerly recognized subspecies (GenBank Accession nos DQ497451–542; Fig. 2; Appendix I). Thirty-nine identical sequences were removed from the data matrix to facilitate phylogenetic analysis, leaving a total of 52 terminals. *Trimorphodon tau* was defined as the outgroup because the relationship of *Trimorphodon* to other colubrid snakes remains completely unresolved, and attempting to root the tree using the outgroup criterion is problematic when the ingroup and outgroup are too divergent (Wheeler 1990; Huelsenbeck *et al*. 2002). Constraining *T. tau* as the outgroup is justified, given that *T. biscutatus* and *T. tau* have long been recognized as distinct species, differing in several diagnostic features and occurring in sympatry at lower altitudes in parts of western Mexico (McDiarmid & Scott 1970). DNA was extracted from tissues (liver, muscle, or shed skin) using phenol:chloroform methods (Hillis *et al*. 1996) or QIAGEN DNeasy tissue kits following the manufacturer's protocol (QIAGEN). An 817 base-pair fragment of mtDNA comprised of the ND4 gene and flanking tRNAs (His, Ser, Leu) was amplified via polymerase chain reaction (PCR) using the primers ND4 and LEU (Arévalo *et al*. 1994). PCRs consisted of 35 cycles of 94 °C for 1 min, 48 °C for 2 min, and 72 °C for 3 min. PCR products were purified using sodium acetate or QIAquick PCR purification
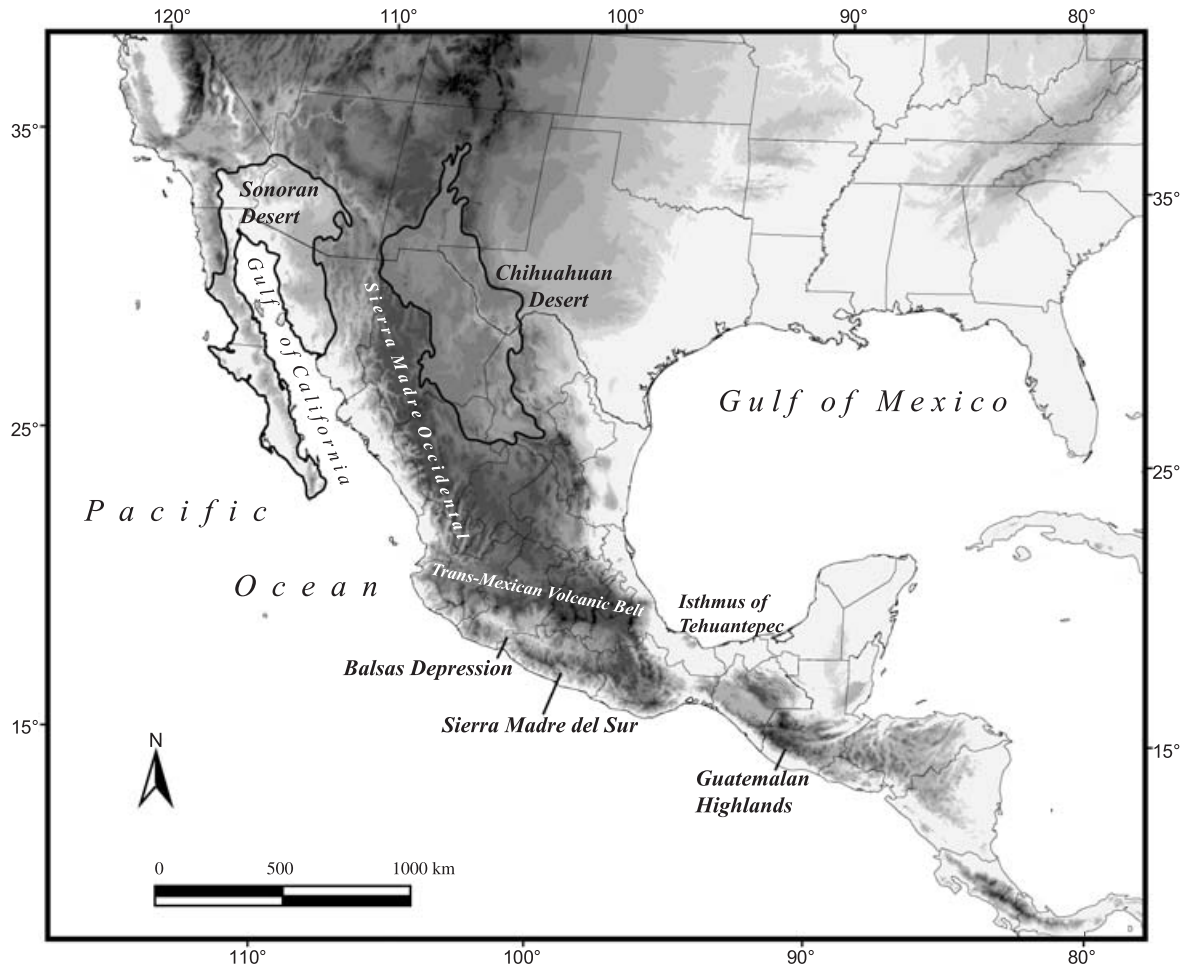
Fig. 1 Map of North America illustrating location of relevant major geographical features discussed in text.

kits following the manufacturer's protocol (QIAGEN). Purified templates were sequenced using dye-labelled dideoxy terminator cycle sequencing on an ABI 377 automated DNA sequencer or an ABI 3730 capillary sequencer (Applied Biosystems). DNA sequences were edited and aligned using SEQUENCHER version 3 (Gene Codes).

Phylogenetic analyses

Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian methods. A hierarchical likelihood ratio test (Huelsenbeck & Crandall 1997) implemented in the program MODELTEST version 3.06 (Posada & Crandall 1998) was used to evaluate the best-fit model of sequence evolution. The general-time-reversible model (six substitution rate categories) with some invariable and variable sites following a discrete gamma distribution with four categories (GTR + I + Γ) was chosen (Appendix II). Maximum-likelihood analysis was performed with PAUP* version 4.0b10 (Swofford 2001) using an initial topology generated under parsimony using a heuristic

search [10 random sequence addition replicates of taxa and tree-bisection–reconnection (TBR) branch swapping] with equal weighting of codon positions and classes of nucleotide substitutions. The model parameters that maximized the likelihood under the GTR + I + Γ model were estimated under the likelihood criterion on the initial parsimony topology, and then fixed and used in a heuristic search (simple stepwise additions of taxa and TBR branch swapping) under the likelihood criterion. This process was reiterated until the same maximum likelihood score was obtained by subsequent searches (Swofford *et al.* 1996).

Bayesian analyses were conducted using MRBAYES version 3.0b4 (Huelsenbeck & Ronquist 2001). Because a single model of sequence evolution may not provide the best fit for a DNA sequence, the data were partitioned into first, second, and third codon positions and tRNAs (four partitions total), and analysed using different models of sequence evolution for each partition (Nylander *et al.* 2004; Brandley *et al.* 2005). A hierarchical likelihood ratio test implemented in MRMODELTEST version 1.1b (Nylander 2002) chose GTR + Γ for the first and third codon positions,
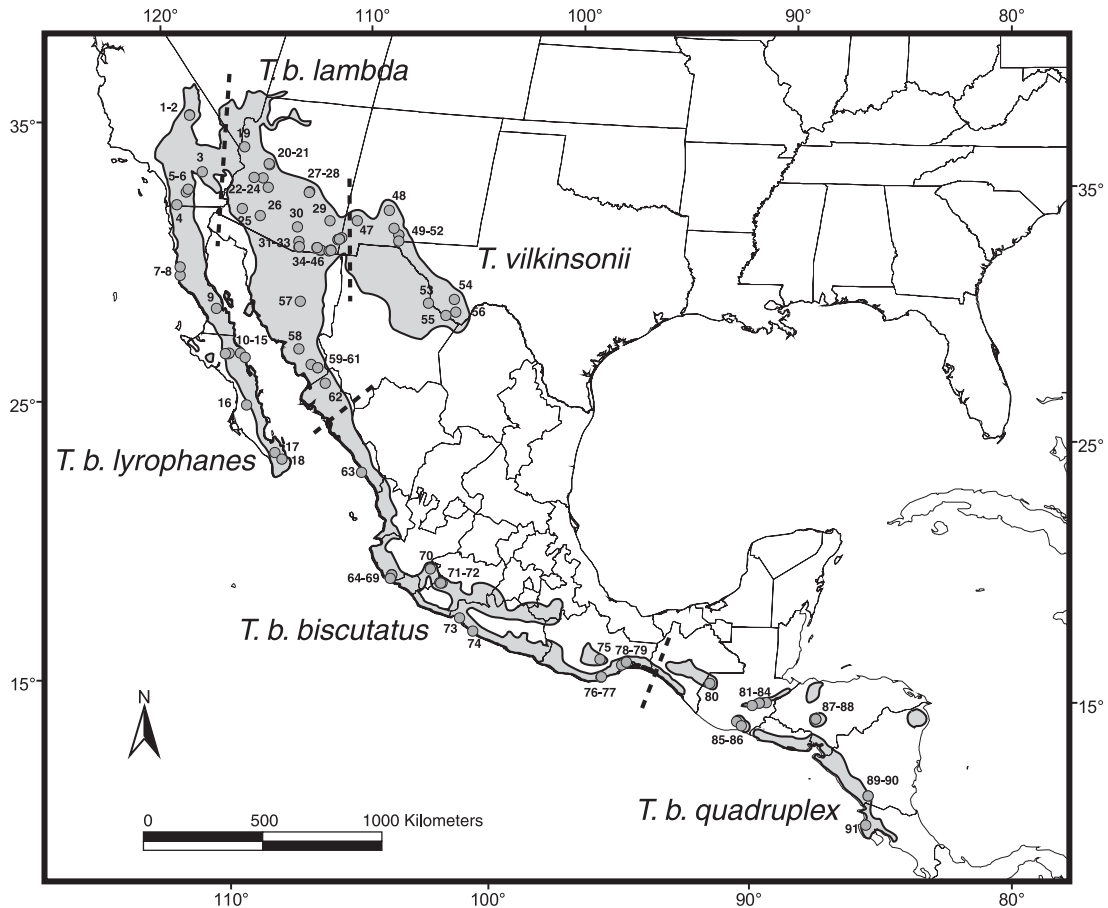
Fig. 2 Distribution of the Western Lyresnake (*Trimorphodon biscutatus*) showing ranges of currently recognized subspecies separated by dashed lines (after Gehlbach 1971; Scott & McDiarmid 1984a). Numbers correspond to localities of sampled populations listed in Appendix A (*N* = 91).

HKY for the second positions, and K80 + I + Γ for tRNAs. To ensure convergence on the global optimal topology, four independent analyses were run for 10^7 generations, each using random starting trees and default priors. In each analysis, four Markov chains (using default heating values) were sampled every 1000 generations. Stationarity was evaluated by examining plots of log-likelihood scores vs. number of generations (Leaché & Reeder 2002). Trees sampled prior to reaching stationarity were discarded as burn-in, and the remainder used to construct a 50% majority rule consensus tree. Posterior probability values were used to assess phylogenetic support (Huelsenbeck & Ronquist 2001); clades with values greater than 95% were considered to be significantly supported (Leaché & Reeder 2002).

Divergence time estimation

Based on concordance between lineage boundaries and geographical barriers (see results below), divergence times were estimated under a vicariance model using Markov chain Monte Carlo (MCMC) methods in a Bayesian relaxed clock approach that allows for different rates among branches, implemented in the software package MULTIDISTRIBUTE (Thorne *et al*. 1998; Thorne & Kishino 2002). Estimates of the transition/transversion rate ratio, κ, and rates for site classes following a discrete gamma distribution with five site classes under the $F_{84} + Γ$ model of nucleotide substitution (the most complex model implemented in the MULTIDISTRIBUTE package) were obtained from the program BASEML in the package PAML version 3.14 (Yang 1997). The program PAML2MODELINF was then used to convert the output from BASEML to a new infile for the program ESTBRANCHES. These parameter estimates and a prespecified tree topology generated using maximum likelihood were used to estimate branch lengths and a variance-covariance structure of branch length estimates using the program ESTBRANCHES. Finally, MULTIDIVTIME was used to generate posterior distributions of substitution rates and divergence times using MCMC methods. Prior gamma distributions for MCMC analyses in MULTIDIVTIME are required for the time separating the ingroup root from the present (age of the ingroup root node) and the rate of molecular evolution at the ingroup root node. The age of the root node was given a mean value

of 11 million years ago (Ma) with a standard deviation set to its maximum value (equal to the mean) in order to generate a diffuse prior. This value was chosen based on concordance between the deepest divergence in the phylogeny and the geographical position of the TMVB (and thus assuming vicariance), the initial uplift of which began 14–7 Ma (Verma & Carrasco-Nunez 2003). The rate at the root node was calculated by taking the mean branch length from the ingroup root to the ingroup tips divided by the time between the tips and the root (= 0.07 substitutions/site/10 million years). Theoretically, the data and node calibrations should determine the age of the root and rate at the root, and thus these priors should have little effect on the posterior distributions (Yang & Yoder 2003). The prior of the Brownian motion constant ν (which determines the amount of rate change allowed between ancestral and descendant nodes) and its standard deviation were both set to 1 following the software author's recommendation. Additional priors required by the Bayesian analysis include one or more internal calibration points in the form of upper and/or lower bounds on node times. In the absence of fossil evidence, I constrained two nodes based on geological data, each with lower and upper bounds: the most basal divergence (node A, Fig. 3) occurs across the TMVB and was constrained to between 14 and 7 Ma based on a range of geological, geochronological, and/or geochemical data (Ferrari *et al*. 1999, 2000; Martinez *et al*. 2000; Verma & Carrasco-Nunez 2003). A second node (node C, Fig. 3) was constrained to between 8 and 4 Ma based on estimates of the timing of the formation of the Gulf of California (reviewed in Carreño & Helenes 2003). These nodes were chosen as calibration points because the geological history of these regions is relatively well characterized (Ferrusquía-Villafranca *et al*. 2005). Default values were used for all other priors in the MCMC analyses. Markov chains were run for 1 million generations and sampled every 100th generation after a burn-in period of 100 000 generations. Analyses were performed at least twice from different random starting points to check for convergence.

Results and discussion

Both Bayesian and ML analyses recovered five well-supported tip clades within *Trimorphodon biscutatus* (Fig. 3). The most basal divergence separates populations across the TMVB into northern and southern groups. Within the southern group, two distinct lineages separated by the Guatemalan highlands exist: one composed of individuals from eastern Guatemala, Nicaragua, Honduras, and Costa Rica (hereafter, the Central American clade), and another composed of individuals from western Guatemala and Mexico south of the TMVB (hereafter, the southern Mexico clade; Fig. 4). The northern group consists of three distinct strongly supported lineages: individuals from California and the Baja California Peninsula (hereafter, the Baja California clade) are strongly supported as sister to the two remaining clades. Samples from the TMVB north through Sinaloa also form a clade (hereafter, the western Mexico clade), as do populations from the Chihuahuan and Sonoran deserts (hereafter, the Sonoran + Chihuahuan Desert clade). Average uncorrected pairwise distances for these five clades are shown in Table 1. Bayesian posterior estimates of divergence times were highly congruent between separate runs, indicating convergence; posterior estimates along with 95% credibility intervals are listed in Table 2. It should be emphasized that because this approach allows calibrations to be defined as ranges rather than as single dates, this method incorporates rate variation among lineages by integrating over a range of possible rates instead of assigning a particular rate to each branch, and thus tends to produce conservative divergence time estimates bounded by broad confidence intervals (Cranston & Rannala 2005). Not surprisingly then, credibility intervals (CI, Table 2) for divergence time point estimates are broad, and thus these estimates are highly conservative. This uncertainty is particularly acute when attempting to accurately estimate very recent divergences within closely related lineages based on a single marker (Arbogast *et al*. 2002). These point estimates should be considered hypotheses that are testable using additional independent loci and/or phylogeographical data from other codistributed taxa.

Phylogenetic patterns

The boundaries of the five well-supported clades are highly concordant with existing geographical and/or climatic barriers (Fig. 4). Coupled with observed levels of divergence, this pattern suggests that regional differentiation is more consistent with a model of Neogene vicariance rather than recent dispersal around pre–existing geographical barriers (see Gehlbach 1971). The majority of these clades exhibit substantial local geographical structure (Table 1; Fig. 4). Below, I discuss geological and climatic events hypothesized to have played a role in the differentiation of *T. biscutatus* and other arid-adapted lineages across western North America, beginning with the most basal divergence and working forward in time to the tips of the phylogeny. Finally, I compare the geographical ranges of the evolutionary lineages recovered in phylogenetic analyses to boundaries of taxa traditionally recognized as subspecies based on morphological data.

Historical biogeography

The major biogeographical provinces of western North America began forming between the late Oligocene and
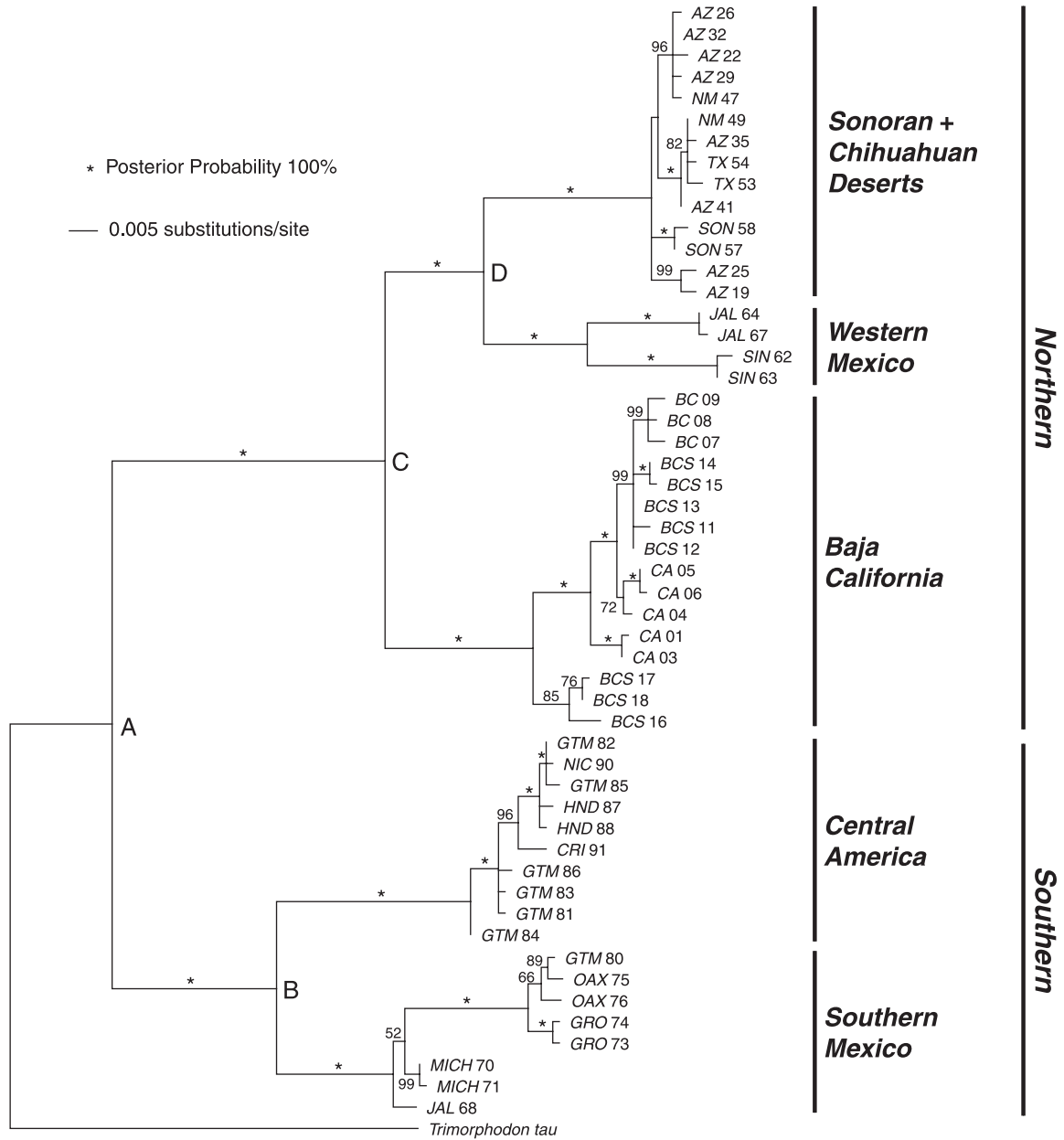
Fig. 3 Phylogenetic relationships of *Trimorphodon biscutatus* based on the 50% majority rule consensus tree from Bayesian analysis under the GTR + I + Γ model of sequence evolution. Numbers and asterisks on branches represent Bayesian posterior probability values and are shown on major nodes only for clarity. The same topology was recovered in the maximum likelihood analysis employing the same model. Letters on nodes correspond to divergence times listed in Table 2. Divergence times were calibrated using upper and lower bounds on nodes A (14–7 Ma) and C (8–4 Ma) based on geological data for the timing of the formation of the TMVB and Baja California Peninsula, respectively.

middle Miocene during a general trend towards increasing aridity resulting from climate change associated with intense Cenozoic tectonics and volcanism that formed the Rocky Mountains, Mexican and Central American Plateaus, and the Sierra Madres Occidental, Oriental, and del Sur (Axelrod 1983; Van Devender 1995; Delgado-Granados *et al.* 2000; Rogers *et al.* 2002). Following the

initial uplift of the Sierra Madre Occidental, the trans-Mexican volcanic belt was formed beginning in the middle Miocene (*c.* 14 Ma) and continuing to the present (Ferrari *et al.* 2000; Verma & Carrasco-Nunez 2003) as a result of subduction of the Cocos and Rivera plates beneath the North American plate (Martiny *et al.* 2000). The formation of the Sierra Madres Occidental and Oriental and Rocky
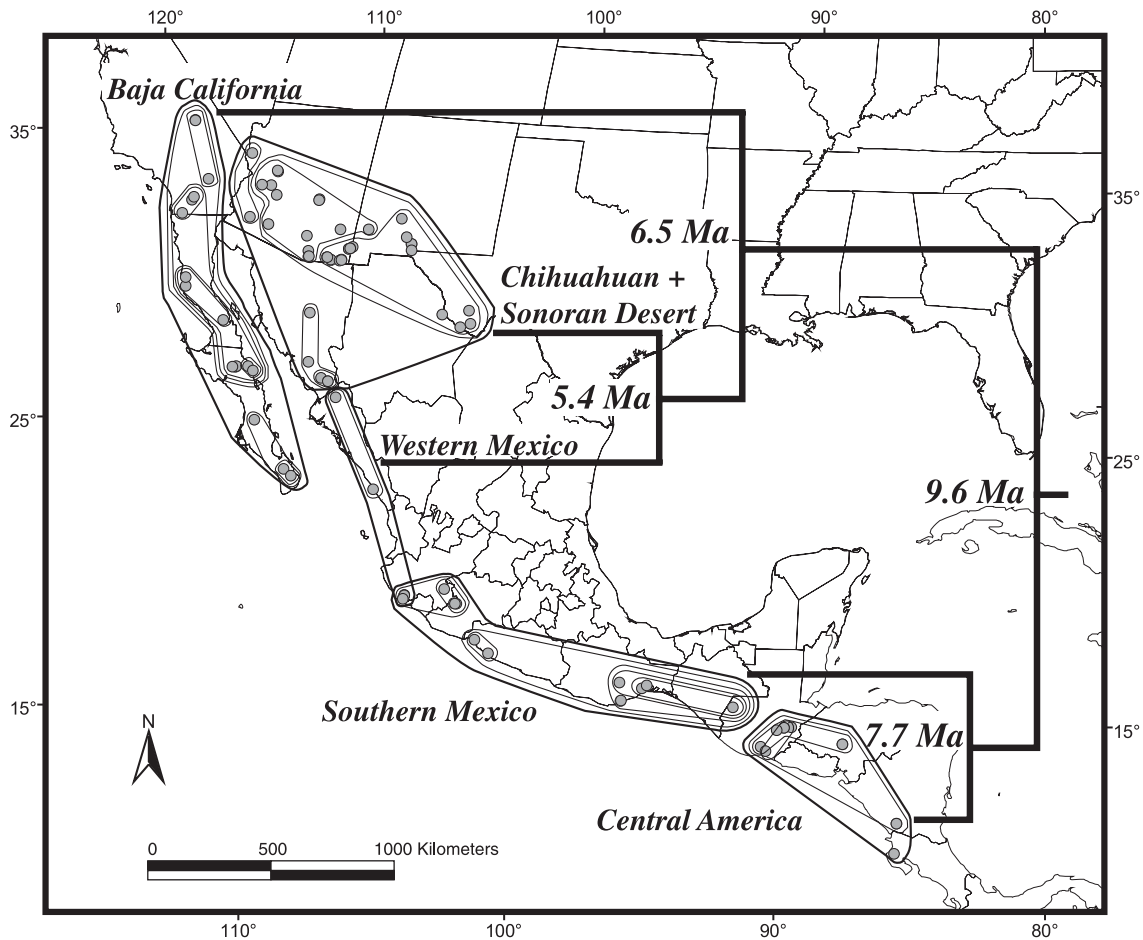
Fig. 4 Area cladogram for the five major clades recovered in phylogenetic analysis. Monophyletic groups are successively circled to indicate geographical patterns of genetic structure. Numbers on nodes indicate divergence time estimates for the five major clades (see Table 2 for credibility intervals).

Table 1 Average uncorrected pairwise sequence divergence percentages for the five clades of *Trimorphodon biscutatus* plus the outgroup *Trimorphodon tau*

	Sonoran + Chihuahuan	Western Mexico	Baja California	Southern Mexico	Central America	*Trimorphodon tau*
Sonoran + Chihuahuan	0.6	—	—	—	—	—
Western Mexico	5.6	2.1	—	—	—	—
Baja California	6.2	6.6	1.3	—	—	—
Southern Mexico	9.7	9.3	9.1	1.7	—	—
Central America	9.8	9.5	9.5	6.4	0.8	—
Trimorphodon tau	10.8	10.4	10.1	8.8	9.7	—

Mountains from the Eocene (*c.* 46 Ma) to middle Miocene (*c.* 23 Ma) interrupted upper atmospheric flow, blocking warm, moist air masses from the Pacific Ocean and Gulf of Mexico, and drying out the Mexican Plateau and Great Plains (McDowell & Keizer 1977; McDowell & Clabaugh 1979; Axelrod 1983; Graham & Dilcher 1995; Van Devender 2000; Aranda-Gómez *et al.* 2003).

This Miocene climate change segregated species along latitudinal and altitudinal environmental gradients, sparking evolutionary radiations in a number of successful modern lineages, including colubrid snakes (Greene 1997; Van Devender 1995). Because the phylogenetic relationships of lyresnakes to other colubrid snakes are not well resolved and fossil evidence is lacking, any inferences about the

Table 2 Bayesian estimates of divergence times in millions of years ago (Ma) including 95% credibility intervals estimated under the $F_{84} + \Gamma$ model

Node	Divergence date (95% CI)	Geographical barrier
A*	9.6 (7.2, 13.1)	Trans-Mexican volcanic belt
B	7.7 (4.8, 11.4)	Guatemalan highlands
C†	6.5 (4.4, 7.9)	Gulf of California
D	5.4 (3.2, 7.4)	Sierra Barabampo/Río Fuerte

*Node time constrained to between 14–7 Ma.
†Node time constrained to between 8 and 4 Ma.

origin of *Trimorphodon* are at this point purely speculative. *Trimorphodon* may have diverged from other colubrid snakes during the late Oligocene–early Miocene. Based on geographical distribution and habitat associations of extant species, the divergence of the *T. biscutatus* group from the *Trimorphodon tau* group may have occurred during the early to mid-Miocene as climate change separated an ancestral lineage into lowland and upland forms, respectively. *T. tau* is widely distributed in upland regions of Mexico north of the Isthmus of Tehuantepec, as well as on the coastal plain (where it is sympatric with *T. biscutatus*) and inhabits areas of rocky relief and pine-oak forest from 100 to 2600 m above-sea-level (a.s.l.) altitude (Scott & McDiarmid 1984b). *T. biscutatus* is found in lowland tropical deciduous forests and thornscrub in Central America and Mexico south of Sonora, while populations in the deserts of northern Mexico and the southwestern United States are found in rocky areas of up to 2400 m a.s.l. (Scott & McDiarmid 1984a). Historically, tropical deciduous forests were widespread across much of the southwestern United States and Mexico during the early Tertiary, but became restricted to lowland areas along the coasts of Mexico following the formation of the Sierra Madres Occidental and Oriental during the middle Tertiary (Axelrod 1983; Graham & Dilcher 1995; Graham 1999). The ancestral *T. biscutatus* lineage probably began differentiating into the lineages represented today during the mid-Miocene.

The trans-Mexican volcanic belt

The deepest division supported by phylogenetic analyses separates populations into northern and southern lineages in the general vicinity of the western edge of the trans-Mexican volcanic belt (TMVB) in southwestern Jalisco (Figs 3 and 4). The divergence date between these northern and southern clades was estimated to have occurred approximately 9.6 Ma (constrained to between 14 and 7 Ma; Fig. 3, Table 2). The TMVB region has been of particular interest to biogeographers (e.g. Marshall & Liebherr 2000; Escalante *et al.* 2004) because it acts as a transitional area between distinct Nearctic and Neotropical

fauna in the interior of the continent (Fig. 1; Ortega & Arita 1998; Morrone & Márquez 2001; Morrone 2006). Recent phylogenetic and phylogeographical studies of lineages spanning this boundary have consistently revealed deep divergence between northern and southern lineages across the TMVB (Zink & Blackwell 1998; Mulcahy & Mendelson 2000; Zink & Blackwell-Rago 2000; Mateos *et al.* 2002; Amman & Bradley 2004; Hulsey *et al.* 2004; Pauly *et al.* 2004; Zaldívar-Riverón *et al.* 2004; Mateos 2005).

The Chiapan-Guatemalan highlands and Stuart's subhumid corridor

The second divergence supported by phylogenetic analyses separates the Central America clade from the southern Mexico clade across the highlands of Chiapas and Guatemala (Figs 1 and 4), estimated to have occurred approximately 7.7 Ma (Table 2). This divergence is generally consistent with the formation of the Chiapan-Guatemalan highlands of northern Central America, which formed during two distinct time intervals (Campbell 1999). The uplift of the extensive northern Central American plateau took place during the late Miocene to early Pliocene, from approximately 10–3.8 Ma (Rogers *et al.* 2002). Beginning in the late Pliocene, a younger chain of volcanoes was formed along the western portion of the Central American plateau (Williams 1960). The formation of this imposing mountain chain had a significant impact on the local biota, both through extinction and resulting climatic change, creating cloud forest conditions on the windward (south) slopes and rain shadow conditions in the interior valleys (Campbell & Vannini 1988; Campbell 1999). An abrupt turnover from xeric, subhumid vegetation to humid forest occurs in south-central Chiapas and extends along the coast to south-central Guatemala (Campbell & Vannini 1988; Campbell 1999). These cooler, wetter conditions along the Pacific coast of southwestern Guatemala and southeastern Chiapas are unsuitable for arid-adapted lineages such as *Trimorphodon*, which are isolated in the arid interior basins of the Ríos Grijalva and Motagua that drain to the Atlantic (Campbell & Vannini 1988). These valleys show a high degree of similarity to tropical deciduous forests of the Pacific foothills of western Mexico, and a number of other arid-adapted reptiles derived from the Pacific coast are also restricted to these arid interior valleys (Campbell & Vannini 1988).

The origin and evolution of these isolated populations of arid-adapted species of reptiles has been of particular interest to biogeographers since Stuart (1954) offered three, nonmutually exclusive historical biogeographical hypotheses to explain their distribution. These hypotheses included: (i) formerly contiguous dry forest through the lowlands of the Yucatan Peninsula; (ii) previously uninterrupted dry forest along the Pacific versant of northern

Central America; and (iii) a past subhumid corridor extending from the Isthmus of Tehuantepec through the dry interior valleys of northern nuclear Central America to the Pacific versant, which Stuart (1954) favoured as the best explanation. While all three scenarios may explain the distribution of arid-adapted species of reptiles inhabiting the 'corridor', the second hypothesis of Stuart (1954) involving a formerly continuous dry forest along the Pacific coast appears most plausible for a number of lineages derived from the Pacific versant (Campbell & Vannini 1988). As with the results presented here, recent phylogeographical analyses of codistributed Middle American dry forest reptiles generally provide support for this scenario (Parkinson *et al*. 2000; Sasa Marin 2000; Hasbún *et al*. 2005).

The Río Balsas

Within the southern Mexico clade, additional phylogeographical structure was found across the Río Balsas (Fig. 1), with populations separated by the river averaging 2.7% uncorrected pairwise sequence divergence, although this relationship was not strongly supported (Fig. 3). Lacustrine sediments in the region suggest that low-lying areas such as the Balsas Depression were periodically inundated historically (Gómez-Tuena & Carrasco-Núñez 2000) and likely served as barriers to dispersal (Marshall & Liebherr 2000; Amman & Bradley 2004). The low-lying Balsas Basin is bounded on its western margin by the Río Balsas, the Sierra Madre del Sur to the south, the TMVB to the north, and the Sierra Norte of Oaxaca to the east (Fig. 1). The uplift of the TMVB and Sierra Madre del Sur isolated the dry forests of the Balsas Depression subprovince (Ferrusquía-Villafranca 1993), resulting in high diversity and endemism across diverse groups (Ayala *et al*. 1993; Llorente-Bousquets & Luis-Martínez 1993; Rzedowski 1993; Becerra & Venable 1999; Zaldívar-Riverón *et al*. 2004).

The Gulf of California

The isolation of the Baja California clade is the next deepest divergence in the phylogeny, estimated to have occurred 6.5 Ma (constrained to between 8 and 4 Ma; Table 2), and consistent with the earliest marine incursions into the northern part of the gulf (Oskin & Stock 2003). The transfer of the peninsula from the North American to the Pacific plate took place gradually (Holt *et al*. 2000), with initial rifting of the continental crust along the San Andreas Fault as early as 14 Ma (Karig & Jensky 1972). The southern mouth of the gulf opened less than 4 Ma (Atwater 1989; Lonsdale 1991). During the late Miocene to early Pliocene (approximately 8–4 Ma), the protogulf transgressed northward into lowland areas of California and Arizona, forming a shallow embayment 40–50 km wide (the Bouse Sea) along

the Colorado River as far north as Lake Mead in southern Nevada (Metzger 1968; Blair 1978; Eberley & Stanley 1978; Buising 1990; Spencer *et al*. 1998), and the San Gorgonio Constriction in the Salton Trough region (Boehm 1984; Ingle 1987; McDougall *et al*. 1999). These marine incursions effectively isolated the peninsula and much of southwestern California west of the Coachella Valley from the remainder of the continent. Genetic discontinuities between peninsular and continental taxa reflecting this isolation have been demonstrated in mammals, birds, amphibians, reptiles, cacti, and arthropods (Murphy 1983; Lamb *et al*. 1992; Upton & Murphy 1997; Orange *et al*. 1999; Riddle *et al*. 2000a, b, c; Rodriguez-Robles & de Jesus-Escobar 2000; Gatenbein *et al*. 2001; Murphy & Aguirre-Leon 2002; Nason *et al*. 2002; Hurtado *et al*. 2004; Hafner & Riddle 2005).

Within the Baja California clade, additional north–south patterns of genetic differentiation were found across the central portion of the peninsula, similar to results reported from other codistributed reptiles, birds, mammals, and near-shore fishes (e.g. Zink & Blackwell 1998; Aguirre *et al*. 1999; Riddle *et al*. 2000a, b, c; Rodriguez-Robles & de Jesus-Escobar 2000; Zink *et al*. 2000; 2001; Zink 2002; Whorley *et al*. 2004; Riginos 2005). The underlying mechanisms responsible for this pattern remain under debate. Following Upton & Murphy (1997), several authors have invoked a Pleistocene (*c*. 1 Ma) mid-peninsular seaway to explain north–south patterns of genetic differentiation in mtDNA lineages (Aguirre *et al*. 1999; Riddle *et al*. 2000a, b, c; Zink 2002; Whorley *et al*. 2004). Others have challenged this view, citing a lack of geological evidence from the appropriate time period, and have suggested instead that the north–south divergence may result from divergent ecological selection across a gradient of environmental conditions, which change abruptly midway down the peninsula (Grismer 2002; reviewed in Jacobs *et al*. 2004). Earlier, well-dated marine rocks (Miocene) have been found in the central Baja California Peninsula suggesting extensive former marine incursions (Delgado-Argote *et al*. 2000; Holt *et al*. 2000), although the hypothetical seaway proposed by Helenes & Carreño (1999) appears to be unnecessary to explain their presence (Oskin & Stock 2003).

The Nearctic–Neotropical transition along the Pacific versant

Phylogenetic results support a final division between the western Mexico clade and the Sonoran + Chihuahuan Desert clade to the north, estimated to have occurred approximately 5.4 Ma (Figs 3 and 4; Table 2). These lineages average 5.6% sequence divergence across a narrow region (~70 km) bounding the Sonora–Sinaloa border. This genetic discontinuity coincides with the Río Fuerte and Sierra Barabampo of northernmost Sinaloa, which may provide effective barriers to dispersal. A

similar break has been demonstrated in arid-adapted rodents across the same region (Hafner & Riddle 2005). Divergent ecological selection may also play a role, given that the environmental transition from the Nearctic to the Neotropical realms along the Pacific versant of Mexico occurs across the same general region (Udvardy 1975; Olson *et al*. 2001). While distinguishing between primary and secondary intergradation is difficult at best (Endler 1977), studies of widespread, geographically variable lineages such as *T. biscutatus* that span the gradient from tropical to temperate environs in Mexico offer the potential for investigating presumptive cases of parapatric ecological speciation, and underscore the need for additional phylogenetic studies of codistributed taxa.

The Chihuahuan–Sonoran desert filter barrier

An unexpected result obtained from phylogenetic analyses is the absence of phylogeographical structure between morphologically distinct lineages (see LaDuc & Johnson 2003) of the *T. biscutatus* group inhabiting the Sonoran and Chihuahuan deserts. In contrast, a number of other widespread reptiles, birds, and mammals show marked genetic discontinuities between Sonoran and Chihuahuan desert populations (Orange *et al*. 1999; Omland *et al*. 2000; Riddle *et al*. 2000a, b, c; Wilgenbusch & de Queiroz 2000; Zink *et al*. 2000; 2001; Zink 2002; Hafner & Riddle 2005). Given the history of recent (Pleistocene-Holocene) climatic shifts that have periodically separated and reconnected the Sonoran and Chihuahuan deserts, recent secondary contact and dispersal may have masked the genetic architecture of earlier vicariance across the Sierra Madre Occidental.

The beginning of the Pleistocene (*c.* 1.8 Ma) marked a sharp transition from warmer tropical conditions to a cooler, more continental climate. During glacial periods, cooler, wetter conditions allowed the expansion of woodland trees and shrubs into areas formerly occupied by desert, restricting arid regions to lowland basins below 300 m a.s.l. at more southerly latitudes, and forming pluvial lakes in lowland areas (Van Devender & Spaulding 1979; Van Devender 1990a, b; Van Devender *et al*. 1994; Holmgren *et al*. 2003). Continuing climate change resulted in a shift from Late Wisconsin pinyon-juniper-oak woodland to early Holocene oak-juniper woodland to middle Holocene desert grassland, and finally, late Holocene Chihuahuan desertscrub (Van Devender 1995). During warm interglacial periods, such as the present one marking the beginning of the Holocene 11 000 BP, isolated desert refugia began to expand. The expansion of the Sonoran and Chihuahuan deserts into their current ranges occurred approximately 9000 BP during the period of maximum warmth since the Last Glacial Maximum (Van Devender 1990a, b, 2000; Holmgren *et al*. 2003). Modern vegetation, climate, and species composition of the Chihuahuan and Sonoran deserts was established by 4000 BP (Metcalfe *et al*. 2000; Van Devender 2000).

The woodlands that dominated during the most recent Wisconsin glacial maximum (10 000–20 000 BP) and for much of the Pleistocene (Van Devender 1995) likely proved effective barriers to dispersal for arid-adapted lineages. Remnants of middle Holocene desert grassland exist today as an ecotone between the Chihuahuan and Sonoran deserts in southeastern Arizona, southwestern New Mexico, and adjacent Mexico. This transitional region is characterized by a broad (200 km wide) 1500-m high plain spanning the continental divide and forming a gap between the Rocky Mountains to the north and the Sierra Madre Occidental to the south, and with several high, discontinuous, north–south mountain ranges (the Chiricahuas, Huachucas, and Peloncillos; Morafka 1977; Van Devender 1995). The area functions as a two-way, east–west filter between arid-adapted species inhabiting the Chihuahuan and Sonoran deserts, as well as between woodland species inhabiting the forests of the Rocky Mountains to the north and the Sierra Madre Occidental to the south (Axtell 1977; Morafka 1977; Hafner & Riddle 2005; Lomolino *et al*. 2005).

Remington's (1968) suture zones revisited

In his seminal paper describing 'suture–zones of hybrid interaction between recently joined biotas', Remington (1968) identified six major (and several minor) areas in North America where hybrid zones in plants, mammals, reptiles, amphibians, birds, and insects appeared to be concentrated. Recently, there has been renewed interest in Remington's work by researchers interested in understanding population divergence and speciation (Coyne & Orr 2004; Hewitt 2000, 2001, 2004; Swenson & Howard 2004, 2005). In *T. biscutatus*, there appears to be strong agreement between putative zones of secondary contact and areas identified by Remington as suture zones. For example, Remington proposed the TMVB region as one potential suture zone where distinct southern and northern biota may meet and hybridize, based in part on the work of Sibley (Sibley 1950, 1954) who documented hybridization between species of towhee (*Pipilo*) across the region. Previous workers suspected two distinct species of the *T. biscutatus* group would be found to occur in sympatry near the western edge of the TMVB in southwestern Jalisco (McDiarmid & Scott 1970). Results presented herein confirm this prediction, and provide compelling evidence that secondary contact has occurred. These morphologically distinct lineages are characterized by ~9–10% uncorrected pairwise sequence divergence (Table 1) and are narrowly sympatric (personal observation; Fig. 4). Morphological intermediates have not been reported, suggesting complete reproductive isolation.

A second suture zone in Mexico identified by Remington (1968) occurs in northern Sinaloa, where distinct mitochondrial lineages of *T. biscutatus* (the western Mexico and Sonoran + Chihuahuan Desert clades) occur in parapatry. This region corresponds generally to the environmental gradient between tropical deciduous forest/foothills thornscrub to Sonoran Desert (the 'Sonoran-Sinaloan transition subtropical dry forest' *sensu* Olson *et al*. 2001), where many Neotropical species reach the northern limit of their distribution along the Pacific versant of Mexico. Populations representing these distinct clades may hybridize in northern Sinaloa and southern Sonora, but phylogenetic results indicate substantial historical isolation across the Río Fuerte and Sierra Barabampo near the Sonora-Sinaloa border. Ecological differences exist between these mitochondrial lineages as well: Sonoran Desert populations are found in rocky areas of relief up to 2400 m a.s.l. in altitude, while populations from south of Sonora inhabit semi-arid tropical deciduous or thornscrub forest where they are restricted to altitudes below 1200 m a.s.l. (Scott & McDiarmid 1984a).

In the United States, one of the suture-zones described by Remington (1968) as a 'minor or little-known' zone lies in the Salton Trough region near the head of the Gulf of California (the 'California Desert-Pacific Slope Suture-Zone'). An abrupt environmental transition from mesic coastal vegetation and climate to xeric Sonoran Desert conditions occurs in this area, and appears to be responsible, in part, for maintaining a hybrid zone between species of quail (*Callipepla*; Gee 2004). Two morphologically distinct lineages of *T. biscutatus* occur across the same ecotone in the Salton Trough region. The recession of the proto-gulf of California to its current formation during the Pliocene may have allowed these formerly allopatric peninsular and continental lineages to experience secondary contact in southeastern California and southern Nevada, although hybrids have not been reported.

A final area Remington identified as a suture zone where morphologically distinct forms of *Trimorphodon* meet and hybridize is located in the borderlands region of southwestern New Mexico, southeastern Arizona, and northern Sonora (the 'Southwestern New Mexico Suture-Zone'). Remington described this area as a suture zone between distinct Sonoran and Chihuahuan desert biota based largely on work detailing hybridization between Sonoran and Chihuahuan forms of the western whiptail lizard *Aspidocelis* (*Cnemidophorus*) *tigris* (Dessauer *et al*. 1962; Zweifel 1962; Wright & Lowe 1967, 1968). Dessauer *et al*. (2000) concluded that narrow hybrid zones in extreme southwestern New Mexico between *Aspidoscelis tigris punctilinealis* of the Sonoran Desert and *A. t. marmoratus* of the Chihuahuan Desert resulted from secondary contact during the most recent connection between the Sonoran and Chihuahuan deserts approximately 4500 BP. Geographic-

ally concordant hybrid zones have subsequently been described between the Mexican and Plains spadefoot toads (*Spea multiplicata* and *S. bombifrons*, respectively) in the nearby San Simon Valley of southeastern Arizona (Pfennig & Simovich 2002; Pfennig 2003), further bolstering support for Remington's predictions. The concordance of putative zones of secondary contact among *T. biscutatus* lineages with areas Remington predicted would contain hybrid zones suggests that these suture zones remain potentially interesting arenas for evolutionary studies.

Evaluating the gene tree against subspecies limits

An overlay of currently recognized taxa on the phylogeny reveals limited congruence between phylogenetic results and the boundaries of currently recognized subspecies (Fig. 5). A variety of causes may be responsible for the discordance, including inaccurate taxonomy, interspecific hybridization, and/or incomplete lineage sorting (reviewed in Funk & Omland 2003). In the case of *Trimorphodon biscutatus quadruplex*, imperfect 'subspecies' limits appear to be responsible (Fig. 5). Previous workers concluded that the break between *Trimorphodon biscutatus biscutatus* and *T. b. quadruplex* occurred at the Isthmus of Tehuantepec (Fig. 2; Gehlbach 1971). Phylogenetic analyses indicate a genetic discontinuity farther south across the Guatemalan highlands, grouping populations from western Guatemala (representing *T. b. quadruplex* as currently recognized; Fig. 5) with populations from southern Mexico (representing *T. b. biscutatus*). Morphological differences mirror this genetic pattern; individuals from Guatemala west of the highlands lack the '*quadruplex*' colour pattern characteristic of populations from eastern Guatemala and the remainder of Central America, instead resembling populations inhabiting Mexico south of the TMVB (Devitt 2003).

In turn, '*T. b. biscutatus*' is comprised of two morphologically distinct, nonsister lineages (the southern and western Mexico clades) that are narrowly sympatric in southwestern Jalisco (personal observation; Fig. 4), confirming previous workers' suspicions that two species of the *T. biscutatus* group would be found to occur in sympatry in that area (McDiarmid & Scott 1970). Although not currently recognized, populations representing the western Mexico clade were formerly considered a distinct species, *Trimorphodon paucimaculatus*, based on morphological differences (Taylor 1938). *T. paucimaculatus* was relegated to subspecific status (Fugler & Dixon 1961) and later subsumed under *T. b. biscutatus* (Gehlbach 1971) based on hypothesized intergradation with Sonoran Desert *Trimorphodon biscutatus lambda* populations in northern Sinaloa and southern Sonora (Fugler & Dixon 1961; Fouquette & Rossman 1963). Genetic evidence, however, indicates substantial historical isolation between populations from
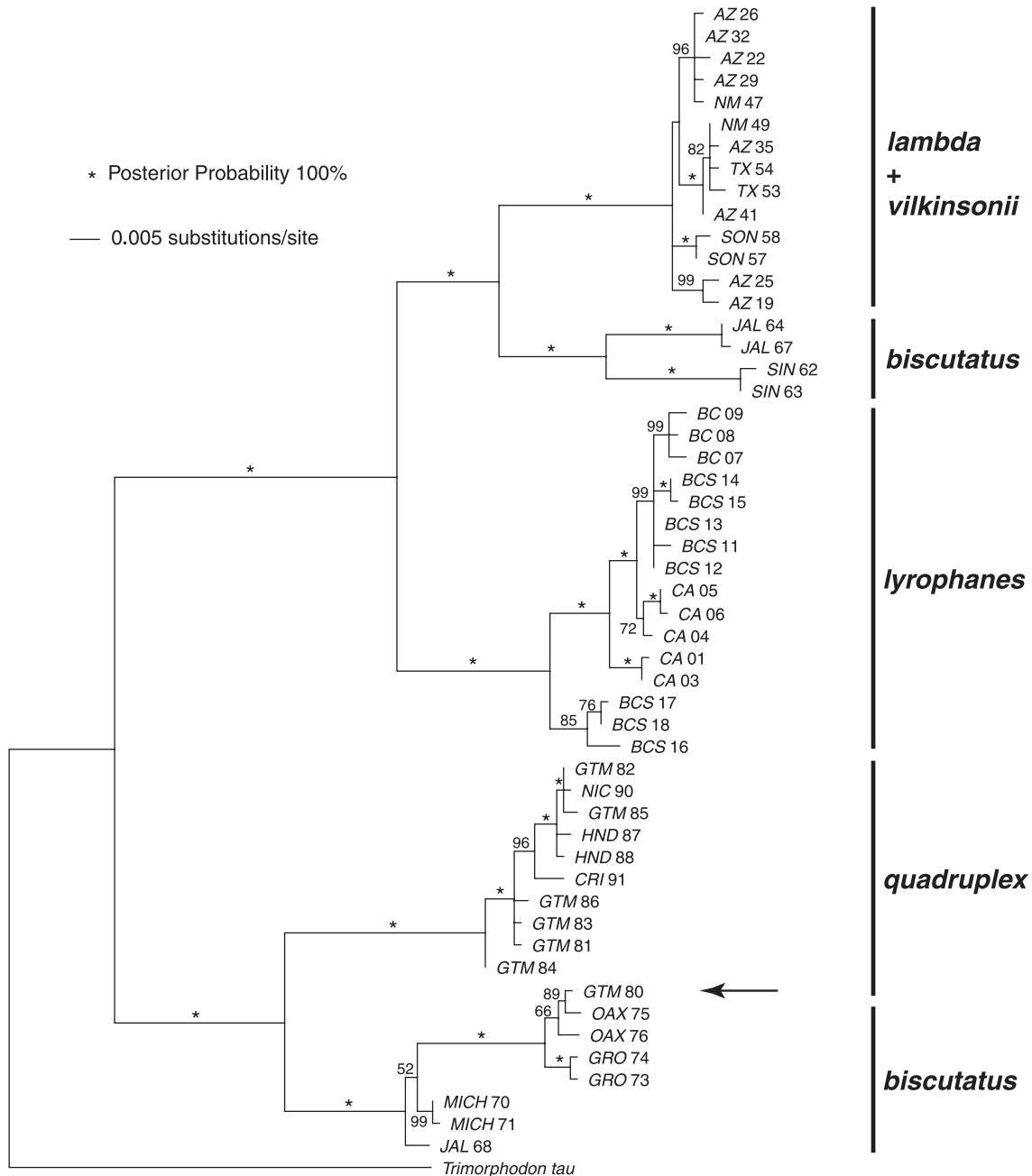
Fig. 5 Subspecies designations based on geographical location (after Scott & McDiarmid 1984a) superimposed on the phylogeny illustrating discordance between current taxonomy and mitochondrial lineages recovered in phylogenetic analysis. Arrow indicates paraphyly of *Trimorphodon biscutatus quadruplex* based on currently defined subspecies boundaries.

southern Sonora and those from northern Sinaloa (Fig. 4).

In the case of *T.b. lambda* and *Trimorphodon vilkinsonii*, the morphological differences demonstrated by LaDuc & Johnson (2003) provide a nuclear background against which the mtDNA gene genealogy may be evaluated (Funk & Omland 2003). Although distinguishing between the effects of incomplete lineage sorting and introgression is difficult from gene genealogies alone (Nielsen &

Wakeley 2001), a pattern of geographically localized, interspecifically shared haplotypes is not expected under incomplete lineage sorting as it is under local introgression (Hare & Avise 1998; Masta *et al.* 2002; Funk & Omland 2003). Sharing of geographically localized haplotypes between morphologically distinct species is usually considered a clear signature of introgression (Funk & Omland 2003). Mitochondrial introgression is not uncommon, and may occur despite very low levels of hybridization

(Takahata & Slatkin 1984; Ballard & Whitlock 2004). Because mtDNA exhibits low levels of linkage with nuclear DNA, mitochondrial alleles are less constrained by indirect selection and may introgress further and more readily than nuclear genes that are linked to other selected loci (Barton & Jones 1983; Harrison 1990; Arnold 1993; Funk & Omland 2003; Ballard & Whitlock 2004). Alternatively, it is possible that the species-level paraphyly exhibited by these morphologically distinct taxa is due to incomplete lineage sorting. Given its restricted geographical range, *T. vilkinsonii* may be a recently derived peripheral isolate that budded off from ancestral populations in the Sonoran Desert and colonized the northwestern margin of the Chihuahuan Desert during Quaternary climatic oscillations and has had insufficient time to sort to monophyly. In cases of peripatric speciation, recognizing the nested and parental lineages as distinct, or conversely, recognizing them as the same, both mask the distinct process of peripheral isolation (de Queiroz & Donoghue 1988; Wiens & Penkrot 2002; Funk & Omland 2003). Regardless of the process that resulted in the differentiation of these two taxa, morphological data indicate that recent gene flow between these taxa has occurred across the desert grassland ecotone between the Chihuahuan and Sonoran deserts in southeastern Arizona and southwestern New Mexico (Gehlbach 1971; LaDuc & Johnson 2003). Hybrids appear to be uncommon and are restricted to a narrow region of extreme southeastern Arizona and southwestern New Mexico (Gehlbach 1971; LaDuc & Johnson 2003), suggesting that these lineages are maintaining their own separate identities despite some recurrent gene flow.

Conclusions, conservation, and taxonomic implications

It seems clear that species-level diversity is currently underestimated within this group (formal taxonomic recommendations will appear separately in an analysis of morphological geographical variation; Devitt & LaDuc submitted). This result is consistent with other phylogeographical investigations of arid-adapted species inhabiting the deserts and subhumid regions of North America that have revealed substantial cryptic diversity and an older, more complex evolutionary history than originally envisaged by most workers. The majority of these studies have focused on the warm, regional deserts of the southwestern United States and northern Mexico, with relatively fewer works including taxa inhabiting the seasonally dry, tropical deciduous forests of Mexico and Central America. Despite an exceptionally rich assemblage of flora and fauna, Neotropical dry forest is often considered the least known of tropical ecosystems as well as the most endangered, due to exceptionally high rates of deforestation and a general lack of conservation areas (Flores-Villela & Geréz 1988; Janzen 1988; Gentry 1995;

Maass 1995; Mooney *et al*. 1995; Martínez-Yrízar *et al*. 2000; Pennington *et al*. 2000; Trejo & Dirzo 2002; Cantú *et al*. 2004). North America's aridlands are disappearing at an alarming rate due to agricultural conversion and other adverse human impacts (Peterson *et al*. 2002; Hafner & Riddle 2005; Riddle & Hafner 2006), and thus, identifying cryptic diversity and regional areas of endemism has significant implications for biodiversity conservation.

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Tom Devitt is interested in ecology, evolution, and conservation. His research focuses on investigating the origin and maintenance of geographic variation among populations to understand evolutionary processes such as local adaptation, population divergence, and speciation. This work represents part of his Master's research conducted at Louisiana State University under the supervision of Jimmy A. McGuire. Tom is currently a Ph.D. candidate at the University of California, Berkeley where he is studying isolating barriers that prevent gene flow between sympatric members of the *Ensatina eschscholtzii* complex.

Appendix I

Locality information for samples used in phylogenetic analyses. Standard abbreviations for museums follow Leviton *et al.* (1985). Personal field series and other museum abbreviations are as follows: AEH: Alan E. Hebbert; CLS: Carol L. Spencer; DGM: Dan G. Mulcahy; ENEPI: Escuela Nacional de Estudios Profesionales Iztacala, ENS: Eric N. Smith, JAC: Jonathan A. Campbell; JMM: Jesse M. Meik; JRO: John R. Ottley; MSM: Mahmood S. Marin; RWM: Robert W. Murphy; TJD: Thomas J. Devitt, TJL: Travis J. LaDuc, TWR: Tod W. Reeder

Sample number	Taxon	Locality	Specimen ID	GenBank Accession no.
1	*T. b. lyrophanes*	USA: California: Inyo County, Pass over Argus Mountains on Trona Road, 29.4 mi S jct. Hwy. 190	JMM 79	DQ497506
2	*T. b. lyrophanes*	USA: California: Inyo County, Trona-Wildrose Rd. crossing Slate Range N of Trona	TJD 085	DQ497516
3	*T. b. lyrophanes*	USA: California: San Bernardino County, Joshua Tree National Park	4/24/00	DQ497509
4	*T. b. lyrophanes*	USA: California: San Diego County, Hwy. 78, Sentenac Canyon	5/6/99	DQ497511
5	*T. b. lyrophanes*	USA: California: San Diego County, 1.0 mi SW of Borrego Springs, on S22 Montezuma Valley Rd	TJD 088	DQ497512
6	*T. b. lyrophanes*	USA: California: San Diego County, off Hwy. 94 on Barret Lake Rd.	SDSU 4102	DQ497513
7	*T. b. lyrophanes*	Mexico: Baja California: El Rosario	ROM 14484	DQ497517
8	*T. b. lyrophanes*	Mexico: Baja California: El Rosario	JRO 266	DQ497505
9	*T. b. lyrophanes*	Mexico: Baja California: 15.0 mi (by road) W of Bahia de los Angeles	BYU 34638	DQ497504
10	*T. b. lyrophanes*	Mexico: Baja California Sur: Hwy. 1, 1.5 km E San Ignacio	JRO 531	DQ497501
11	*T. b. lyrophanes*	Mexico: Baja California Sur: Hwy. 1, 4 km E San Ignacio	JRO 536	DQ497507
12	*T. b. lyrophanes*	Mexico: Baja California Sur: 3.1 mi N (by Mex. Hwy. 1) San Ignacio	MVZ 233299	DQ497508
13	*T. b. lyrophanes*	Mexico: Baja California Sur: Hwy. 1, 5.4 mi W San Ignacio	13580	DQ497500
14	*T. b. lyrophanes*	Mexico: Baja California Sur: 5 mi NW Santa Rosalia	ROM 14482	DQ497502
15	*T. b. lyrophanes*	Mexico: Baja California Sur: Isla San Marcos	RWM 1920	DQ497503
16	*T. b. lyrophanes*	Mexico: Baja California Sur: Hwy. 1, 29.2 mi W Ciudad Constitution	13608	DQ497510
17	*T. b. lyrophanes*	Mexico: Baja California Sur: 0.5 mi S El Triunfo	ROM 34073	DQ497514
18	*T. b. lyrophanes*	Mexico: Baja California Sur: 3 mi from San Dionysus	ROM 34074	DQ497515
19	*T. b. lambda*	USA: Arizona: Mohave County, Black Mtns. 4.5 mi E of Oatman Road/US Route 66 jct, on Route 66	UTA-R 50702	DQ497493
20	*T. b. lambda*	USA: Arizona: Yavapai County, 13 mi SE Bagdad on Hwy. 96	MVZ 232829	DQ497485
21	*T. b. lambda*	USA: Arizona: Yavapai County, 8 mi SE Bagdad on Hwy. 96	MVZ 232830	DQ497486
22	*T. b. lambda*	USA: Arizona: Maricopa County, 0.5 mi (rd) W Eagle Eye Rd on Hwy. 60	UAZ 54127	DQ497460
23	*T. b. lambda*	USA: Arizona: La Paz County, Harcuvar Mountains, Alamo Dam Rd., 10 rd. mi N Wenden, Cunningham Pass	UTEP 15989	DQ497481
24	*T. b. lambda*	USA: Arizona: Maricopa County, 9 mi North of I-10 on Wickenburg-Hassayampa Rd. at Coyote Wash	TJD 084	DQ497482
25	*T. b. lambda*	USA: Arizona: Yuma County, Old Hwy. 80, 0.4 mi w of Mohawk Exit (No. 54)	DGM 847	DQ497462
26	*T. b. lambda*	USA: Arizona: Maricopa County, Hwy. 85 N of Ajo	JMM 101	DQ497451
27	*T. b. lambda*	USA: Arizona: Gila County, AZ 288, 3.0 mi N jct. with AZ 88	LACM 131374	DQ497478
28	*T. b. lambda*	USA: Arizona: Gila County, 13.9 mi NW (by Hwy. 88), Claypool	MVZ 232612	DQ497479
29	*T. b. lambda*	USA: Arizona: Graham County, Hwy. 266, 2.7 mi W of Hwy. 191, Pinaleno Mountains	UTA-R 52925	DQ497480

Appendix I *Continued*

Sample number	Taxon	Locality	Specimen ID	GenBank Accession no.
30	*T. b. lambda*	USA: Arizona: Pima County, Tucson Mountains	TJL 770	DQ497483
31	*T. b. lambda*	USA: Arizona: Santa Cruz County, Santa Rita Mountains, Mount Hopkins, 0.3 mi N of Whipple Observatory picnic area	UAZ 54125	DQ497461
32	*T. b. lambda*	USA: Arizona: Santa Cruz County, AZ Hwy. 82 at 0.7 mi (rd) S Patagonia Lake State Park entrance	UAZ 54128	DQ497459
33	*T. b. lambda*	USA: Arizona: Santa Cruz County, jct of E Three R Rd. and Hwy. 82, Patagonia Mountains	DGM 767	DQ497484
34	*T. b. lambda*	USA: Arizona: Cochise County, 16.5 mi east of Douglas (AZ 80 east and north intersection)	LSUMZ 83695	DQ497465
35	*T. b. lambda*	USA: Arizona: Cochise County, Mule Mountains, Hwy. 80, 3.2 mi E of AZ 92	UAZ 53614	DQ497468
36	*T. b. lambda*	USA: Arizona: Cochise County, Mule Mountains, Hwy. 90, 0.6 mi W of AZ 80	UAZ 53615	DQ497470
37	*T. b. lambda*	USA: Arizona: Cochise County, Mule Mountains, 3.0 mi E of jct. AZ 80/AZ 92	UAZ 53616	DQ497471
38	*T. b. lambda*	USA: Arizona: Cochise County, Mule Mountains, Hwy. 80, 4.0 mi E of jct. AZ 80/AZ 92	UAZ 53617	DQ497469
39	*T. b. lambda*	USA: Arizona: Cochise County, Hwy. 80, ~8 mi N of Douglas, Perilla Mountains	LSUMZ 84797	DQ497466
40	*T. b. lambda*	USA: Arizona: Cochise County, Hwy. 80, 27.1 mi S of Price Canyon Road	CLS 430	DQ497463
41	*T. b. lambda*	USA: Arizona: Cochise County, Hwy. 80, 1.0 mi N Hwy. 90 (14.5 mi S of Tombstone)	DGM 774	DQ497456
42	*T. b. lambda*	USA: Arizona: Cochise County, Chiricahua Mountains, Cave Creek Rd., 0.9 mi E of Southwestern Research Station	LSUMZ 83684	DQ497467
43	*T. b. lambda*	USA: Arizona: Cochise County, Chiricahua Mountains, Cave Creek Rd., 1.2 mi E of Southwestern Research Station	UTEP 11409	DQ497472
44	*T. b. lambda*	USA: Arizona: Cochise County, Chiricahua Mountains, Cave Creek Rd., 2.7 mi E of Southwestern Research Station	LSUMZ 83685	DQ497464
45	*T. b. lambda*	USA: Arizona: Cochise County, Portal Rd., 1 mi E Portal	MVZ 229993	DQ497457
46	*T. b. lambda*	USA: Arizona: Cochise County, Chiricahua Mts, Portal Rd at 1.1 mi (rd.) N Herb Martyr Rd	UAZ 54126	DQ497458
47	*T. vilkinsonii*	USA: New Mexico: Grant County, off Tyrone-Redrock Rd.	TJD 086	DQ497491
48	*T. vilkinsonii*	USA: New Mexico: Sierra County, NM 51, 2.7 rd. mi ESE Rio Grande bridge in Truth or Consequences	UTEP 15597	DQ497473
49	*T. vilkinsonii*	USA: New Mexico: Doña Ana County, Organ Mountains	CL02	DQ497452
50	*T. vilkinsonii*	USA: New Mexico: Doña Ana County, Organ Mountains	CL03	DQ497453
51	*T. vilkinsonii*	USA: New Mexico: Doña Ana County, Doña Ana Mountains	CL04	DQ497455
52	*T. vilkinsonii*	USA: Texas: El Paso County, Franklin Mountains	TJL 882	DQ497477
53	*T. vilkinsonii*	USA: Texas: Presidio County, Chinati Mountains	TJL 338	DQ497492
54	*T. vilkinsonii*	USA: Texas: Brewster County, about 30 mi S of Alpine	TJL 883	DQ497474
55	*T. vilkinsonii*	USA: Texas: Presidio County, TX 170, 11.5 mi NW Lajitas	UTEP 10762	DQ497475
56	*T. vilkinsonii*	USA: Texas: Brewster County, TX 118, 8 mi N jct. with TX 170, foot South Luna Vista Hill	UTEP 12146	DQ497476
57	*T. b. lambda*	Mexico: Sonora: 7.8 mi NE of Ures on Hwy. 14	TWR 1342	DQ497490
58	*T. b. lambda*	Mexico: Sonora: Hwy. between Hornos and Obregon	JAC 24856	DQ497454
59	*T. b. lambda*	Mexico: Sonora: Hwy. 1, 18 km ENE Navojoa	JRO 683	DQ497487
60	*T. b. lambda*	Mexico: Sonora: Alamos, Rio Cuchijaqui	ROM 14897	DQ497488
61	*T. b. lambda*	Mexico: Sonora: Alamos, Rio Cuchijaqui	ROM 14945	DQ497489
62	*T. b. biscutatus*	Mexico: Sinaloa: Carretera El Fuerte-Choix (MEX 32), 8.3 mi (rd.) N El Fuerte	UTA-R 52929	DQ497498

Appendix I *Continued*

Sample number	Taxon	Locality	Specimen ID	GenBank Accession no.
63	*T. b. biscutatus*	Mexico: Sinaloa: playa N of Mazatlan on road to Alamos	ROM 14959	DQ497499
64	*T. b. biscutatus*	Mexico: Jalisco: Municipio La Huerta, Arroyo Colorado, 6 km NE Estación de Biología, Reserva Chamela-Cuixmala	UTA-R 52654	DQ497494
65	*T. b. biscutatus*	Mexico: Jalisco: Carretera Melaque-Autlán (MEX 80)	UTA-R 52918	DQ497497
66	*T. b. biscutatus*	Mexico: Jalisco: Carretera Melaque-Autlán (MEX 80)	JAC 23893	DQ497495
67	*T. b. biscutatus*	Mexico: Jalisco: Carretera Melaque-Autlán (MEX 80)	UTA-R 52919	DQ497496
68	*T. b. biscutatus*	Mexico: Jalisco: Carretera Melaque-Autlán (MEX 80)	UTA-R 52932	DQ497523
69	*T. b. biscutatus*	Mexico: Jalisco: Carretera Melaque-Autlán (MEX 80)	UTA-R 52933	DQ497524
70	*T. b. biscutatus*	Mexico: Michoacan: Carretera Coalcoman-Apatzingan	JAC 24050	DQ497519
71	*T. b. biscutatus*	Mexico: Michoacan: Carretera Tapalcatepec-Apatzingan	UTA-R 52927	DQ497522
72	*T. b. biscutatus*	Mexico: Michoacan: Carretera Tapalcatepec-Apatzingan	UTA-R 52928	DQ497520
73	*T. b. biscutatus*	Mexico: Michoacan: Rio Chilta Bridge	LSUMZ 39531	DQ497529
74	*T. b. biscutatus*	Mexico: Guerrero: Carretera Zihuatanejo-Acapulco	UTA-R 52926	DQ497521
75	*T. b. biscutatus*	Mexico: Oaxaca: Carretera El Camarón, San Carlos Yautepec	ENEPI 6749	DQ497528
76	*T. b. biscutatus*	Mexico: Oaxaca: Carretera Huatulco-Salina Cruz, 41 m	JAC 24309	DQ497525
77	*T. b. biscutatus*	Mexico: Oaxaca: Carretera Huatulco-Salina Cruz, 103 m	UTA-R 52930	DQ497526
78	*T. b. biscutatus*	Mexico: Oaxaca: 2.3 mi S Tehuantepec on road to Salinas Cruz	MVZ 143524	DQ497527
79	*T. b. biscutatus*	Mexico: Oaxaca: 13.7 mi N on Hwy. 185 from junction with Hwy. 190	MVZ 164979	DQ497530
80	*T. b. quadruplex*	Guatemala: Huehuetenango: 14.5 km S Nenton	UTA-R 42306	DQ497518
81	*T. b. quadruplex*	Guatemala: Zacapa	ENS 10800	DQ497541
82	*T. b. quadruplex*	Guatemala: Zacapa: Cabañas, Aldea El Arenal, *c.* 1.0 km NNE from El Arenal Quebrada Seca	UTA-R 42222	DQ497531
83	*T. b. quadruplex*	Guatemala: Zacapa: Cabañas, Aldea El Arenal, *c.* 1.0 km NNE from El Arenal Quebrada Seca	UTA-R 42221	DQ497536
84	*T. b. quadruplex*	Guatemala: El Progreso: Carretera Cobán	UTA-R 42299	DQ497539
85	*T. b. quadruplex*	Guatemala: Escuintla: Guanagazapa	ENS 10798	DQ497540
86	*T. b. quadruplex*	Gutaemala: Santa Rosa: Carretera Escuintla-Taxisco	UTA-R 44717	DQ497535
87	*T. b. quadruplex*	Honduras: Comayagua: Aldea Las Mesas	MSM 450	DQ497533
88	*T. b. quadruplex*	Honduras: Comayagua: Aldea Las Mesas	UTA-R 41247	DQ497534
89	*T. b. quadruplex*	Nicaragua: Isla de Ometepe, San Ramon, Volcán Madera	AEH 026	DQ497538
90	*T. b. quadruplex*	Nicaragua: Isla de Ometepe, San Ramon, Volcán Madera	AEH 021	DQ497532
91	*T. b. quadruplex*	Costa Rica	MVZ 164854	DQ497537
outgroup	*T. tau*	Mexico: Guerrero: Sierra Madre del Sur, Carretera Milpillas, Filo de Caballo	ENEPI 6378	DQ497542

Appendix II

The GTR + I + Γ model parameters estimated from maximum likelihood analysis. The G↔T transformation rate is fixed at 1

Log likelihood of best tree: −2994.37430.
Base frequencies: A: 0.366711, C: 0.286555, G: 0.109954, T: 0.236781.
Rate matrix R: AC: 2.46304, AG: 41.26953, AT: 2.55929, CG: 1.0946e-12, CT: 21.69317, GT: 1.00000.
Proportion of invariant sites: 0.618140.
Shape parameter for gamma distribution: 1.506596.