EFFECTS OF A PLEISTOCENE BARRIER ON HERPETOFAUNAL DISTRIBUTIONS IN SOUTHWESTERN NORTH AMERICA

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ABSTRACT

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Lake Cabeza de Vaca existed in the northern portion of the Chihuahuan Desert during the Pleistocene, possibly limiting taxon ranges and driving diversification of taxa by sundering once continuous populations occurring across this region. I reevaluate phylogenetic studies in light of evidence of Cabeza de Vaca and present molecular data that addresses the biological influence of the lake. Supplementing our reevaluations of previous studies, we identified taxa likely to show genetic differentiation consequent of the lake and tested them. Specimens from the colubrid snake *Rhinocheilus lecontei* and the phrynosomatid lizard taxa *Phrynosoma cornutum* and *Urosaurus ornatus* were collected, followed by DNA sequencing of a mitochondrial gene region that included Nicotinamide Adenine Dinucleotide subunit 4 (ND4), and the tRNAs Serine, Histidine, and Leucine. Phylogenetic analyses using maximum likelihood and Bayesian methods were used to construct evolutionary relationships between individuals of each taxo

based on the obtained nucleotide sequences. In order to determine if correlations existed between sequence data variation and geography, haplotype networks were constructed and nested clade analysis was performed on *P. cornutum* using a combined data set that included other available sequence of the ND4 gene region. Deep molecular divergences were found within each taxon from specimens collected across the northern Chihuahuan Desert. Application of a molecular clock using three calibration rates suggests that the divergences predate the age of the lake. Orogeny of the southern extension of the Rocky Mountains in the Miocene and Pliocene likely produced the molecular signatures recovered in each taxon. These findings corroborate a more widely known hypothesis invoked to account for the split of the Sonoran and Chihuahuan Desert faunas.

INTRODUCTION

Fluctuations in global climate during the Pleistocene epoch produced temporary barriers within and between the geographic ranges of species and populations (Brown and Lomolino 1998). Because of the recency of these events, their effects on current distributions and gene pools is thought to have been immense, particularly in the northern hemisphere (Webb and Bartlein 1992, Hewitt 2000). The geographic distribution of many extant North American taxa support geologic evidence of recent glacial and lacustrine barriers (Davis 1986). The phylogenetic consequences of these vicariant events continue to be investigated with the use of molecular systematic analyses (e.g., Kidd and Friesen 1998).

Cabeza de Vaca was a Pleistocene pluvial lake situated in the northern Chihuahuan desert and was the ancestor to the modern channel of the Rio Grande River. The range boundaries of several vertebrate sister taxa appear to coincide with the location of the ancient lake. Molecular phylogenies produced by two recent biogeographic studies demonstrate marked divergences between pairs of mammalian taxa with ranges that coincide with the eastern and western boundaries of Cabeza de Vaca (Lee et al. 1996, Walpole et al. 1997). These findings may support the hypothesis that ancient Cabeza de Vaca was a major regional force shaping vertebrate differentiation by restricting gene flow.

The current study tested the prediction that Cabeza de Vaca was a source of vicariance for vertebrate taxa inhabiting the Chihuahuan Desert during the Pleistocene. Phylogenetic relationships of specimens from four reptilian taxa (two lizards and two snakes) that occur across this desert region were assessed using mitochondrial DNA (mtDNA) characters. Results suggest that the prehistoric lake may have been an important force shaping the diversification of biota in this desert region. The influences of other local biogeographic forces, as well as previous biogeographic hypotheses, are evaluated given the current findings. Taxonomic and systematic consequences of the results are discussed, as well as problems encountered that could affect the interpretation of the data.

Pleistocene Environment. The Pleistocene epoch began nearly two million years before present (b.p.) and was marked by rapid and severe oscillations in climate. Fluctuations in temperature have been determined by measuring stratigraphic variation of oxygen isotopes in ice and seabed cores; the cyclical nature of these temperature fluctuations has been correlated to periodic changes in the earth's orbit, tilt, and orientation by comparing the timing of these planetary fluctuations to measurements of decay time of radioactive isotopes also recovered from ice and seabed cores (Gates 1993). This period of climatic reversals produced a series of glacial-interglacial cycles in the northern hemisphere. Glacial deposits (tillites) and geomorphology of subglacial strata indicate the size, location, and direction of glacial movement (Brown and Lomolino 1998). The formation of huge lakes due to accumulated precipitation during periods of lower temperature and increased rainfall occurred in several regions of the southwestern United States (MacDonald 2003). Evidence of these pluvial lakes comes primarily from

sedimentary profiles of basin and range topographies, and examination of ancient shorelines. Measured depths of accumulated lacustrine-derived sedimentation provide estimates of their age and duration (Flint 1957, Gustavson 1991).

The responses of North American biota to these changes in climate and geography were significant and variable. The distribution of habitats shifted in latitude and elevation concordant with climate change, with most species following a general southward trend. Species from higher latitudes and elevations were pushed southward and to lower elevations during periods of glacial maxima, with some species and communities regressing northward and to higher elevations following the retreat of colder temperatures (Graham 1986). This movement of species and communities during and since the Pleistocene has been determined primarily by palynological and paleolimnological evidence (Gates 1993), as well as from analysis of macrofossils, such as those found in packrat middens (Betancourt et al. 1990, Van Devender 1986) and caves (Elias 1999). Species that were unable to follow the spatial movement of prime habitats were forced to adapt to new biomes or faced extinction (Brown and Lomolino 1998).

The ranges of many species were sundered by glacial and climatic barriers during the Pleistocene, and the ranges of some extant species may still represent the outcomes from an influence. Several pairs of extant North American avian taxa appear to be the result of such barriers, in which the ancestral species were pushed into eastern and western glacial refugia and subsequently differentiated due to loss of gene flow (Avise and Walker 1998, but see Klicka and Zink 1997 *cf.* Johnson and Cicero 2004). Similarly, refugia in southern Texas and peninsular Florida are thought to be associated with the

distributions of a number of pairs of sister taxa (Avise 2000). The "sky islands" of southern Arizona and New Mexico represent small-scale refugia that are mountaintop remnants of forested areas; the desert "seas" that now separate them have been adequate barriers to gene flow, as indicated by divergent populations on disjunct mountain ranges (e.g., Masta 2000). A large literature now exists that provides strong molecular evidence for the influence of Pleistocene and late Pliocene climates in structuring the current assemblages and relationships of biota across the landscape.

Cabeza de Vaca. Another potential Pleistocene barrier, but one relatively unknown, existed in the southwestern United States and northern Mexico. Geological activity during the early Cenozoic that included volcanic activity, block faulting, and tectonic uplifting occurred at the convergence of the present borders of Texas, New Mexico, and Mexico, creating a topography typical of the Basin and Range physiographic province in which this area lies (Strain 1970, Morrison 1991). Bolsons (flat bottomed depressions surrounded by mountains) in this area are characterized by lacustrine and erosional sediments (Gustavson 1991). In the early Quaternary (2 million years b.p.), the Rio Grande River flowed more directly southward compared to its current course (Kottlowski 1958), and filled this series of bolsons during cycles of heavy precipitation (Seager et al. 1984). During periods of maximum inundation these water bodies merged into a massive lake known as Cabeza de Vaca (Strain 1966). Reeves (1965, 1969) identified later, lower stages of the lake, which he called Pluvial Lake Palomas. The lake system began to drain mid-Pleistocene, flowing south into a large basin in the northern Chihuahuan Desert, and, when finally breached, created the modern channel of the Rio Grande River. At its maximum the lake covered an area of 23,000-26,000 km^2; at such a

size its effect on terrestrial organismal distributions would have been immense (Figure 1). Evidence of the lake has come from examination of ancient beach lines and the presence of lakebed depositions and evaporite basins in the area (Reeves 1965, 1969, Strain 1966,

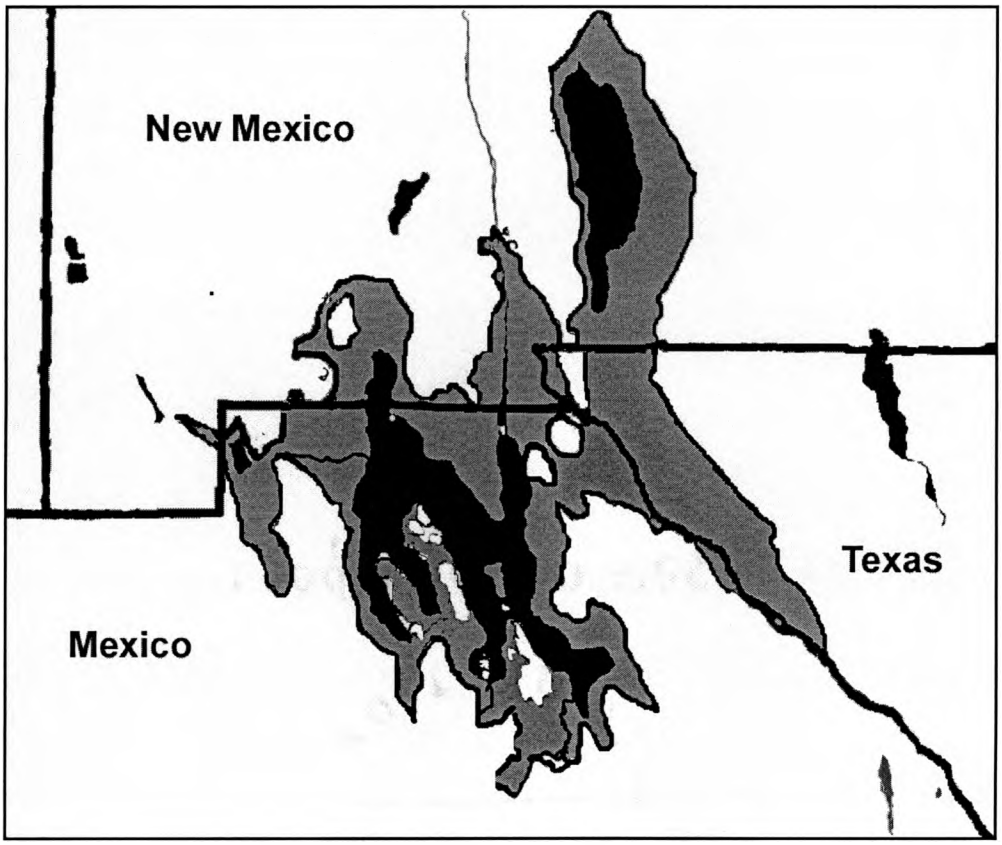

Fig. 1. Lake Cabeza de Vaca covered an area of 23,000 – 26,000 km^2 during peak lake levels, and may have had a hand in shaping the genetic structure of populations in its vicinity. Vertical gray line is Rio Grande River. Light gray areas represent the lake at maximum inundation during early Pleistocene. Black areas depict later Wisconsin remnants. Adapted from Axtell, 1974.

1970, Morrison 1991). Axtell (1978) found multiple vertebrate taxon pairs whose current range boundaries converge at the lake site and thus may be remnants of a period of allopatry during the existence of Cabeza de Vaca (e.g., Figures 2-4). Additionally, the

ranges of many other vertebrates that occur in the area may well have been influenced by the presence of Cabeza de Vaca (Figures 5-8). The ranges of these taxa are representative

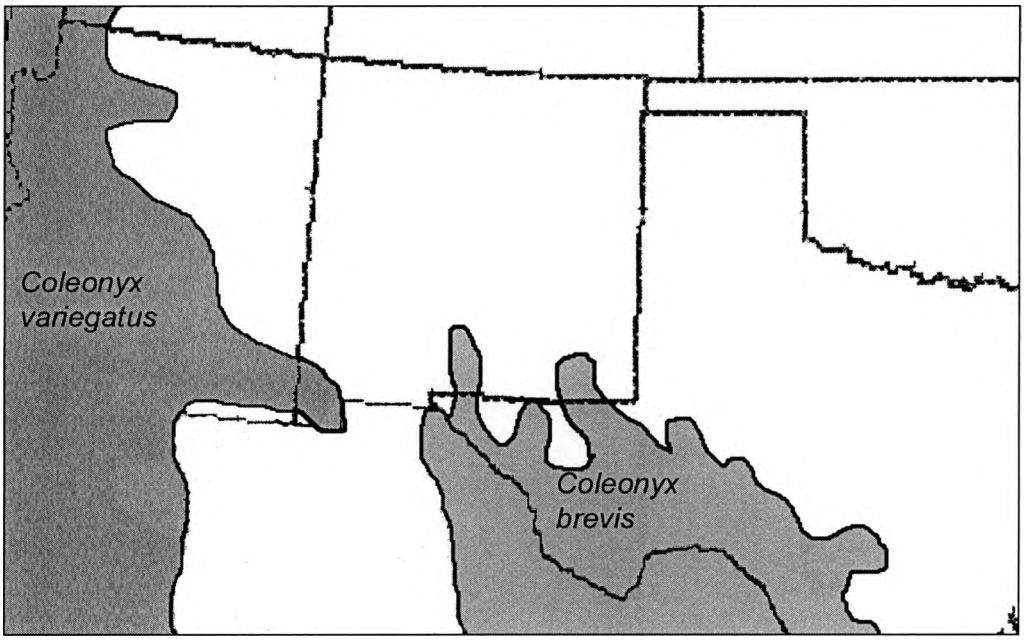

Fig. 2. Eastern and western ranges of two species of banded geckos (*Coleonyx*) nearly touch in the ancient lake bed. Axtell (1978), using inductive reasoning, concluded that Cabeza de Vaca was the likely biogeographic barrier.

of a trend common to many southwestern reptiles and mammals. Axtell (1978) found that 47% of reptiles and 43% of nonvolent mammals presently occurring in the Cabeza de Vaca region demonstrate western or eastern range limitations in the area and approximately 46% of reptiles and 20% of mammals occurring in this region demonstrate specific or subspecific differentiation that may be associated with the lake. These findings suggest that Cabeza de Vaca may have acted as a lacustrine barrier within the range of many ancestral terrestrial taxa and persisted long enough for differentiation at the species or subspecies level to occur. Recent studies utilizing mitochondrial and nuclear DNA fragments have shown that the pattern of genetic divergence in other species from this region. Walpole et al.

(1997) looked at variation in mitochondrial DNA (mtDNA) restriction fragments between Sonoran and Chihuahuan populations of the cactus mouse,

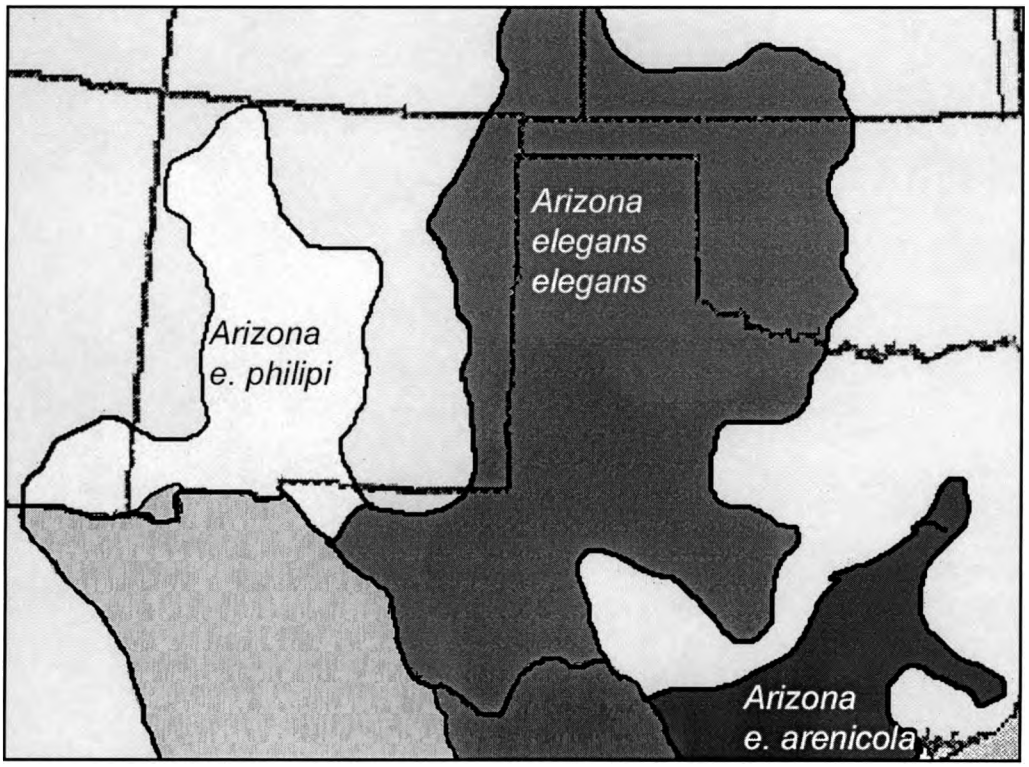

Fig. 3. Subspecies of the colubrid snake, *Arizona elegans*, converge at the site of Cabeza de Vaca; a similar pattern across multiple taxa would suggest that a local event was the source.

Peromyscus eremicus, and found that mtDNA haplotypes were not shared between the populations, suggesting an absence of gene flow. They proposed that the divergence was due to a Pleistocene-era separation and called for taxonomic re-evaluation of the genus. Examination of their data reveals that the divergence they found coincides with the vicinity of Lake Cabeza de Vaca (Figure 9), however, they failed to mention the possible influence of the Pleistocene lake.

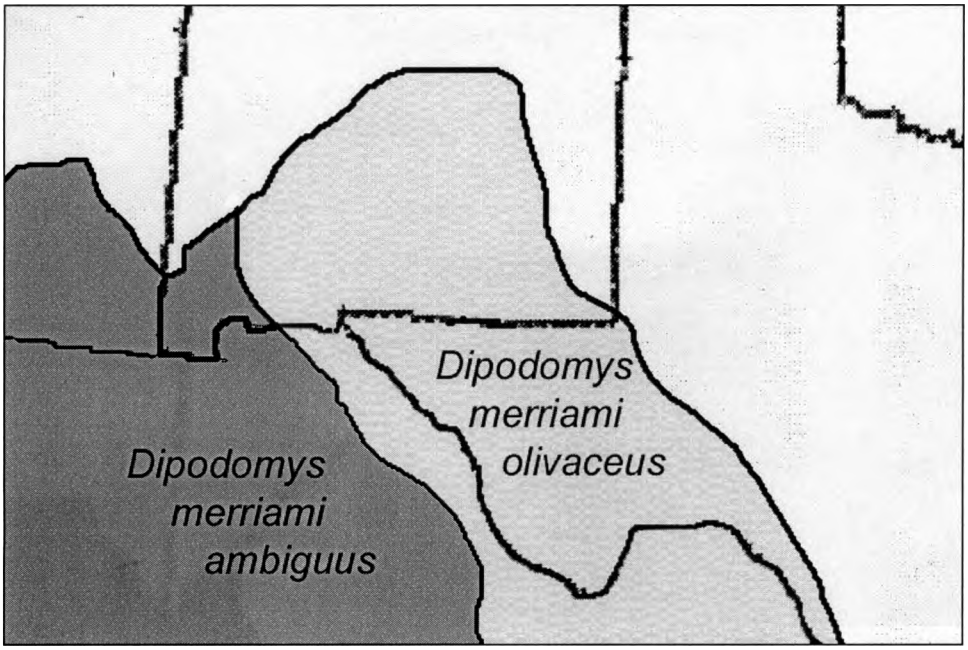

Fig. 4. Northeastern range limits of Merriam's kangaroo rat, *Dipodomys merriami*, shows subspecific differentiation in the vicinity of Cabeza de Vaca; a biogeographic barrier such as the ancient lake could have been the source of such a differentiation.

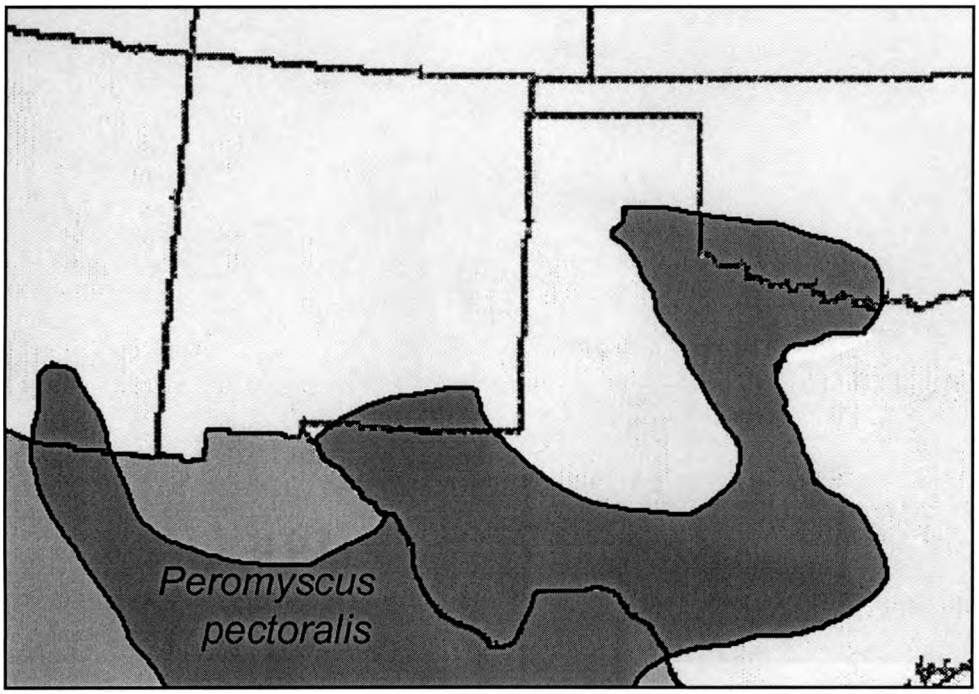

Fig. 5. The western half of the northern range limit of the white-ankled mouse, *Peromyscus pectoralis*, showing possible influence of Cabeza de Vaca. A northward range expansion during the Pleistocene would have been impeded by the presence of the lake.

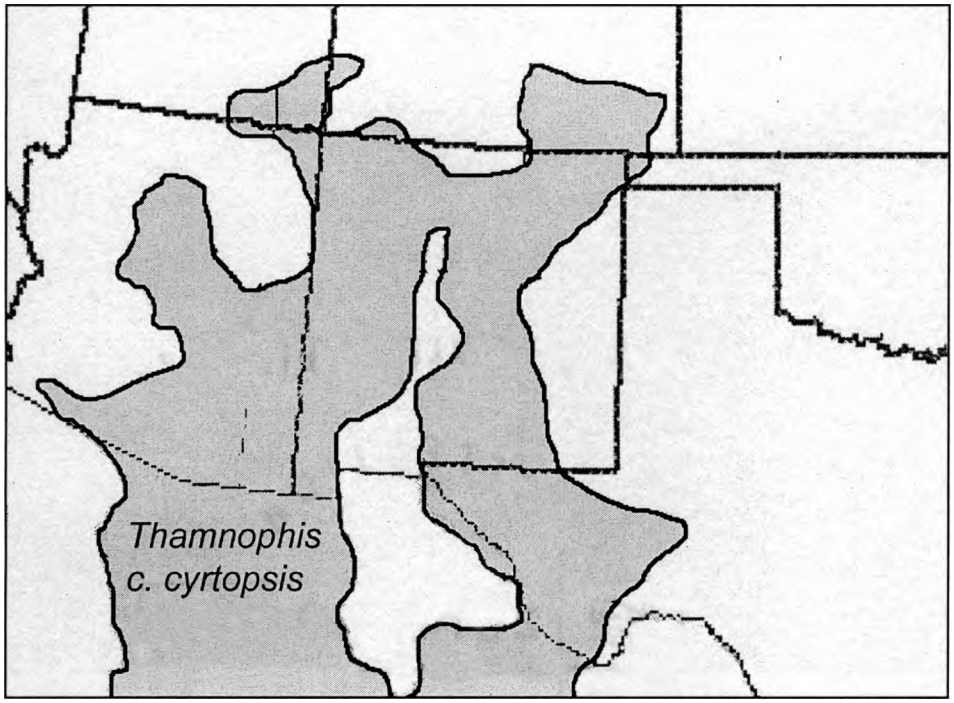

Fig. 6. The garter snake, *Thamnophis cyrtopsis cyrtopsis*, does not occur in an area that approximates the location of Cabeza de Vaca. The lake may have been a barrier to dispersal, later reinforced by desertification of the region.

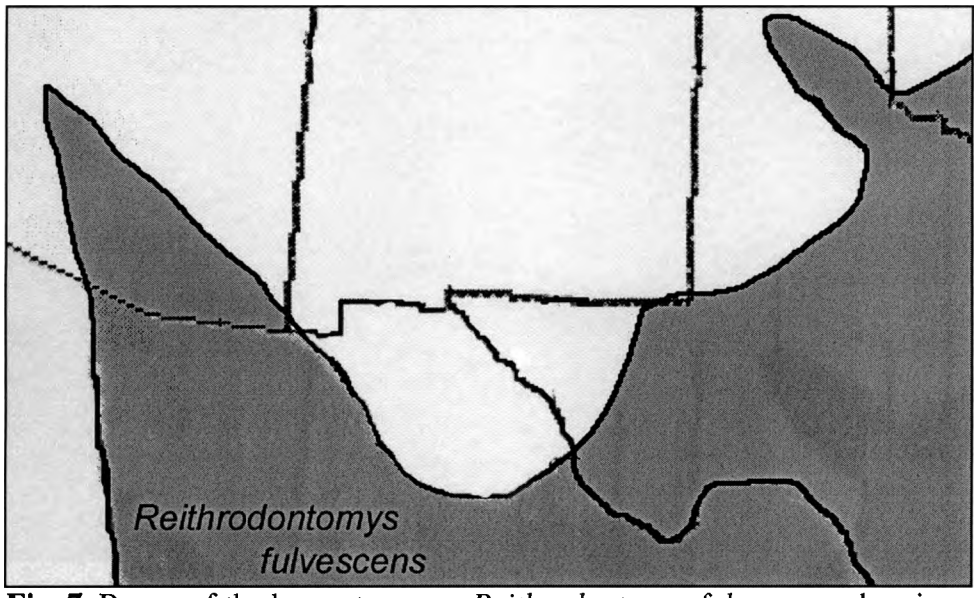

Fig. 7. Range of the harvest mouse, *Reithrodontomys fulvescens*, showing dip in northern range limit. Cabeza de Vaca may have acted as a vicariant barrier within the species' range during the Pleistocene.

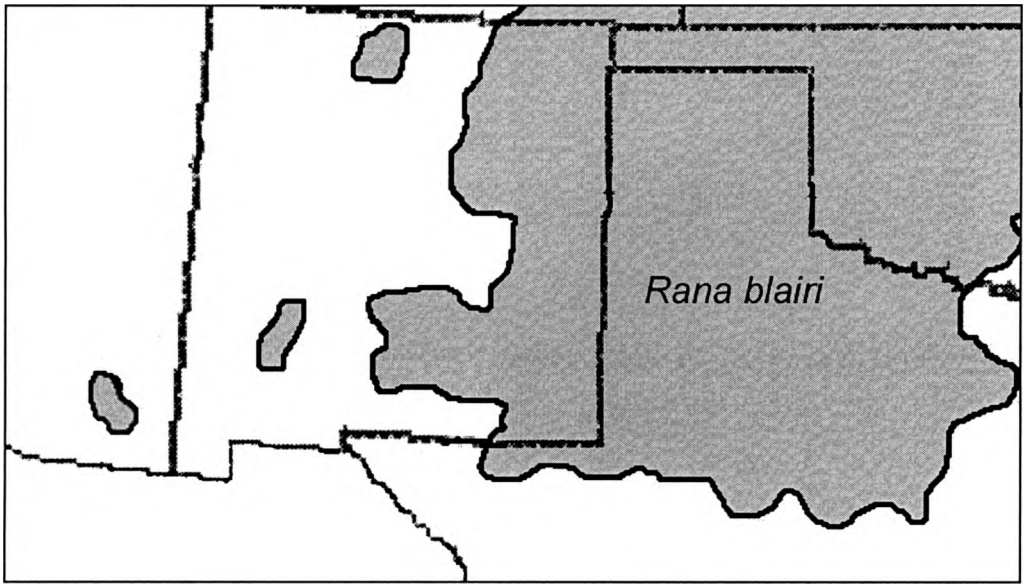

Fig. 8. Satellite populations of the leopard frog, *Rana blairi*, possibly were isolated when Cabeza de Vaca formed in the early Pleistocene. Genetic analysis of the populations may reveal their historical relationships.

Similar results were obtained in a study of the desert pocket mouse, *Chaetodipus penicillatus* (Lee et al. 1996), which also utilized mtDNA restriction fragments as the operational phylogenetic units. The authors found a significant break in haplotypes between populations from the Sonoran and Chihuahuan desert scrub biotas (Figure 10) and thus recommended that the subspecies *C. p. eremicus* be elevated to species status.

Not only mammals, but reptile species as well, show genetic breaks in the vicinity of Cabeza de Vaca. Mitochondrial and nuclear analyses of the Texas horned lizard, *Phrynosoma cornutum* from across its range of Texas to Arizona (Guerra 1998) was conducted to assess how genetic heterogeneity could affect conservation management. Guerra found a significant genetic divergence between samples taken in New Mexico

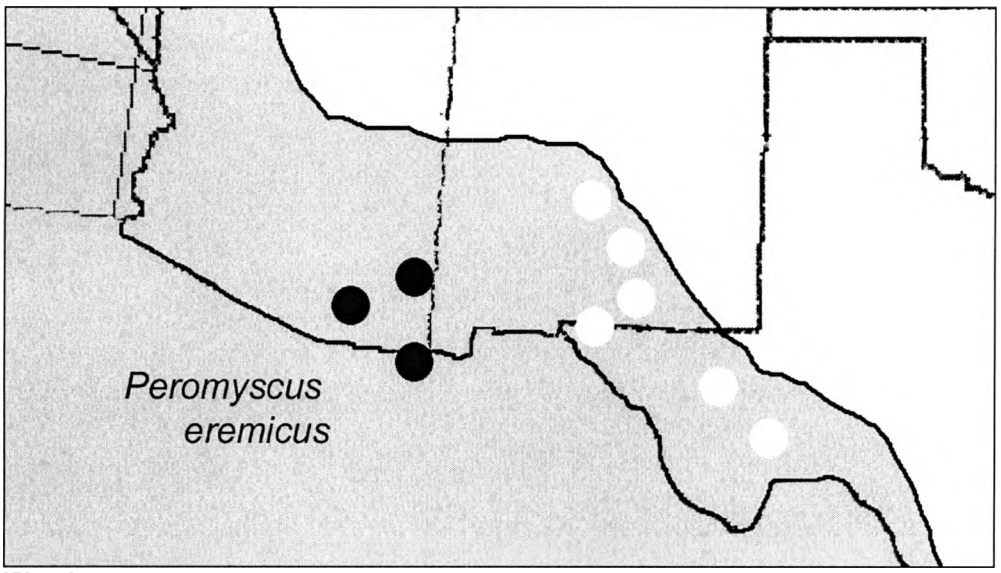

Fig. 9. Collection localities of *Peromyscus eremicus* (Walpole et al.1997); no mtDNA haplotypes observed in the study were shared between black and white localities. Such a disparity in the midst of a continuous range could be the genetic legacy of a Pleistocene barrier.

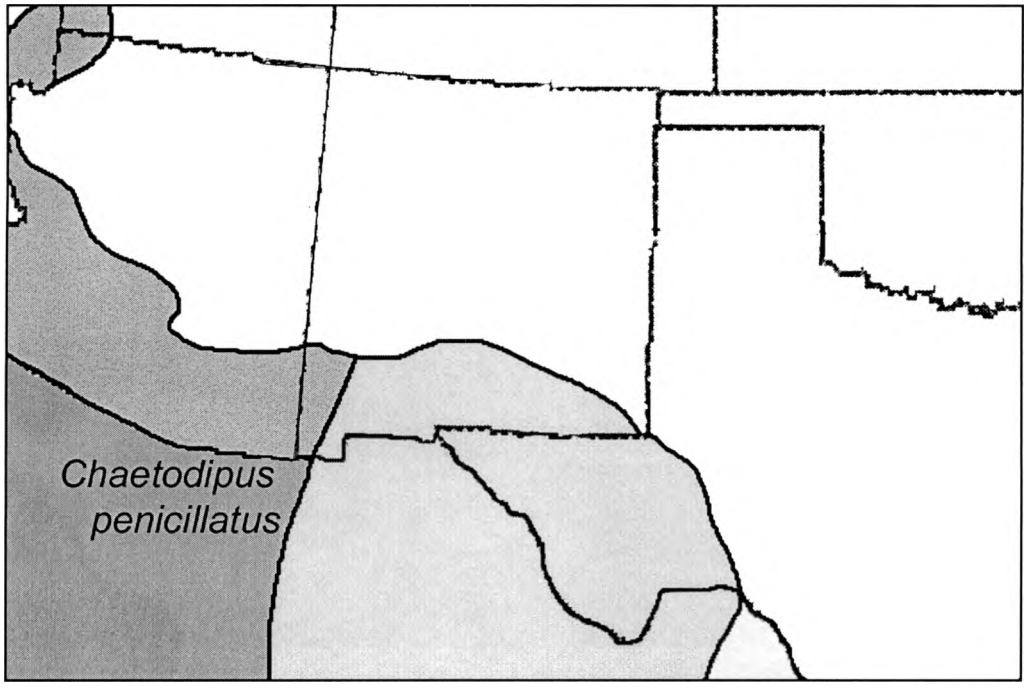

Fig. 10. Ranges of two *Chaetodipus penicillatus* subspecies; Lee et al. (1996) proposed that *C. p. eremicus* (lighter gray) be elevated to species status based on differences in mtDNA haplotypes. Further molecular phylogenies of species in this area may clarify evolutionary relationships not resolvable by morphological characters, and may thus shed light on biogeographic trends in the area.

and Arizona and those taken in Texas (Figure 11). She attributed this divergence to Lake Cabeza de Vaca and proposed that populations in New Mexico and Arizona be recognized as a separate species.

Additionally, a phylogenetic examination of barking frogs (*Eleutherodactylus augusti*), a species whose range expands from southern Mexico northward into Texas, New Mexico, and Arizona (Goldberg et al. 2004). Based on

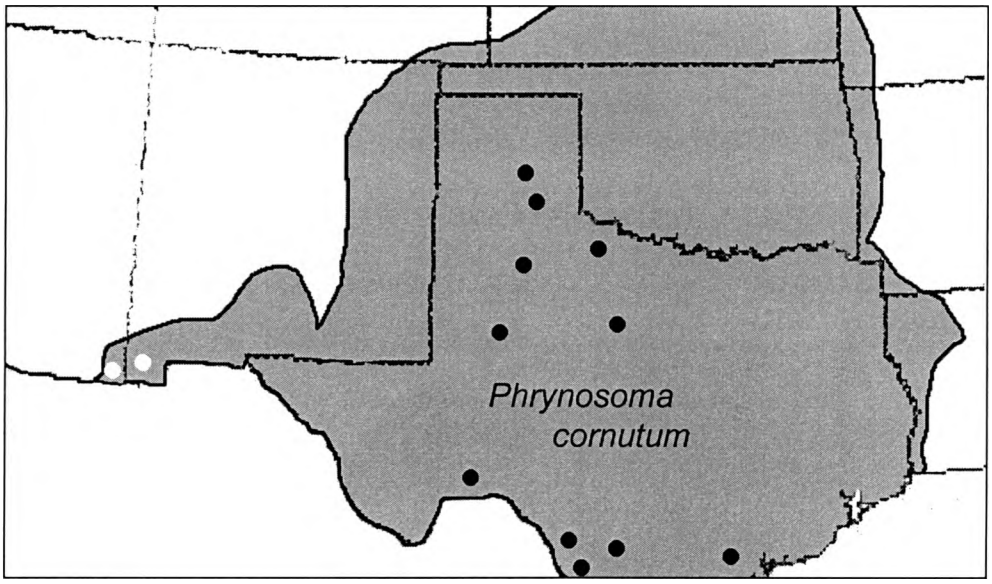

Fig. 11. Guerra (1998) found a significant break in haplotype frequencies between white and black collection localities of the Texas horned lizard and thus called for a taxonomic revision of the species. Guerra concluded that Cabeza de Vaca was the likely source of the genetic divergence.

differences in advertising call frequency, duration, and pulse duration, coloration, and DNA sequence variation, the authors recognized a clade containing the Texas and New Mexico samples as being a distinct and likely reproductively isolated lineage from the population sampled in Arizona. The authors presumed that the population structure was the result of divergent post-Pleistocene northward expansions of the species, but they did not assign the sharp differences between

the Texas/New Mexico and Arizona clades to any particular historical, climatic or geological activity.

Finally, a recent morphological analysis of the lyre snake, *Trimorphodon biscutatus,* found significantly divergent characters between subspecies whose ranges

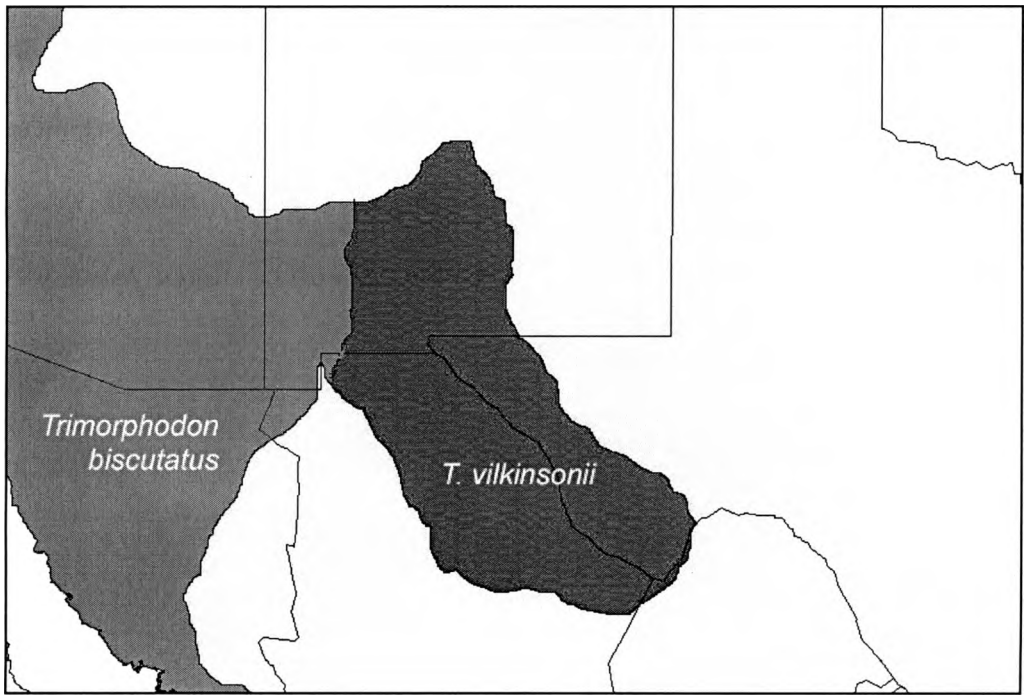

Fig. 12. Ranges of *Trimorphodon biscutatus* (lighter gray) and *T. vilkinsonii.* LaDuc and Johnson (2003) found that morphological differences justified the elevation of *T. vilkinsonii* from subspecies to species status.

converge in the area of the prehistoric lake (Figure 12). Using both univariate and multivariate statistical approaches, the authors were able to assign specimens from East and West of the ancient lake to the recognized subspecies classification (LaDuc and Johnson 2003). Furthermore, the authors found that specimens collected in a putative hybrid zone (in the vicinity of lake Cabeza de Vaca) showed no evidence of clinal variation and were consistently identified as the more western of the two subspecies, *T. b.*

lambda; due to the strength of their results, the easternmost subspecies, *T. b. vilkinsonii* has been elevated to species status (LaDuc and Johnson 2003).

These findings together provide support for the hypothetical role of Cabeza de Vaca as a source of vicariance. That several other taxa have demonstrated morphologically cryptic, but significant, genetic differentiation across this region (Lee et al., 1996) suggests that further investigation is warranted. Taxa influenced by this potential barrier would not necessarily be limited to members of any particular clade, but rather could be species that occur across this region. Three reptilian taxa were chosen for the current study (one snake and two lizard taxa), based on their distribution and the availability of geographically distributed samples.

Study Organisms. *Rhinocheilus lecontei* Baird and Girard, the Longnose Snake, is a member of the family Colubridae, however, its position within this family, and in particular its relationship to or within the tribe Lampropeltini, remains unclear (Keogh 1996, Dowling et al. 1996, Lopez and Maxson 1995, Rodriguez-Robles and De Jesus Escobar 1999). *Rhinocheilus* fossils at least 2.85 million years old have been found in North America (Parmley and Walker 2003), and *R. lecontei* fossils found in the northern Chihuahuan Desert date to at least 10,000 years b.p. (Harris 1978). The species ranges from northern California southwest to Oklahoma and Texas and south into Mexico (Conant and Collins 1998, Stebbins 1985), across which four subspecies are recognized, two of which have converging ranges in the vicinity of Cabeza de Vaca (Figure 13). *R. l. lecontei* occurs in Arizona and the extreme southwestern edge of New Mexico. *R. l. tessellatus* occurs in Texas, New Mexico, and Oklahoma, and both subspecies ranges

continue southward into Mexico. A recent reexamination of morphological characters did not demonstrate consistent or

significant levels of differentiation between the two subspecies, and further systematic studies and possible taxonomic revision was suggested (Manier 2004). The species is nocturnal and occurs in dry prairies, deserts, and rocky canyons (Conant and Collins 1998, Stebbins 1985) and feeds primarily on lizards of the genus *Cnemidophorus*, though other prey items are often taken (Rodriguez-Robles and Greene 1999). A recent study found that radiotelemetered *R. lecontei* had home ranges from 15,337 m^2 to 73,494 m^2 and tended to remain in areas that contained suitable habitat, which included shrub and burrow refugia (Beck and Peterson 1995).

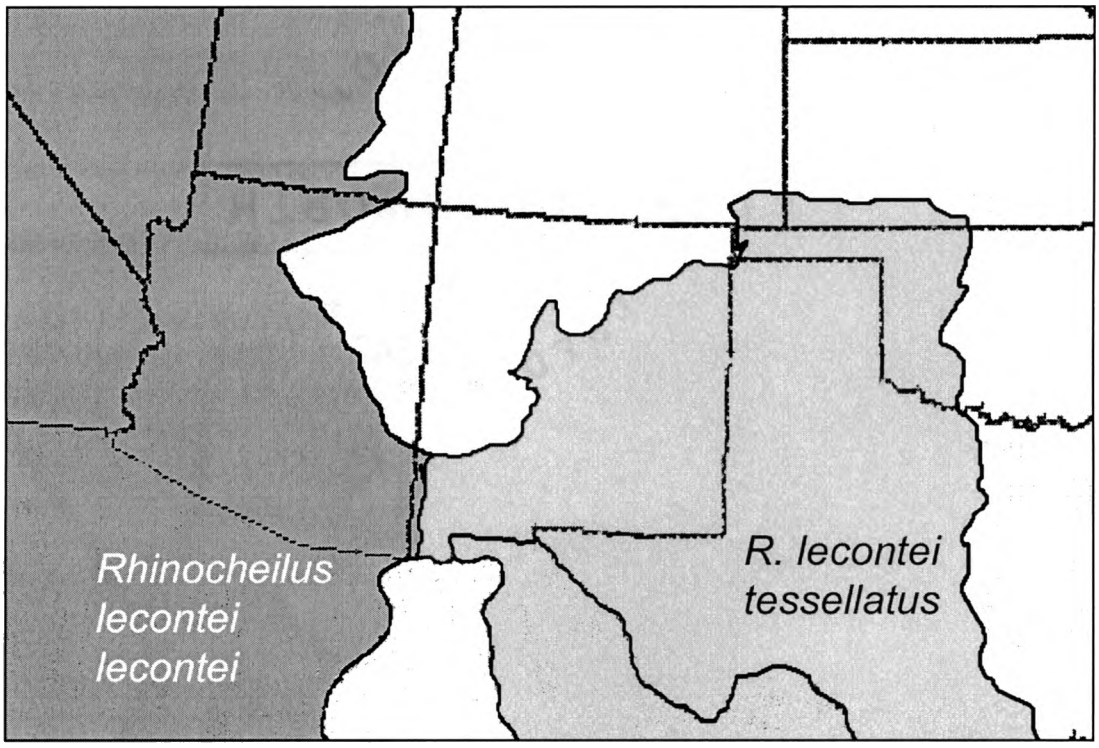

Fig.13. Range limits of longnose snake, *Rhinocheilus lecontei*, subspecies meet in an area once occupied by Lake Cabeza de Vaca. Perhaps drainage of the lake allowed the subspecies to reconverge.

The Tree Lizard, *Urosaurus ornatus* Baird and Girard, is a member of the Phrynosomatidae (Frost and Etheridge 1989) and occurs from Nevada and Colorado

south and east into New Mexico, Texas, and Mexico, and is composed of eight (Behler and King 1985) or six (Smith 1949) subspecies, though other authors do not recognize the validity of subspecies in this taxon (Stebbins 1985, Wiens 1993). The most recent phylogenetic analysis of the genus (Wiens 1993) utilized mostly morphological characters (plus three ecological (terrestrial, saxicolous, or arboreal) and allozyme data (1 protein from only 4 species)) and differed from a previous phylogenetic study (Mittleman 1942) in the placement of some taxa, however both studies supported a basal placement of the species *U. ornatus*. Following Behler and King (1985), three subspecies of *U. ornatus* occur in the area under study (Figure 15). *U. ornatus* may be either a terrestrial, saxicolous, or tree dwelling species,

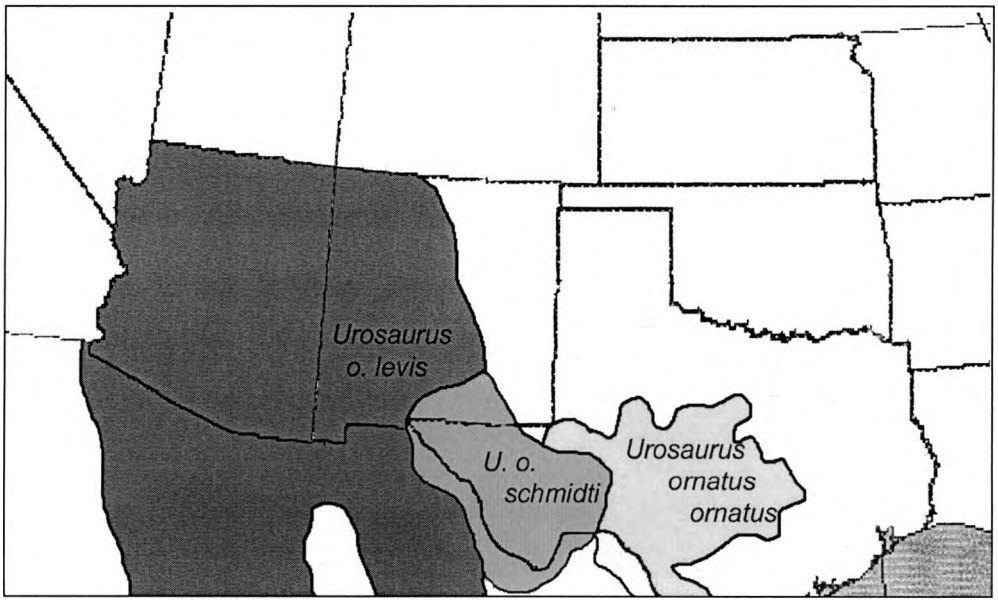

Fig.14. A period of allopatry may have driven subspecific differentiation in *Urosaurus ornatus*. Cabeza de Vaca, as well as other barriers, likely were biogeographic forces that shaped its distribution and differentiation.

depending on habitat availability and competition with congeners (Wiens 1993), but is prefers above ground habitats (Conant and Collins 1998). The species appears to follow current or extinct river drainages (Axtell 1997), and has an extremely patchy distribution

that is seemingly only partially dependent on available habitat, at least in Texas (F. L. Rose – pers. comm. to J.R. 2003); groups of individuals are often found in unevenly distributed clumps within apparently prime expanses of available habitat. Assuming weak gene flow and small effective population size, reduced heterozygosity within population islands might be expected, with local or demic adaptation possible. Desertification has enlarged the size of the Chihuahuan Desert since the retreat of the last (Wisconsinan) ice age (Anderson et al. 2002, Holmgren et al. 2003, but see Krider 1998), causing concomitant changes in many species distributions (Elias 1999). Hence, *U. ornatus* may be experiencing range contractions as available habitat disappears, and increased genetic heterogeneity as dispersal corridors disappear, due to both climatically and anthropogenically induced environmental changes. Such fragmentation could potentially blur or distort the signature left by Cabeza de Vaca. Further, fine scale population genetic analyses are thus warranted for the species. Higher resolution methods might help discern the forces, both historic and current, that influence this species, especially if phylogenetic relationships between populations can be correlated with geographic variation in behavioral, physiological, or morphological environmentally mediated traits (e.g. Sanders et al. 2004, Schulte et al. 2002). While the species has been the focus of physiological (Congdon et al. 1982), behavioral (M'Closkey et al. 1987), and ecological (Dunham 1980) studies, only one published study of intraspecific molecular variation exists (Haenel 1997). Though phenotypic plasticity has been invoked to explain observed levels of variation in behavior (M'Closkey et al. 1987) and ecology (Ballinger 1977), Haenel (1997), using mtDNA restriction-site analysis, found high levels of genotypic diversity (G = 0.950), with a G_{ST} value of 0.856, which estimates that 85% of

the observed variation was due to differences among populations. This is consistent with the species' patchy distribution on typically isolated stretches of habitat and suggests that reduced gene flow may in fact be a characteristic of modern *U. ornatus* populations.

P. cornutum Harlan, (family Phrynosomatidae) the Texas Horned Lizard, occurs further east than any of the twelve other species of the genus, from Arizona, Colorado and New Mexico east into Kansas, Oklahoma, and Texas, and south into the northeastern states of Mexico (Sherbrooke 2003) (Figure 11). Fossils identified as *P. cornutum* found in the northern Chihuahuan Desert have been dated to up to 25,000 years b.p. (Harris 1978). It occurs in a variety of arid habitats characterized by open, flat areas with scattered trees and shrubs and otherwise sparsely vegetated with grasses and forbs (Conant and Collins 1998). The State Reptile of Texas (Donaldson et al. 1994), this diurnal species is a dietary specialist, eating primarily harvester ants of the genus *Pogonomyrmex*, but also preying on a variety of small insects (Conant and Collins 1998). The ecological adaptations of this species are well known and studied (Sherbrooke 1990, 1991, Middendorf and Sherbrooke 1992), however geographic variation in such traits have not been described, but would be interesting given the range of habitat types in which it occurs. Research on Texas horned lizards found that individual home ranges ranged from 291 m^2 to 14,690 m^2 and the annual survival rate ranged from 9% to 54% (Fair and Henke 1999). A recent morphological analysis of *P. cornutum* examined geographic variation in body size across the entire species range in order to test the hypothesis that the species demonstrates decreased body size with increased latitude (Montgomery et al. 2003). In order to detect clinal variation, the authors assumed that the range of this species contained no physical barriers or isolating mechanisms, thus they

ignored the potential genetic effects of any historic biogeographic barriers on the species. They found an association between decreased size and increased latitude, but no significant association between body size and longitude.

Mitochondrial DNA. Mitochondrial DNA has been a useful molecule for testing predictions about the origins of historical and current distributions. MtDNA is a circular, extranuclear molecule that is maternally inherited by both genders, exhibits significant intraspecific variation, and does not recombine during meiosis (Graur and Li 2000). Therefore, it is transmitted across generations clonally; the same molecule found in extant taxa would have been present in the ancestors of the lineage, except for mistakes in replication, in which one base is substituted with another. Quantification of these mutations is useful for deducing if or when cladogenesis occurred, as well as for measuring the degree of difference between lineages (Avise 2000). Studies of base substitutions in mitochondrial gene sequences suggest that such sequence evolution (base substitution) is more rapid than in nuclear genes and occurs at a consistent rate, at least within classes of organisms (Avise 2000, Britten 1986, Hewitt 2001). Comparison of the number of substitutions within a particular gene region between two lineages allows calibration of a molecular clock that may provide an estimate of the amount of time the lineages separated from their common ancestor. While molecular clocks have proven to be highly variable between classes of organisms (Freeman and Herron 2001, Avise 2000), two percent sequence divergence between animal sister species per million years is a commonly accepted measure for mtDNA protein-coding regions (Graur and Li 2000; a review of mutation rates for various DNA markers can be found in Hewitt 2001). If the separation of clades can be attributed to a historical event, such as the formation of a

pluvial lake, and the barrier can be dated with the use of geological evidence and/or radioisotopes, then such a molecular clock can be calibrated to absolute time and the sequence mutation rate can be verified. Because the use of a standard mtDNA mutation rate is contentious (Avise 2000), further data on sequence divergence rates would be useful.

With the incorporation of polymerase chain reaction (PCR) (Saiki et al. 1988) and methods for generating nucleotide sequence data, extremely detailed relationships between organisms or groups of organisms based on mtDNA are now recoverable, and such techniques have revolutionized not only phylogenetics and phylogeography, but also medicine, agriculture, and biotechnology.

Overall, favorable attributes of mtDNA have made it the cornerstone of phylogeographic studies, although several authors have pointed out that sole dependence on it as a molecular marker can skew biogeographic and phylogenetic interpretations by overlooking potential drawbacks, such as direct and indirect selection on the marker (Ballard and Whitlock 2004, Gerber et al. 2001, Lightowlers et al. 1997).

Phylogenetic Inference. Methods for determining the evolutionary history of groups of organisms have grown tremendously over the past several decades; this growth has been facilitated by several factors, including an increase in the understanding of the mechanisms of molecular evolution, an increase in processing ability of computers, and an increase in the body of literature from which patterns of evolution can be empirically derived. Discourse on the relative benefits of particular methods of evolutionary inference for given types of character data is common in evolutionary journals (e.g.,

Bollback 2002, Alfaro et al. 2003, Huelsenbeck et al. 2002), and determining the correct model from amongst the available models and model parameters for a given data set can be an overwhelming experience (Huelsenbeck and Crandall 1997).

The four dominant methods of phylogenetic inference, in order of their popular inception in systematics, are maximum parsimony, distance methods (e.g. neighbor-joining), maximum likelihood, and Bayesian inference (Graur and Li 2000, Huelsenbeck and Ronquist 2001). The relative benefits and drawbacks of these methods in terms of both their philosophical underpinnings as well as their technical execution are discussed elsewhere (Felsenstein 1978, Nei and Kumar 2000, Huelsenbeck and Crandall 1997, Douady et al. 2003), however such topics are reviewed here.

Dialogue on the strengths and weaknesses of these phylogenetic methods is common in phylogenetic journals (e.g. Hillis and Huelsenbeck 1992, Yang 1997). This suggests that there remains much to be learned about the ability of these methods to infer true evolutionary relationships based on character data. Parsimony methods are based on an assumption of evolution that likely does not reflect the true nature of evolutionary change. Parsimony may not recognize homoplasies as well as other methods, and long-branch attraction may be more prevalent in this model; weighted parsimony may ameliorate some of these problems (Graur and Li 2002). Parsimony also requires every possible tree to be analyzed in order to find the shortest tree, which is not possible for large data sets; parsimony also does not make use of every character (Felsenstein and Sober 1986). For this reason, distance methods may be preferred because they build trees based on overall levels of similarity (Felsenstein 1984), however, if the sequence data is short, methods such as neighbor-joining may be subject to sampling error (Saitou and Nei

1987). Maximum likelihood methods may work well when there is confidence in the assumptions of its substitution model (Huelsenbeck and Crandall 1997), but the method is time-consuming and computationally expensive. Maximum log-likelihoods and Bayesian methods often resolve topologies with similar support values, but in simulations Markov chain Monte Carlo sampling using posterior probabilities was more accurate (Alfaro et al. 2003). Additionally, support for Bayesian analyses is easy to determine while obtaining bootstrap support for large data sets in maximum likelihood is almost impossible (Hall 2001). The Bayesian method is growing in popularity due perhaps in part to the simplicity of its underlying logic (Ross 1998) and because of its relatively short computation time, though a drawback is that it is not easy to compare posterior probabilities directly to bootstrap measures (Douady et al. 2003) when comparing conflicting topologies. Additionally, Bayesian methods may not be as reliable in their ability to approximate tree probabilities (Huelsenbeck et al. 2002). Ideally, several methods of phylogenetic inference should be utilized when attempting to extract taxon history from character data; congruence across multiple methods may be the strongest measure of a given topologies' accuracy in recovering true lineage history.

Phylogeography. Historical biogeography attempts to correlate present and past geographic conditions to current and historic populational distributions and to draw conclusions about how environmental change shapes the structure and function of living systems. Understanding the historical dynamics of biotic and abiotic interactions is useful for illuminating the nature of evolutionary forces, explaining natural phenomena such as convergence and divergence, and predicting future events, such as reactions of species to anthropogenic global and regional environmental changes. Due to the

application and incorporation of phylogenetic methods into biogeography, the field of phylogeography has emerged as a subdiscipline that seeks to make inferences about such processes via molecular data (Avise 1989, Avise 2000). Mapping intraspecific phylogenies onto the geographic areas from which the operational taxonomic units originate can provide phylogeographers with the hypothetical routes of dispersal and other causes of differentiation such as reduced gene flow, bottlenecks, and vicariant events, thus enabling tests of historical biogeographical hypotheses (but see Humphries 2000). However, the accuracy of phylogeographic analyses is dependent upon utilization of specimen samples from well-distributed sample sites that adequately cover the entire range of the species or populations under study. Obtaining sequence data from multiple individuals from the same locale assures that the variation observed is a true reflection of the natural variation present in the populations (Baverstock and Moritz 1996).

Use of haplotype networks (Templeton et al. 1987) provides not only a visual representation of the exact relationship between haplotypes (often measured by number of nucleotide base substitutions), but when associated with the geographic distribution of the haplotypes, can illustrate a demographic pattern that does not presume a particular phylogenetic history (Emerson et al. 2001). Nested clade analysis (NCA) (Templeton 2004), which makes use of haplotype networks, is perhaps the best tool for determining if and what historical events have shaped current structure of genetic populations (but see Knowles and Maddison 2002). This method employs a hierarchical nesting of clades, in which group of more closely related haplotypes are assembled based on a parsimony criterion of relatedness applied to nucleotide differences of haplotypes. By incorporating actual geographic distances into analyses, this technique first determines if a statistically

significant correlation exists between haplotypes and geography, and then, using an inference key, allows a user to find the most reasonable combination of historical events (e.g., restricted gene flow or population fragmentation) that could explain the observed distribution of haplotypes.

Comparative phylogeography can be accomplished by comparison of congruent area cladograms from unrelated, co-occurring taxa presents compelling support for shared histories and may also provide biological evidence of the presence of such historical barriers (Brown and Lomolino 1998). Comparative phylogeography thus shares similarities with traditional historical biogeography, in particular, using parsimony to determine the most likely explanation for patterns of distribution or geographic congruence of characters within unrelated taxa (Arbogast and Kenagy 2001). Since the inception of phylogeography as a method of fine scale biogeography, several studies have found such evidence of shared patterns of geographic subdivision from molecular character data (Avise 2000, Walker and Avise 1998, Riddle et al. 2000), though other studies have not (Lamb et al. 1992, Zink 1996), perhaps a reminder that regional biotas have been shaped by the sum total of environmental factors that vary across space and time. Comparative phylogeographic methods have attempted to be formalized in order to standardize the approaches that researchers take when analyzing phylogeographic data from multiple groups of taxa (Arbogast and Kenagy 2001, Bermingham and Moritz 1998, Zink 2002, Knowles and Maddison 2002, Edwards and Beerli 2000). However, perhaps the most important overriding message present in such papers is the need to recognize factors that can affect correct interpretation of phylogeographic data, such as heterogeneity in mtDNA mutation rate, both across taxa and within taxa across lineages.

This can be compounded by difficulty in selecting appropriate molecular clock rates for specific taxa, as well as by realization that gene divergence and species divergence are not always congruent. Effects of variation in ancestral population size (*N*) and recognition of multiple versus single events (e.g. effects of multiple Pleistocene glacial cycles versus only the Wisconinan) further complicate conclusions drawn solely from mitochondrial markers.

Alternative Biogeographic Hypotheses. The formation of the modern North American deserts and concomitant effects on the biota of these areas has received a great amount of attention by researchers, and has resulted in several hypotheses that address the timing of the separation of these deserts and subsequent diversification of respective desert biotas. While the fragmentation of the large super desert Mojavia into the modern North American deserts began in the late Miocene and early Pliocene (Morafka 1977), most biogeographic analyses of Chihuahuan Desert biota have focused on the timing and causes of its split with Sonoran Desert taxa (e.g. Riddle and Honeycutt 1990). Because the western edge of the Chihuahuan Desert meets or nearly meets with the eastern extension of the Sonoran Desert (discussed in Morafka 1977), it is possible that some of the differentiation of taxa in the region of Cabeza de Vaca is due to the earlier effects of the divergence of these deserts, rather than to the influence of the lake. In addition, the northern Chihuahuan Desert does not represent a unique or geographically isolated region – this area contains dispersal routes in every direction to neighboring regions, unlike another North American desert province, the Baja Peninsula (Savage 1960, Riddle et al.

2000). Therefore, critical evaluation of alternative explanations is necessary, as is a framework for falsifying all alternative hypotheses.

While earlier hypotheses suggested that differentiation between Sonoran and Chihuahuan desert biota occurred across the Rocky Mountains during Pleistocene glacial maxima (Findley 1969), recent analyses suggest that an early split occurred between these ecoregions, dating to the Late Pliocene (Riddle and Honeycutt 1990, Riddle 1995, Lamb et al. 1989, Lee et al. 1996, Zink 2002, Walpole et al. 1997, Pook et al. 2000, but see Ayoub and Reichert 2004). Under this latter scenario, a Late Pliocene vicariance between biotas occurred due to the uplift of the southern extension of the Rocky Mountains, followed by the development of the Chihuahuan Desert and subsequent Late Pliocene – Early Pleistocene separation of the Sonoran and Chihuahuan Deserts (Lee et al. 1996).

An abundance of fossil evidence from the Chihuahuan Desert indicates that climate change over the last 40,000 years has been a major influence on species distributions (Betancourt et al. 1990) and thus could have led to diversification of evolutionary lineages. Masta (2000), found that divergence between some populations of the Jumping Spider *Habronattus pugillis* may have occurred as recently as 30,000 years ago, suggesting that the glacial-post-glacial period at the end of the Pleistocene may be responsible for isolation of such populations, restricting gene flow. Goldberg et al. (2004) hypothesized that variation between populations of the Barking Frog, *E. augusti*, was due to post-Pleistocene expansion, and Camper and Dixon (1994) conclude that Wisconsin glaciation was most likely responsible for divergence in the *M. taeniatus* species group during periods of allopatry due to habitat changes.

Cabeza de Vaca began to fill in the early Pleistocene (1.8 m.y.a.) and persisted at least until mid-Pleistocene, with many of its bolsons likely forming pluvial lakes during periods of heavy precipitation up until the close of the Pleistocene. Many pairs of sister taxa are thought to have originated during this time frame (Avise 2000), which falls temporally between the two above mentioned hypotheses. By using a molecular clock to determine the age of any recovered divergences between DNA sequences within the focal taxa, I am able to accept or reject the above hypotheses.

Objectives. Three vertebrate taxa in the southwestern United States have range limits that approximately coincide with the historic location of Lake Cabeza de Vaca, suggesting that Cabeza de Vaca was a major biogeographic barrier during the Pleistocene. The purposes of this study were to 1) utilize mtDNA genetic markers to determine the intraspecific and geographic relationships of three taxa whose ranges occur in the region that Cabeza de Vaca once occupied by using phylogenetic systematics and phylogeographic techniques, 2) test the hypothesis that Cabeza de Vaca was an important genetic barrier to taxa of the region by determining if the results, including molecular clock divergence estimates, are best explained by the presence of Cabeza de Vaca or alternate biogeographic hypotheses for the Chihuahuan Desert, and 3) reevaluate the systematics of these taxa in light of the molecular dataanalyze any taxonomic consequences of the results.

MATERIALS AND METHODS

Taxon Sampling. Blood and tissue samples were obtained from field collected specimens of the three focal species by either ventral scale clips in the case of the snakes, toe clips of the lizards, or via caudal veins for all taxa. All field samples were stored in plastic cryotubes in 95% ethanol in the case of tissue samples and blood buffer in the case of blood samples, following Dessauer et al. (1996). Documentation of samples consisted of labeling each cryotube with the collection date, and assignation of a collection number, which was referenced in a field notebook, where county, map coordinates, collection date, and potentially useful information, such as time of day, temperature, or morphometric data was recorded. Collection sites included public and private lands, as well as samples salvaged from road-killed individuals (see Appendix I for permits and license information for samples obtained by the author). Additional samples were obtained from other institutions or were already present in the Forstner tissue collection, Texas State University – San Marcos. All samples that were obtained for the current study were assigned an MF number and placed into the Forstner tissue collection. Outgroup samples were obtained in a similar manner or from GenBank. Outgroup members are typically chosen from nearest lineages outside of the monophyletic group under study (Graur and Li 2000).

DNA Extraction, Amplification, Sequencing. DNA was extracted from samples with the Qiagen DNeasy extraction kit per manufacturer's instructions. Amplification of

samples was performed in 100 μL reactions utilizing a 20μL Taq buffer, 0.5 μL Taq polymerase, 1.0 μL dNTPs, forward and reverse primers, and 75.4 μL ddH2O. Primers appropriate for amplifying mtDNA fragments containing partial ND4, tRNA Histidine, Serine, and Leucine genes were obtained (Arevalo 1992), or designed (see Table 1 for list of primers and Figures 16 and 17 for relative locations of primers on mitochondrial genome). The area that contains these genes has been used successfully in prior reptilian (Raxworthy et al. 2002, Rodriguez-Robles et al. 1999, Zamudio et al., 1997) and mammalian (Pastorini et al. 1998) phylogenetic analyses and has been shown to be superior to other gene regions commonly used to estimate species level relationships in reptile genera (Creer et al. 2003, Nagy 2004). Amplification proceeded under the following parameters: initial denaturation for 5 minutes at 94°C and then 35 cycles of denaturation (94°C, 30 seconds), annealing (45°C, 30 seconds), and extension (72°C, 1 minute) followed by a final extension at 72°C for 5 minutes were performed with GeneAmp PCR System 9700 (Perkin Elmer). Amplified products were cleaned of unincorporated oligonucleotides and reagents using Concert Rapid PCR purification system following manufacturer's instructions. Big Dye 3.0 was used for cycle sequencing the cleaned PCR samples. The cycle sequenced products were electrophoresed in a polyacrylamide gel on a ABI PRISM 377XL DNA sequencer.

Phylogenetic Analyses. Resultant mtDNA sequences were converted into a Nexus file format and aligned using Sequencher™ 4.1 (Gene Codes Corp.). Contigs composed of forward, reverse, and internal sequences were created and subsequently hand aligned and edited if needed. A consensus sequence was then generated for each individual. Subsequent refinement was completed by comparison to published reference

sequences, translation to amino acids, or structural folding, as appropriate in each situation. Aligned sequences were exported into the phylogenetic analysis software program PAUP (Swofford 2000). The datasets were examined with MrModeltest 2.2 (Posada and Crandall, 1998) to analyze transition/transversion ratios, base frequencies, proportion of invariable sites, and substitution (gamma) distribution. For each dataset, an appropriate model of evolution was chosen based on the results from MrModeltest. Maximum likelihood and Bayesian inference were used to infer phylogenetic relationships, and the best resolved or well supported final topology or consensus topology was selected as the most likely evolutionary

Table 1. Primer Sequences used in amplification and sequencing of the mtDNA ND4-Leu gene fragments. Primers ND4 and Leucine were the forward and reverse primers, respectively, used for all taxa. Remaining primers were used to sequence internal fragments as illustrated in Figures 16 and 17. Asterisks refer to Arevalo et al. 1992.

Primer Name	Primer Sequence 5' – 3'
ND4*	CACCTATGACTACCAAAAGCTCATGTAGAAGC
Leucine*	CATTACTTTTACTTGGATTTGCACCA
ccND4*	TCGTTCGTAGTTTGTGTTTGC
Gram A*	CATCAGGTGGCTATTAGTGGAA
LiasisND4 #2	CTACAACAAACAGACCTAAAATCCCT
Lpri #2	CTCTACATATTCATYACAACACA
Lpri #2 Reverse	AGGTGTTCTCGTGWGTGTGTTGG
PhryEm*	ACAGGATTAGGTACACTAATTTACAGC
Npri	TAAGCCCATGTGGCTTAYTGATGA

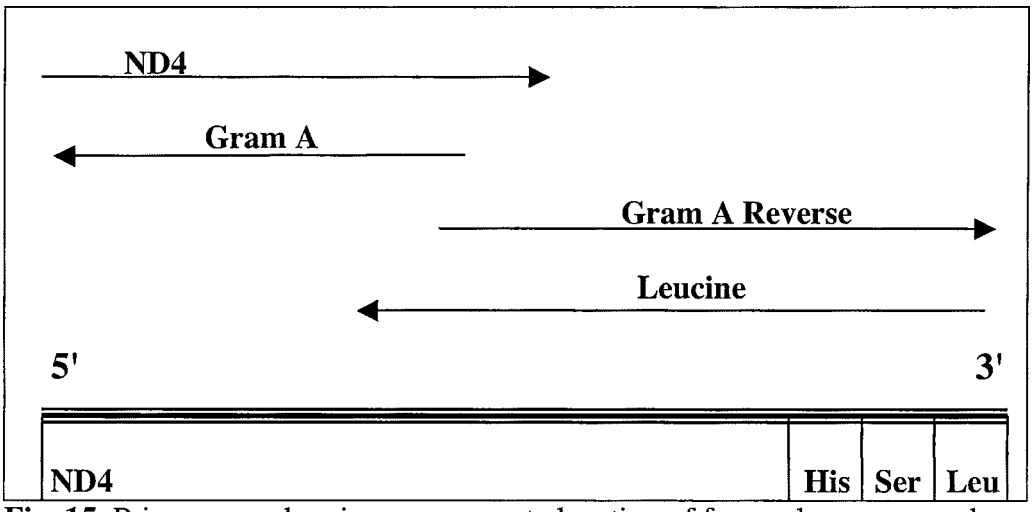

Fig. 15. Primer map showing approximate location of forward, reverse, and internal primers used to amplify mtDNA and attach fluorescent labeled, chain-terminating dideoxynucleotides in the phrynosomatids *U. ornatus* and *P. cornutum*. Placement of the ND4 gene in relation to the tRNAs Histidine, Serine, and Leucine is presented at bottom.

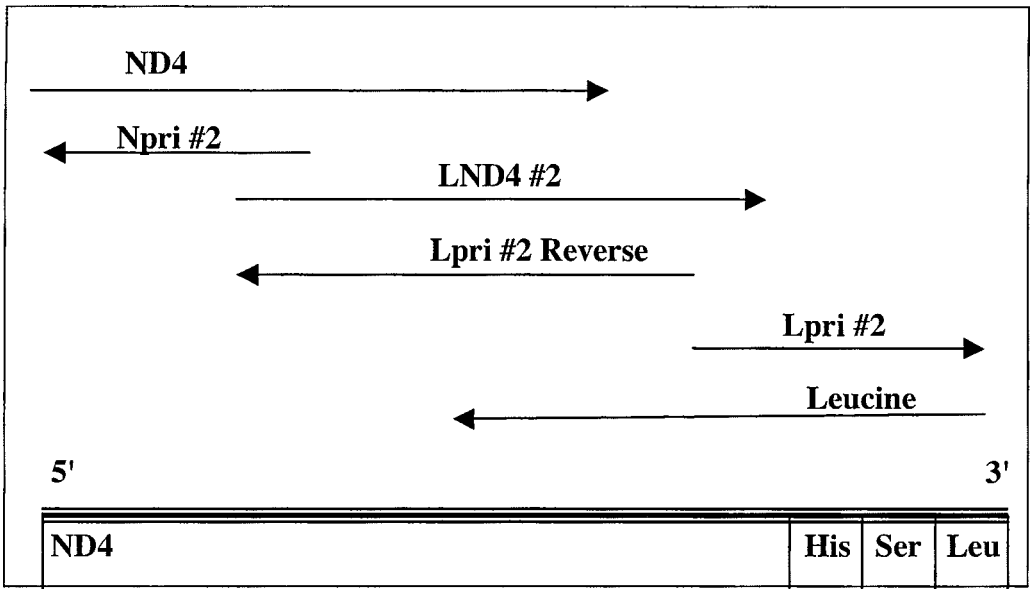

Fig. 16. Primer map showing approximate location of forward, reverse, and internal primers used to amplify mtDNA and attach fluorescent labeled, chain-terminating dideoxynucleotides in the snakes *R. lecontei* and *M. taeniatus*. Placement of the ND4 gene in relation to the tRNAs Histidine, Serine, and Leucine is presented at bottom.

relationship of each dataset's taxa (Swofford et al., 1996, Hall, 2001). Maximum likelihood analyses were performed with PAUP* 4.0 (Swofford 2002). Bootstrap searches were executed with 1000 replications under a heuristic search to determine confidence levels. MrBayes version 3.0 (Huelsenbeck 2000) was used to perform Bayesian analysis. A Markov Chain Monte Carlo set for a 1,000,000 generation run time with a sample frequency of 10 and an initial burn in at 10,000. Results were analyzed in PAUP* and a consensus topology was produced.

Phylogeographic Analyses. Because ND4 sequence data was available for thirty-one other *P. cornutum* specimens (Guerra 1998), a statistical parsimony haplotype network was created by combining data sets and using the software program TCS Version 1.06 (Clement et al. 2000). Haplotypes were nested into increasingly inclusive groups following the rules of Templeton et al. (1987) and Templeton and Sing (1993).

Geodis Version 2.0 (Posada et al. 2000) was utilized to test for significant associations between haplotypes and geography and to calculate clade distances and nested clade distances. Coordinates were estimated for samples in which only road miles or other locality information was known, using the software ArcView 3.1. Nested clade analysis calculated the geographic range of haplogroups, called the clade distance (D_c). It also determined how the haplogroups are distributed in relation to all the haplotypes within nested haplogroups, called the nested clade distance (D_n), by taking the average distance of haplotypes from the geographic center of the next highest nested clade. Random permutation tests were conducted to assess the statistical significance of D_c and D_n. The inference key of Templeton (2004) was used to interpret the results in terms of

most likely historical or contemporary events that shaped current gene geography (Templeton 2004).

Molecular Clock Calibration. While nested clade analysis may be able to reveal geographic associations between taxa and the presence of a vicariant, the percentage of sequence divergence between clades putatively separated by the lake should at least approximate estimated levels of divergence based on mitochondrial sequence evolution. Several published rates of sequence evolution were used to determine expected rates of sequence divergence between specimens collected on opposite sides of the lake (MacRae and Anderson 1988, Zamudio and Greene 1997). If geographical congruence exists between these multiple taxa in regard to Cabeza de Vaca, and genetic divergence is approximately equivalent within each taxon, then support for the Cabeza de Vaca biogeographic hypothesis as an important force influencing the geographic variation present in the taxa under study in the northern Chihuahuan Desert region is warranted.

Zamudio and Greene (1997) have produced evidence that overall rates of mtDNA divergence for small to medium-sized vertebrate ectotherms range roughly from 0.47% to 1.32% per million years, calibrated by comparing observed levels of sequence divergence of the cytochrome b and ND4 genes to known geologic events. These rates, as well as the more general "universal" mtDNA clock rate of 2.00% sequence divergence per million years (MacRae and Anderson 1988) were used in order to interpret resultant rates of sequence divergence in terms of the above listed scenarios. Using the slower clock rate, divergences that can be attributed to Pliocene (5.6-1.8 m.y.a.) or earlier events should demonstrate rates of sequence divergence of $> 0.846\%$; divergences dating to the early to mid Pleistocene (1.8 - 1.2 m.y.a.) should demonstrate between 0.846 - 0.564%,

and divergences dating to the Late Pleistocene (2.5 x 10^4 y.a.) should demonstrate < 0.118% sequence divergence. Using the faster clock, the rates are > 2.376%, 2.376 – 0.1.584%, and < 0.330%, respectively. Using the more general rate of 2.00% yields rates of > 3.600%, 3.600% - 2.400%, and <0.500%, respectively. For all molecular clock analyses, average uncorrected sequence divergence between major clades was utilized following the methods of Zamudio and Greene (1997). Taking the averaged uncorrected sequence divergence between clades provides an overall view of divergence between major groups, though it is not as conservative as using minimum rates or using corrected rates. These estimates will be used for subsequent analyses in order to determine if results obtained conform to any of these scenarios.

Table 2. Predicted sequence divergences for mtDNA based on three estimated rates of sequence divergence.

Clock Rate (% divergence/ million years)	**Pliocene** > 1.8 m.y.a.	**Early - Mid Pleistocene** 1.8-1.2 m.y.a.	**Late Pleistocene** < .25 m.y.a.	**Source**
0.47	0.846	0.846 - 0.564	0.118	Zamudio & Greene 1997
1.32	2.376	2.376 – 1.584	0.330	Zamudio & Greene 1997
2.00	3.600	3.600 – 2.400	0.500	MacRae & Anderson 1988

RESULTS

Taxon Sampling. 9 samples of *U. ornatus*, 6 samples of *P. cornutum*, and 8 samples of *R. lecontei* were obtained (Table 4, Figures 18, 20, 22, 24) from across west Texas, New Mexico, and southeast Arizona and used for all analyses.

Dataset Characteristics. Mitochondrial fragments containing ND4, tRNALeu, tRNAHis, and tRNASer were successfully sequenced from individuals of each taxon under study. The timing of divergence of major clades in taxa in light of estimated rates of sequence divergence are given in Table 3.

Table 3. Inferred times of divergence between major East-West clades identified in each taxon estimated from MtDNA ND4 nucleotide sequence data, using three estimated rates of nucleotide substitution.

		mtDNA clock (% my^{-1})		
Evolutionary Divergence of Major East-West Clades	Sequence Divergence (%)	0.47	1.32	2.00
P. cornutum	6.05	12.87	4.58	3.03
U. ornatus	11.35	24.15	8.60	5.68
R. lecontei	4.47	9.51	3.38	2.24

Phrynosoma cornutum. A fragment 764 bases long, containing 42 variable and 41 informative sites was analyzed from the *P. cornutum* dataset. MrModeltest 2.2 found that the best fit DNA correction model was GTR + G based on hierarchical

Table 4. Locality data for all specimens used in the analyses. Numbers in first column refer to respective sample numbers illustrated in Figures 18, 21, 24, 27-30.

	MF	Species	Collection Location/Coordinates	County	St.
1	6128	*Phrynosoma cornutum*	21.1 Rd mi N Jct US 285 and State Hwy 20, on 20	DeBaca	NM
2	6102	*Phrynosoma cornutum*	Bitter Lake National Wildlife Refuge; 7.0(air) mi NE of Roswell	Chaves	NM
3	9621	*Phrynosoma cornutum*	32 40.256 - 107 31.237 (N. of Las Cruces)	Sierra	NM
3	9622	*Phrynosoma cornutum*	33 40.256 - 107 31.237 (N. of Las Cruces)	Sierra	NM
4	9623	*Phrynosoma cornutum*	32 26.584 - 107 55.358 (NE of Deming)	Luna	NM
5	7453	*Phrynosoma cornutum*	31 53.25 - 109 44.13	Cochise	AZ
1	9435	*Urosaurus ornatus*	29 57.000 - 99 57.500 Eagle's Nest Ranch	Real	TX
1	9436	*Urosaurus ornatus*	29 57.000 - 99 57.500 Eagle's Nest Ranch	Real	TX
2	8879	*Urosaurus ornatus*	29 53.504 - 100 59.482	ValVerde	TX
3	8846	*Urosaurus ornatus*	30 36.114 - 103 54.772	Jeff Davis	TX
4	5008	*Urosaurus ornatus*	CDRI	Jeff Davis	TX
5	8862	*Urosaurus ornatus*	30 48.743 - 103 56.501	Jeff Davis	TX
4	1619	*Urosaurus ornatus*	11.2 mi W. jct. 166/17 Davis Mtn Resort	Jeff Davis	TX
6	9433	*Urosaurus ornatus*	Southwestern Research Station	Cochise	AZ
6	9434	*Urosaurus ornatus*	Southwestern Research Station	Cochise	AZ
1	8215	*Rhinocheilus lecontei*	Hwy 90, 15 mi. S. of Seminole Canyon S. P.	ValVerde	TX
2	6246	*Rhinocheilus lecontei*	Cannon Ranch	Terrell	TX
3	8867	*Rhinocheilus lecontei*	Hwy 90, approx. 10 mi. N. of Marathon	Brewster	TX
3	8866	*Rhinocheilus lecontei*	Hwy 90, Between Alpine & Marathon	Brewster	TX
4	9056	*Rhinocheilus lecontei*	Hwy 118 S. of EMWMA	Brewster	TX
5	9609	*Rhinocheilus lecontei*	115 at spur 247 @ Red Bluff Lake	Luna	NM
6	417	*Rhinocheilus lecontei*		Grant	NM
7	419	*Rhinocheilus lecontei*	Arizona	Cochise	AZ

likelihood ratio tests (hLRT) and GTR + I based on the Akaike Information Criterion (AIC); it found base frequencies of A= 0.3536, C= 0.2623, G= 0.1132, T= 0.2709, transition/transversion ratio of 7.20, and gamma distribution of 0.1623. *P. asio* (Long Spined Horned Lizard) was chosen as an outgroup and used to root resultant trees based on the most recent published phylogeny of the genus *Phrynosoma*, which estimated that *P. asio* was a likely sister taxon to *P. cornutum* (Hodges and Zamudio 2003). Maximum likelihood analysis implementing the GTR+G correction model produced one tree with a –ln L of 1398.8317 (Figures 19 and 20) and tree length of 124 steps (CI=1.000, RI=1.000, RC=1.000). A g1 statistic of –2.092862 based on evaluation of 1000 random trees suggests that the topology recovered from the Maximum Likelihood analysis is due to structure in the data set. A bootstrap analysis with 1000 replicates was conducted in order to determine branch support (Figure 19). The topology recovered from Bayesian analysis was identical to that produced by Maximum Likelihood, with posterior probability values similar to the bootstrap values (Figure 19).

Comparison of the topology of the phylogenetic tree to the geographic location of the samples as well as Lake Cabeza de Vaca demonstrates the presence of a clade east of the lake composed of the samples from Chaves and DeBaca counties in New Mexico, and a clade west of the lake composed of all other samples (Figure 18 and Figure19). Average uncorrected sequence divergence between these two clades was calculated (Table 4) based on three rates of sequence divergence.

Statistical parsimony as implemented by TCS resulted in formation of two unconnected haplotype networks of the *P. cornutum* data set comprised of Guerra's (1998) 31 ND4 sequences and the six sequences obtained in the current study. Under a

95% confidence criterion, up to 11 mutational steps were allowed for network formation. Nested clades are depicted in Appendix 2. Of the two resulting networks, the largest number of mutational steps (single nucleotide substitutions) connecting closest haplotypes was three, however the vast majority of haplotypes were separated by only single base substitutions (Appendix 2). The two unconnected networks were separated by at approximately 40 mutational steps. Haplotype network 1 (Appendix 2) was comprised of the haplotypes that compose the western clade from the current study (Figures 18 and 19), as well as all of Guerra's (1998) western clade samples (Appendix 3), totaling six haplotypes. Haplotype network 2 (Appendix 2) was comprised of the haplotypes that compose the eastern clade from the current study (Figures 18 and 19), as well as all of Guerra's (1998) eastern clade samples (Appendix 3), totalling 16 haplotypes. Haplotype network 2 had one circular connection that joined clade 1-1, 1-9, and 1-5. Following the criteria for deciding among alternative solutions for breaking the loops (Pfenninger and Posada 2002) produced the network presented in Appendix 2.

Haplotype network 1 (Appendix 2) consisted of only one 1-step clade; Geodis found no significant association between haplotype relationships and geography, hence it was not considered for nested clade analysis. Haplotype network 2, the eastern clade (Appendices 2 and 3) yielded two nested haplotype groups with significant associations of haplotype and geography ($p < 0.05$): the tip subclade 2-1, and the interior subclade 2-2. This pattern is inferred to be the result of restricted gene flow with isolation by distance (Templeton 2004).

Urosaurus ornatus. A fragment 867 bases long, containing 103 variable and 99 informative sites was analyzed from the *U. ornatus* dataset. MrModeltest 2.2 found that

the best fit DNA correction model was GTR based on hLRT and GTR + G based on the AIC; it found base frequencies of A= 0.3608, C= 0.2620, G= 0.1166, T= 0.2606, transition/transversion ratio of 4.75, and gamma distribution of 0.5367. *U. microscutatus* (Small-scaled Lizard) was chosen as an outgroup and used to root resultant trees based on the most recent published phylogeny of the lizard family Phrynosomatidae (Reeder 1995). Maximum likelihood analysis implementing the GTR correction model produced one tree with a –ln L of 2065.0102 (Figures 21 and 22) and tree length of 206 steps (CI=0.7136, RI=0.2133, RC=0.1522). A g1 statistic of –0.489974 based on evaluation of 1000 random trees suggests that the topology recovered from the Maximum Likelihood analysis is due to structure in the data set. A bootstrap analysis with 1000 replicates was conducted in order to determine branch support (Figure 21). The topology recovered from Bayesian analysis was identical to that produced by Maximum Likelihood, with posterior probability values similar to the bootstrap values (Figure 21).

Comparison of the topology of the phylogenetic tree to the geographic location of the samples as well as Lake Cabeza de Vaca demonstrates the presence of a clade west of the lake composed of the two samples from Cochise county in Arizona, and a clade east of the lake composed of all other samples (Figure 21 and Figure 22). Average uncorrected sequence divergence between these two clades was calculated (Table 4) based on three rates of sequence divergence.

Rhinocheilus lecontei. A fragment 853 bases long, containing 53 variable and 12 informative sites was analyzed from the *R. lecontei* dataset. MrModeltest 2.2 found that the best fit DNA correction model was HKY+G based on hLRT and GTR+I+G based on AIC; it found base frequencies of A= 0.3486, C=0.2570, G=0.1252, T=0.2692,

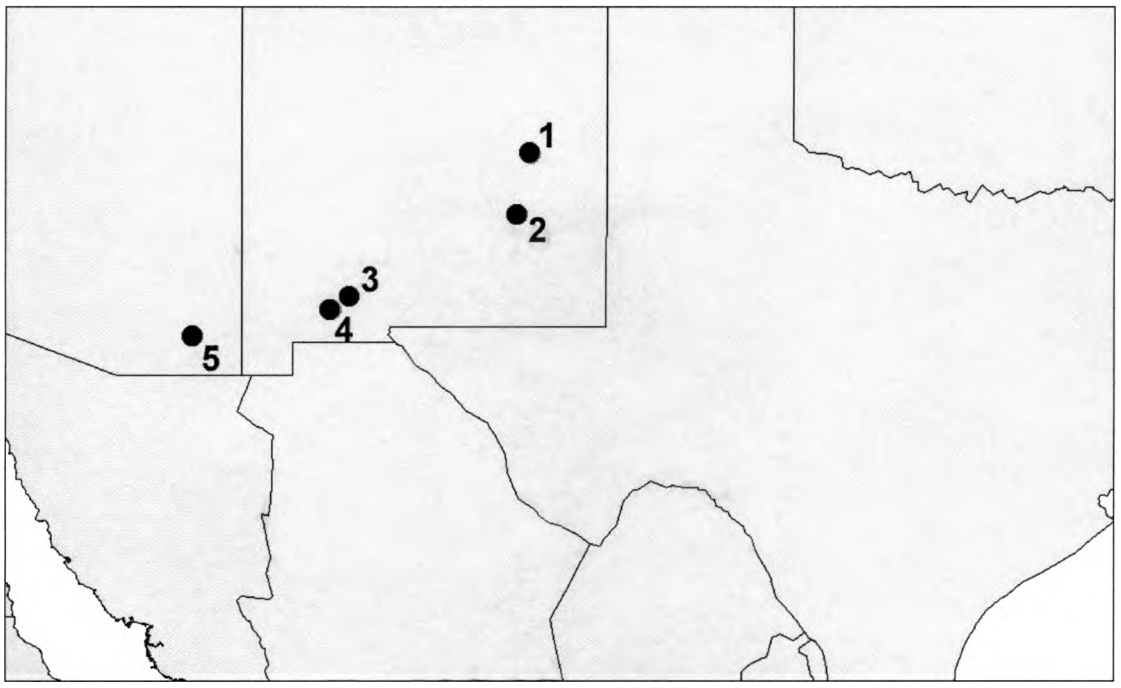

Fig. 17. Collection localities for *Phrynosoma cornutum*. Numbers refer to Table 3 specimen numbers and locality data.

transition/transversion ratio of 30.52, and gamma distribution of 0.0662. *Lampropeltis zonata zonata* (California Mountain Kingsnake) was chosen as an outgroup and used to root resultant trees based on the most recent published phylogeny of the New World colubrid tribe Lampropeltini, which estimated that the genus *Lampropeltis* was a sister taxon to *Rhinocheilus* (Rodriguez-Robles and DeJesus-Escobar 1999). Maximum likelihood analysis implementing the GTR+I+G correction model produced one tree with a –ln L of 1986.71384 (Figure 25 and Figure 26) and tree length of 206 steps (CI=0.7136, RI=0.2133, RC=0.1522). A g1 statistic of –0.489974 based on evaluation of 1000 random trees suggests that the topology recovered from the Maximum Likelihood analysis is due to structure in the data set. A bootstrap analysis with 1000 replicates was conducted in order to determine branch support (Figure 25 and Figure 26). The topology recovered from Bayesian analysis was identical to that produced by Maximum

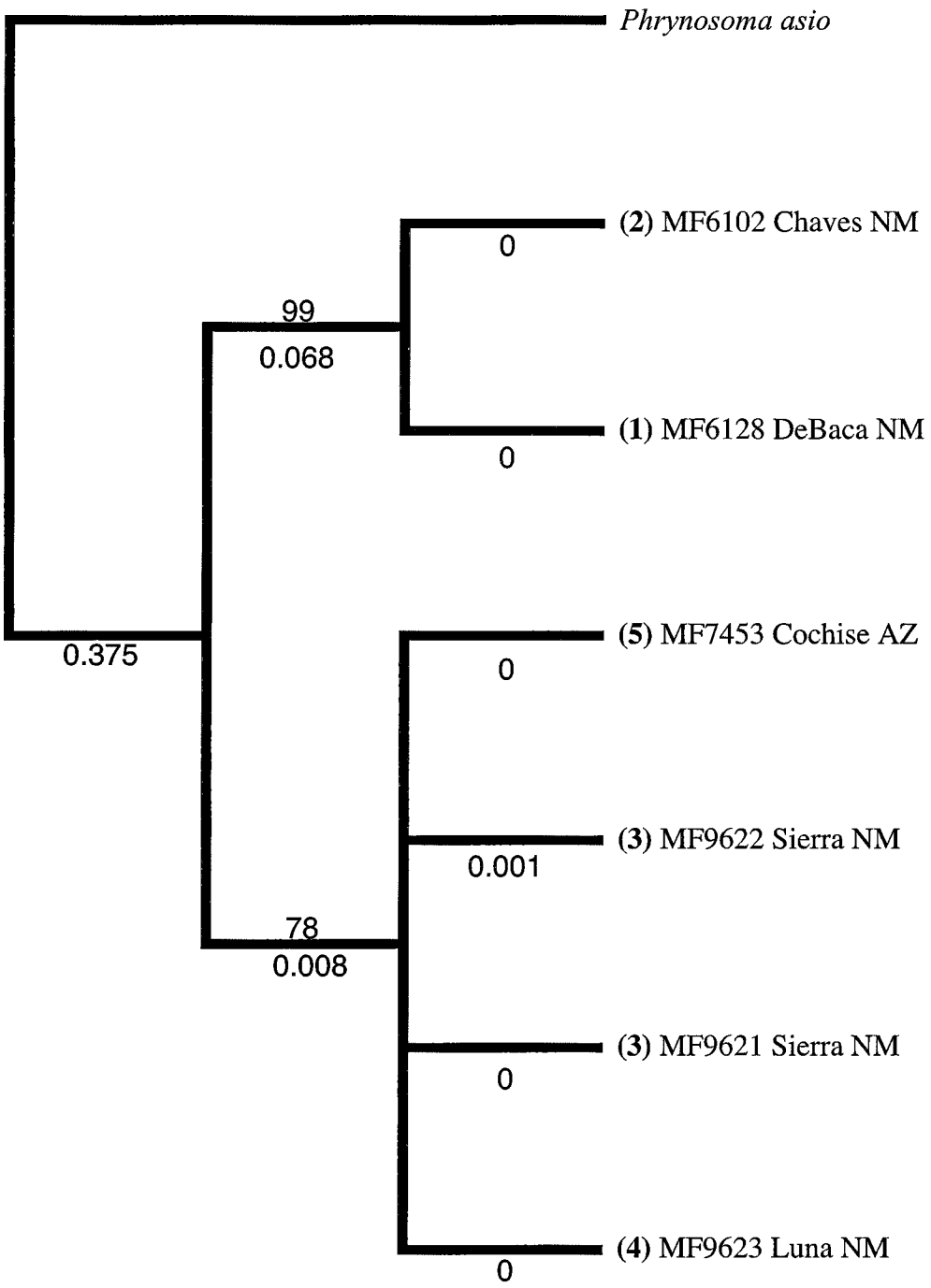

Fig. 18. Maximum Likelihood analysis from *P. cornutum* data set produced one tree with a –ln L of 1407.2340 following an exhaustive search with 1,000 replicates incorporating the GTR+G evolutionary model selected by MrModeltest 2.0 using the Akaike Information Criterion. Branches with 50% or greater bootstrap values are shown. Bootstrap support is listed above each branch and branch lengths are given below each branch. Terminal node labels correspond to MF tissue catalog numbers and county and states from which samples were collected. *P. asio* was used as an outgroup. Numbers in parentheses refer to locality data in Table 3 and Figure 18.

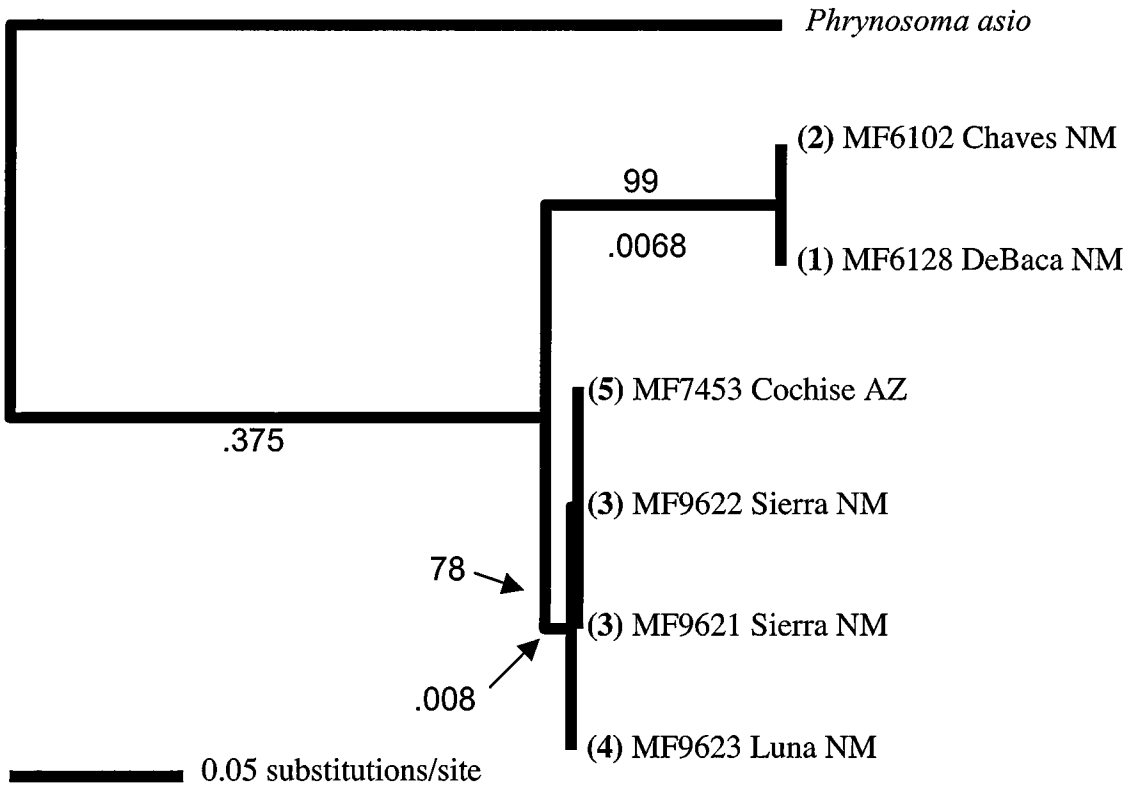

Fig. 19. Phylogram of Maximum Likelihood results from *P. cornutum* data set. One tree was produced with a –ln L of 1407.2340 following an exhaustive search with 1,000 replicates incorporating the GTR+G evolutionary model selected by MrModeltest 2.0 using the Akaike Information Criterion. Bootstrap support is listed above each branch and branch lengths are given below each branch. Terminal node labels correspond to MF tissue catalog numbers and county and states from which samples were collected. *P. asio* was used as an outgroup. Numbers in parentheses refer to locality data in Table 3 and Figure 18.

Likelihood, with posterior probabilities that equaled or exceeded the bootstrap values provided in Figure 25.

Comparison of the topology of the phylogenetic tree to the geographic location of the samples demonstrates the presence of a western clade composed of the sample from Cochise county in Arizona, and an eastern clade composed of all other samples (Figure 24 and Figure 25). Average uncorrected sequence divergence between these two clades was calculated (Table 4) based on three rates of sequence divergence.

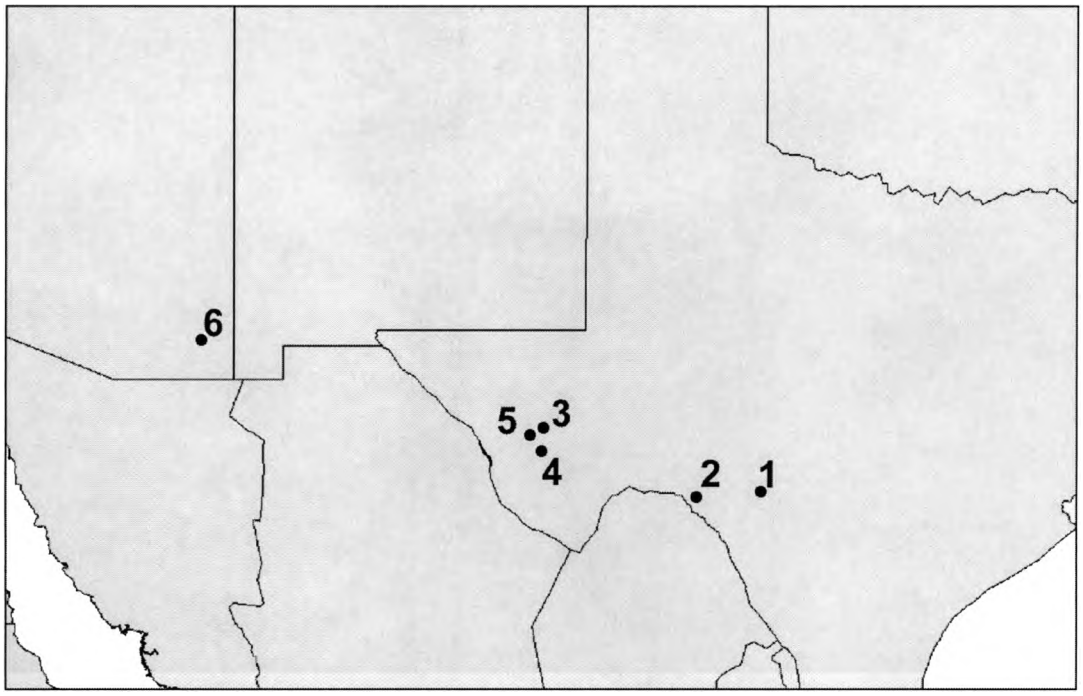

Fig. 20. Collection localities for *Urosaurus ornatus*. Numbers refer to Table 3 specimen numbers and locality data.

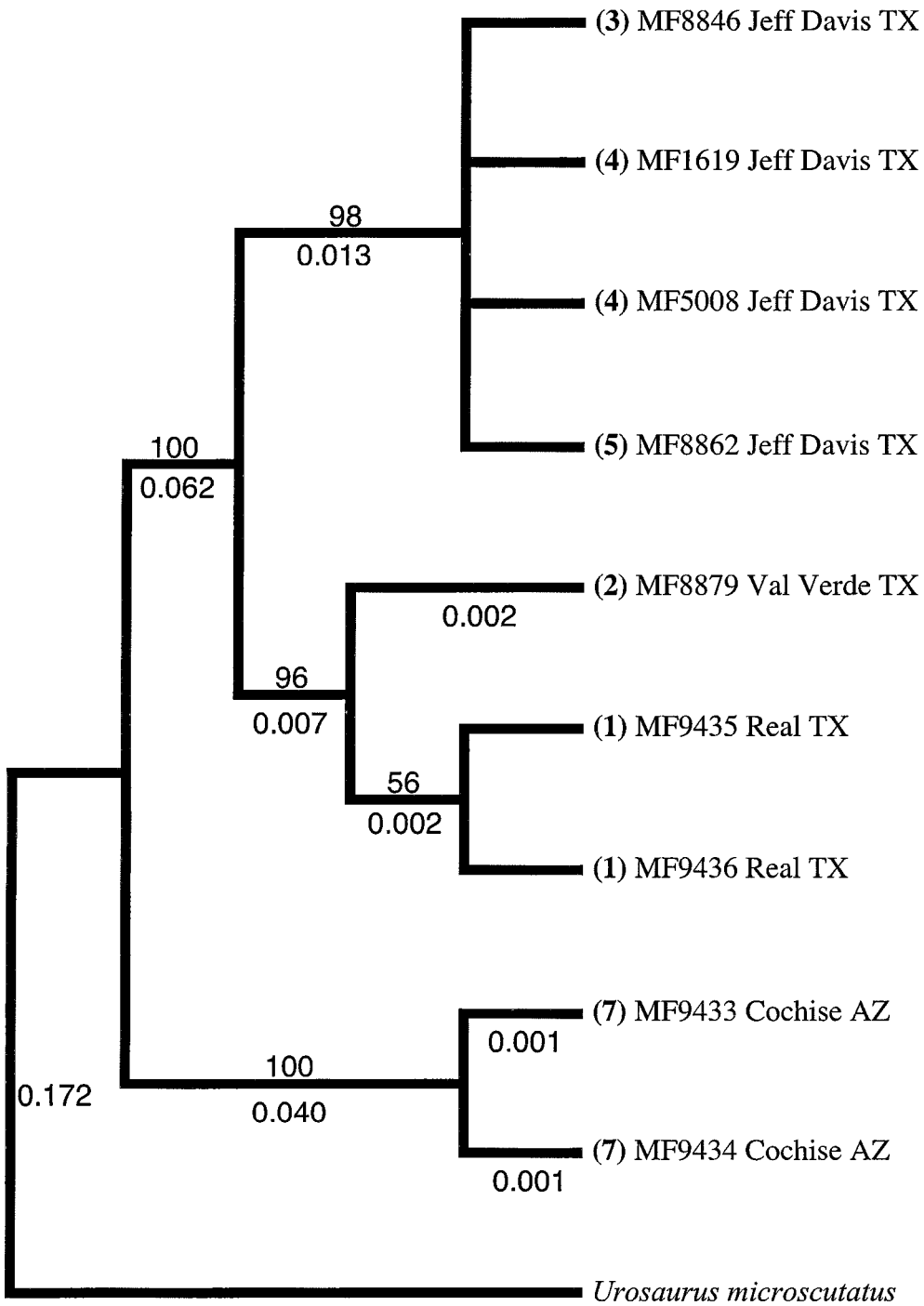

Fig. 21. Maximum Likelihood analysis from *U. ornatus* data set produced one tree with a –ln L of 2065.0102. Bootstrap support is listed above each branch and branch lengths are given below each branch. Terminal node labels correspond to MF tissue catalog numbers and county and states from which samples were collected. *U. microscutatus* was used as an outgroup. Numbers in parentheses refer to locality data in Table 3 and Figure 21.

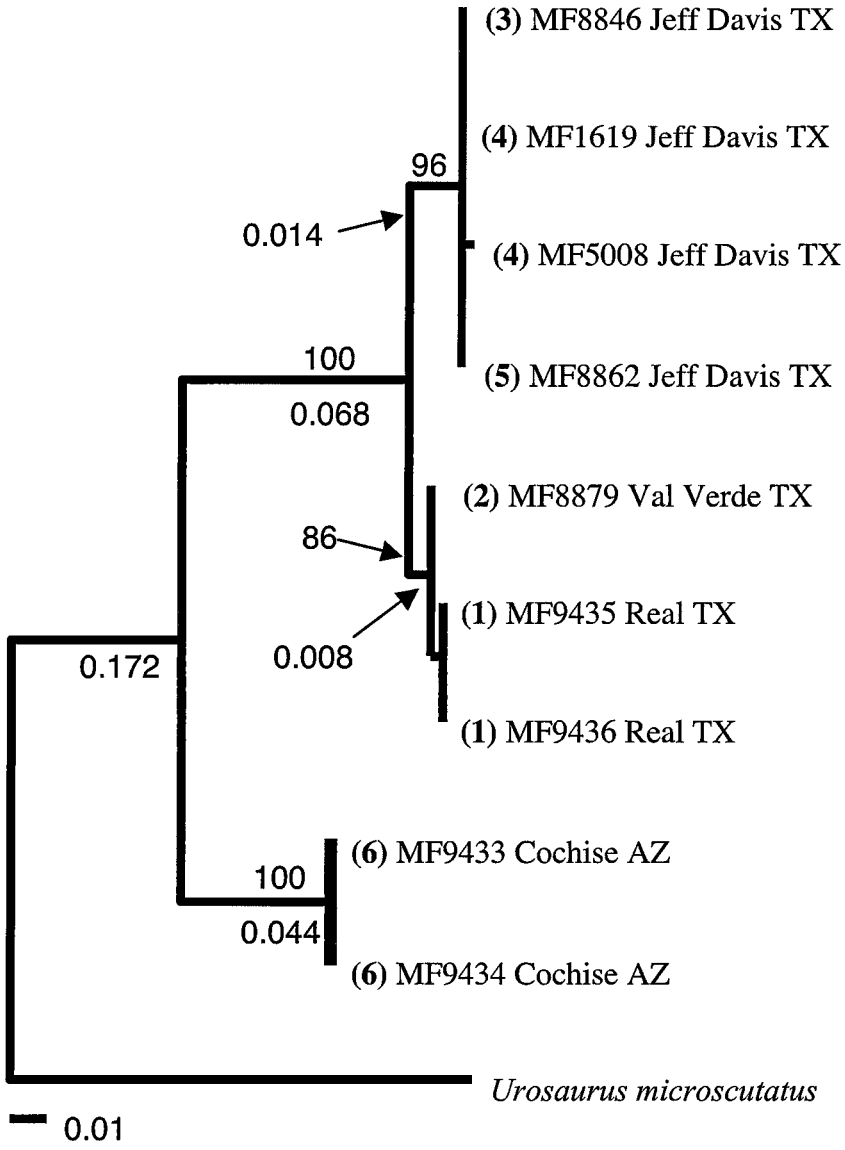

Fig. 22. Phylogram illustrating results of Maximum Likelihood analysis from *U. ornatus* data set. One tree was produced with a –ln L of 2065.0102. Bootstrap support is listed above each branch and branch lengths are given below each branch. Terminal node labels correspond to MF tissue catalog numbers and county and states from which samples were collected. Numbers in parentheses refer to locality data in Table 3 and Figure 21. *U. microscutatus* was used as an outgroup.

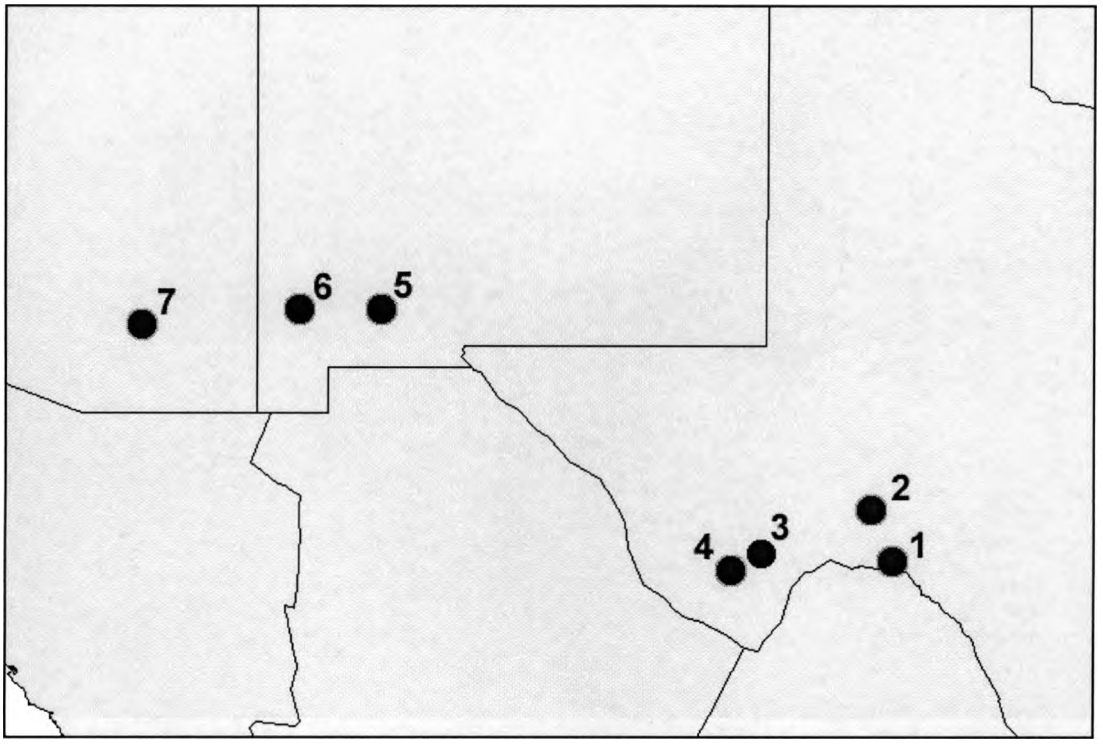

Fig. 23. Collection localities of *Rhinocheilus lecontei*. Numbers refer to Table 3 specimen numbers and locality data.

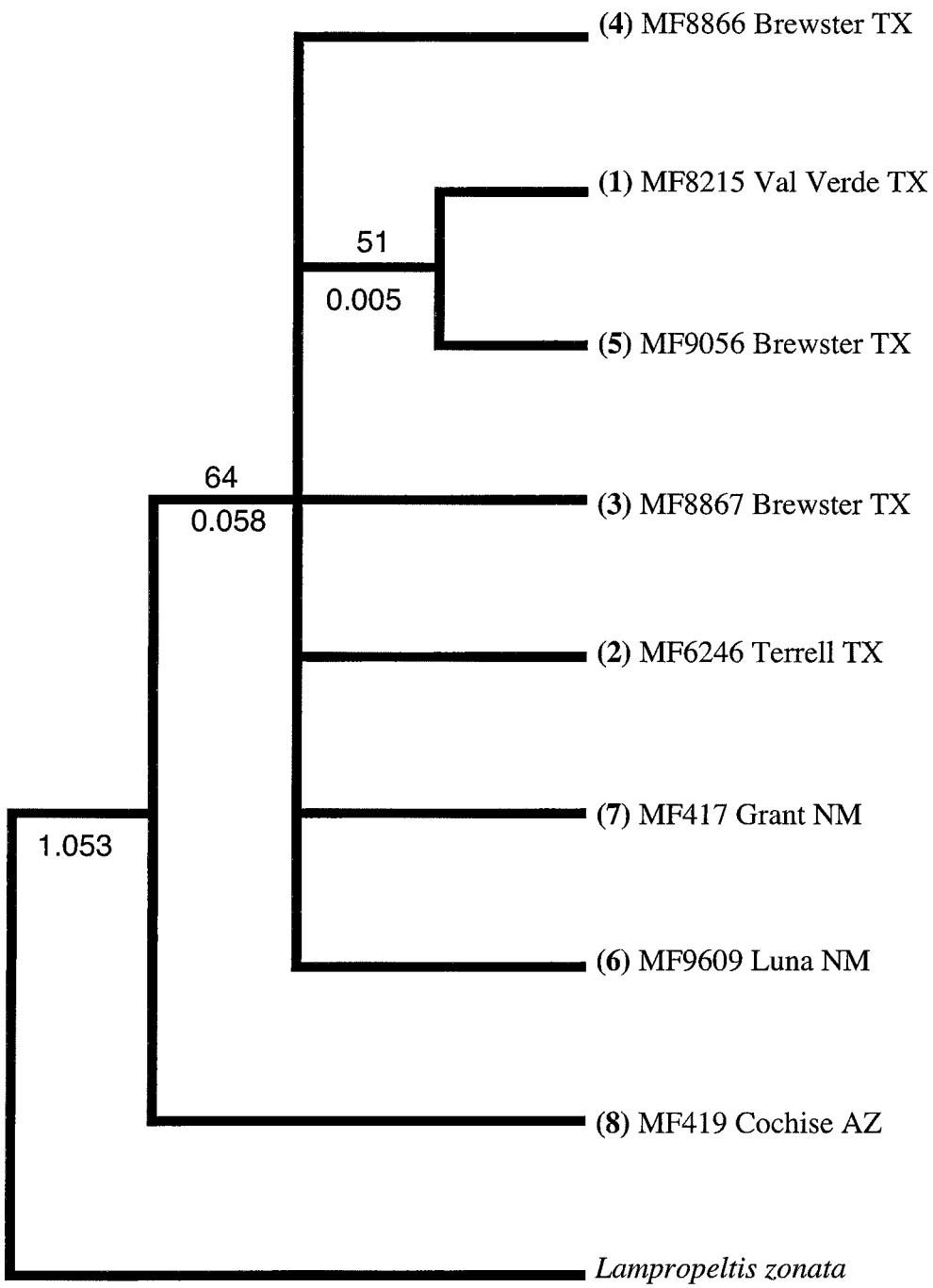

Fig. 24. Maximum Likelihood analysis from *R. lecontei* data set produced one tree with a –ln L of 1795.87165 following an exhaustive search with 1,000 replicates incorporating the GTR+I+G evolutionary model selected by MrModeltest 2.0 using the Akaike Information Criterion. Only branches with 50% or greater bootstrap values are shown. Bootstrap support is listed above each branch and branch lengths are given below each branch. Terminal node labels correspond to MF tissue catalog numbers and county and states from which samples were collected, numbers in parenthesis refer to localities in Fig. 24 and Table 3. *L. z. zonata* was used as an outgroup.

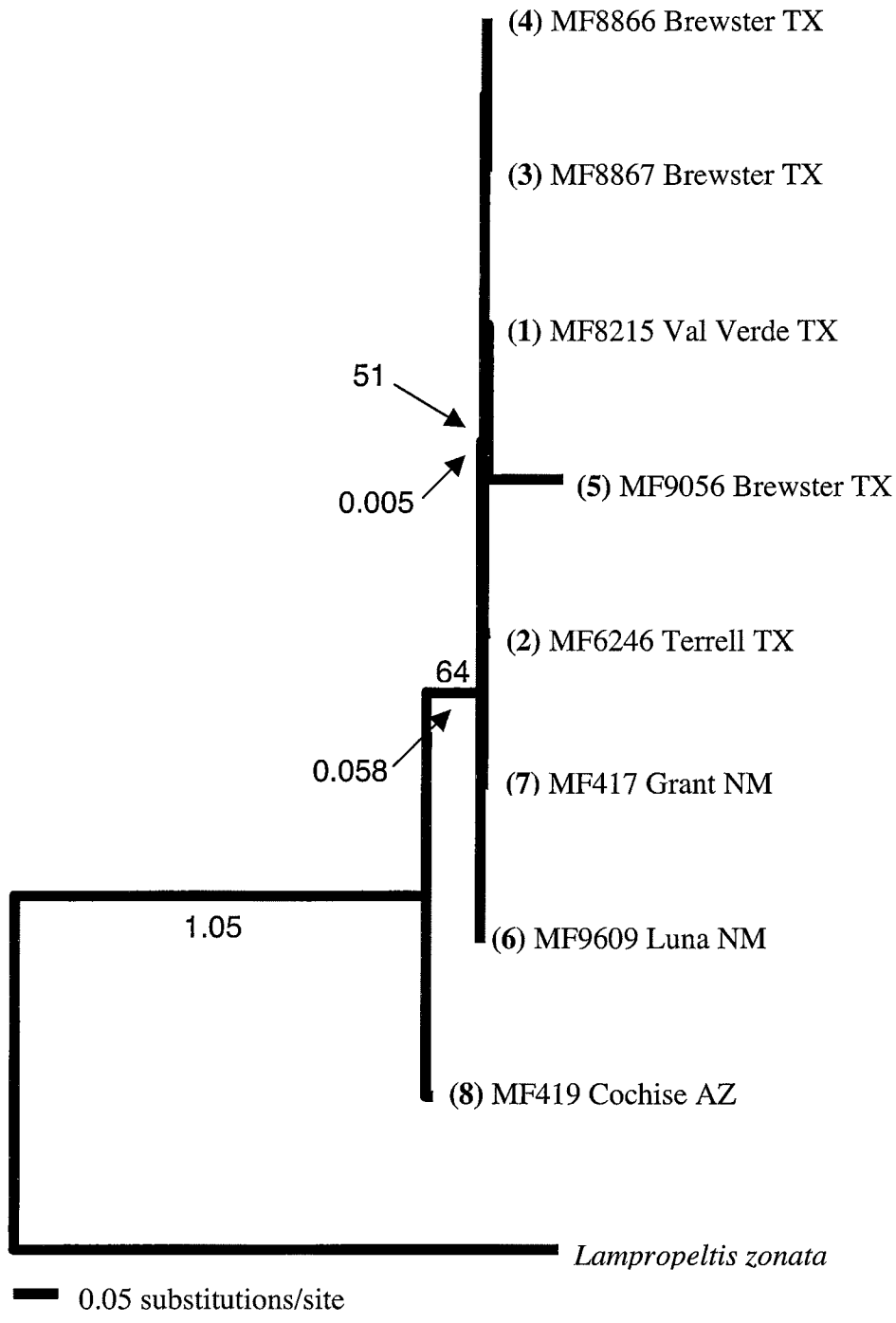

Fig. 25. Phylogram illustrating results of Maximum Likelihood analysis from *R. lecontei*. One tree with a –ln L of 1795.87165 following an exhaustive search with 1,000 replicates incorporating the GTR+I+G evolutionary model selected by MrModeltest 2.0 using the Akaike Information Criterion. Bootstrap support is listed above each branch and branch lengths are given below each branch. Terminal node labels correspond to MF tissue catalog numbers and county and states from which samples were collected. *L. z. zonata* was used as an outgroup.

DISCUSSION

This is the first study to explicitly test the hypothesis that the great Pleistocene pluvial lake, Cabeza de Vaca, influenced the genetic structure of northern Chihuahuan Desert biota. Concordant genetic structuring in multiple taxa was anticipated that would further demonstrate Axtell's (1977) hypothesized role of Cabeza de Vaca as a powerful vicariant sponsor. No published research has specifically tested the validity of this hypothesis. Guerra (1998) invoked the Cabeza de Vaca hypothesis to account for a large geographic break in genetic variation in populations of the Texas Horned Lizard, *Phrynosoma cornutum,* coincident with the location of the lake. The results presented here do not support a Cabeza de Vaca vicariance, instead providing support for another barrier, the Rocky Mountains.

Evaluation of recent, published molecular and morphology-based studies of taxa occurring in this region suggests that, despite the lack of recognition of the Cabeza de Vaca hypothesis, many taxa demonstrate divergence in the portion of the Chihuahuan Desert once occupied by the ancient lake.

The prospect that other taxa in the area bear the genetic imprint of this historical vicariant event was the objective of the current study. In order to differentiate between the effects of both older and more recent biogeographic phenomena, *a priori* predictions as to the amount of sequence divergence expected in focal taxa taken from the eastern and western peripheries of the ancient lake were constructed based on several assumed

rates of molecular evolution. Phylogenetic and phylogeographic analyses were then performed on recovered sequence data; the implications of these results are discussed individually for each taxon.

Phrynosoma cornutum. Results of phylogenetic analysis of *P. cornutum* demonstrate a genetic divergence of the ND4-Leucine gene region between individuals collected in the western portion of the lakebed and from a population northeast of the lakebed (Figure 26). The two samples collected from the eastern side of the lake shared one haplotype, which differed from all other samples by 6.05%, whereas samples collected from the western portion of the lakebed and further west differ from each other by a maximum of 2.95% and an average of 1.96% (Figure 26). Based on the estimated rates of nucleotide sequence evolution, this dates to a mid-Miocene to Pliocene era phenomenon, possibly coinciding with the final uplift of the Rocky Mountains. Because three of the four samples found in the western clade were collected East of the Rocky Mountains, it appears that western populations of *P. cornutum* may have dispersed eastward across the mountains, possibly through the Cochise filter barrier (Morafka 1977) long after the major east-west split. The Cochise filter barrier is a low-lying pass within the Rocky Mountains and is thought to have been a suture zone between populations East and West of the Rocky Mountains during interglacial periods.

Nested clade analysis of the combined data set similarly demonstrated the presence of two distinct clades (Appendix 2) separated by at least 40 nucleotide substitutions, further suggesting that the eastern and western clades are the result of population fragmentation, perhaps due to Rocky Mountain orogeny. The two sequences obtained in the current study that comprise the eastern clade (Figure 26) represent

samples from a geographic area not sampled in Guerra's (1998) analysis and demonstrate that the eastern clade she found may occur up to several hundred miles north and west of her eastern clade samples. While the combined eastern clade contains nearly three times as many haplotypes as the combined western clade, it also has almost twice as many samples. The geographic structure that the nested clade analysis recovered within the combined eastern clade is most likely due to restricted gene flow with isolation by distance based on the inference key of Templeton (2004), but should be interpreted with caution as the two nested clades found to have significant nested clade distances were composed of a total of only five samples, and geographically intermediate populations were not well sampled (Templeton 2004).

Overall, the phylogenetic and nested clade analyses presented here agree with the phylogenetic results obtained by Guerra (1998), however, implementation of molecular clocks refutes her suggestion that the large break was due to the Pleistocene lake, Cabeza de Vaca.

Urosaurus ornatus. Maximum likelihood analysis of *U. ornatus* similarly demonstrates a genetic break between individuals collected east and west of the lakebed (Figure 30). Individuals collected in southeastern Arizona exhibit an average sequence divergence of 11.35% from those collected in Texas; this value is substantially greater than the divergences found in the above taxa. Such a large break has likely been due not to the influence of Lake Cabeza de Vaca but to Miocene era phenomena, such as the orogeny of the Rocky Mountains. The results presented here corroborate the high level of genotypic diversity between populations previously reported for this species (Haenal 1997), suggesting that reduced gene flow between islands of populations is the norm for

this species. Based on the analyses presented here, taxonomic reclassification may be warranted in this taxon if further analyses corroborate the findings presented here.

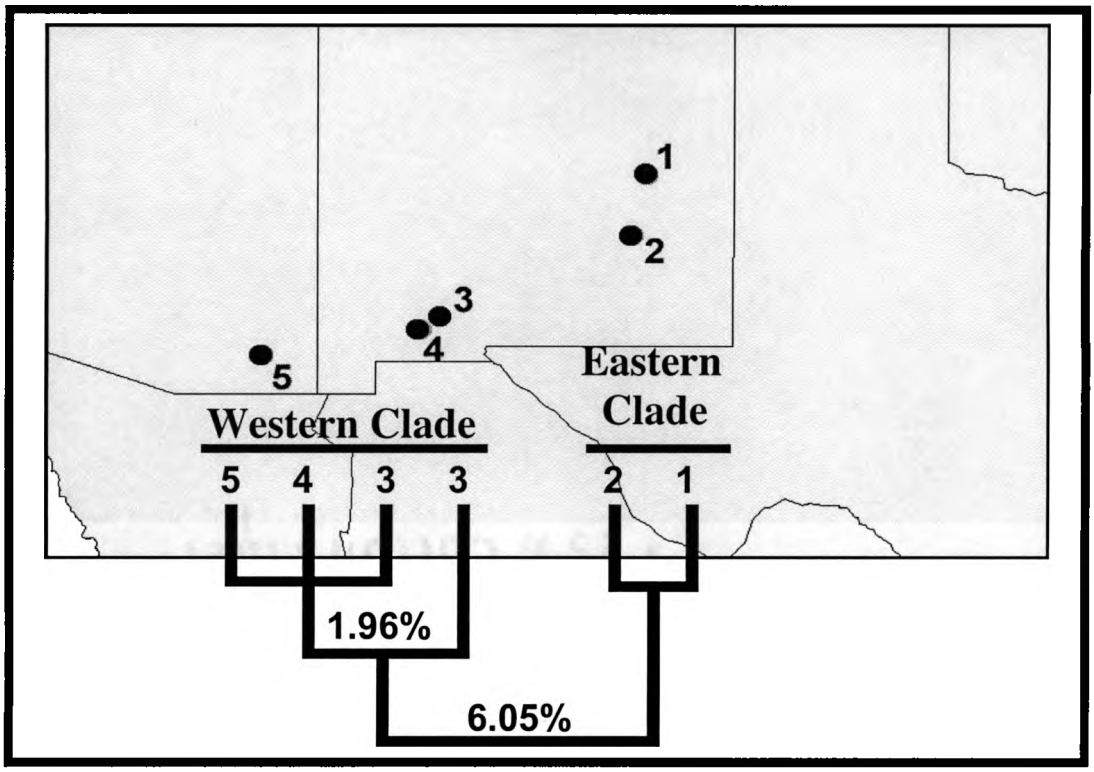

Fig. 26. Geographic depiction of phylogenetic relationships of *P. cornutum* sampled in the current study. Percentages given above branches represent averaged uncorrected sequence divergence between clades.

Rhinocheilus lecontei. Examination of the tree obtained by maximum likelihood and Bayesian analysis revealed that geographic structure exists in this taxon (Figure 26), and that a large divergence exists between samples from Texas and New Mexico and the sample from southeast Arizona, thus exhibiting a genetic break that coincides with the current morphology-based subspecies classifications, as evidenced by an average of4.47% nucleotide divergence between specimens collected from either side of the geographic species boundary (Figures 13, 28); the sample that is geographically closest to

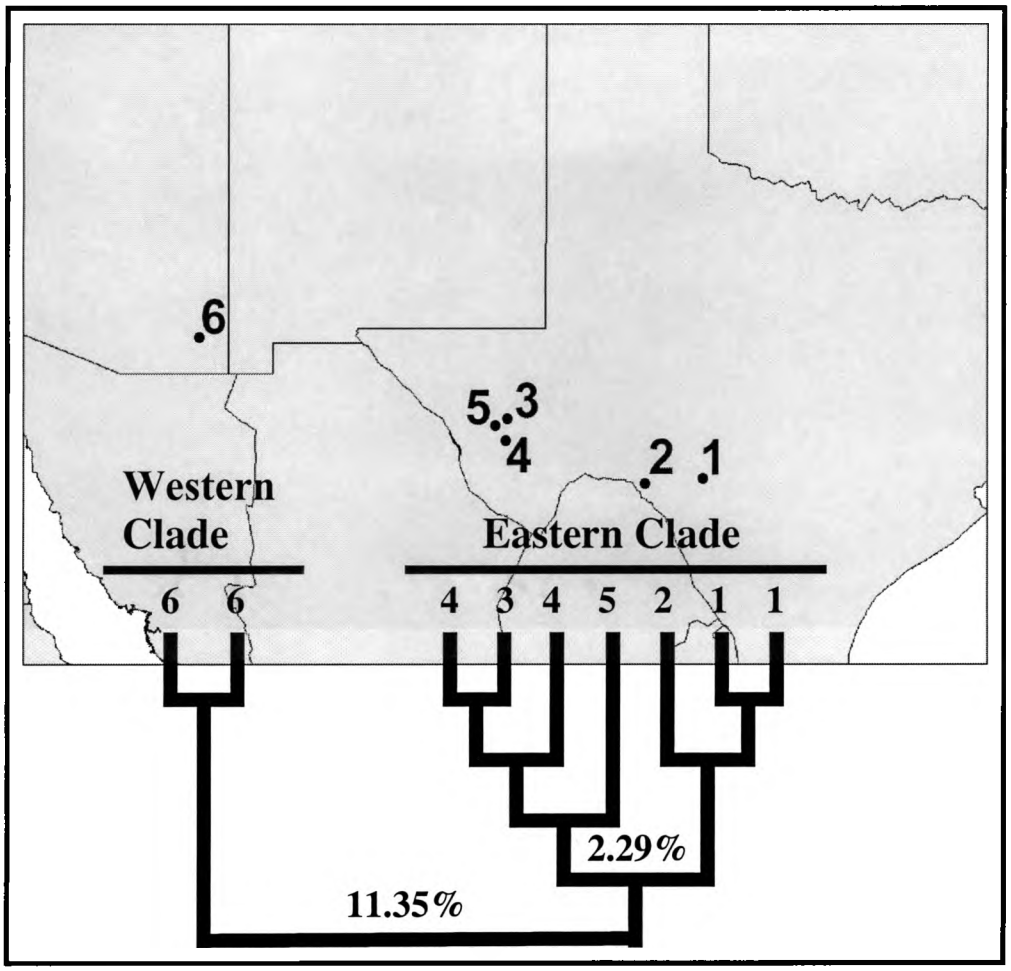

Fig. 27. Geographic depiction of phylogenetic relationships of *U. ornatus* sampled in the current study. Percentages given above branches represent averaged uncorrected sequence divergence between clades.

the Arizona sample differs from it by 4.47% (greater than the average difference between the two major clades), whereas the same sample (Grant NM) exhibits only a 0.59% divergence from the sample that is geographically most distant from it (Val Verde TX). Based on the comparison to predicted amounts of sequence divergence using all molecular clock values, the large break between the western and eastern clades is likely due to Late Miocene to Pliocene era phenomena (Table 3) and thus not a result of the

Pleistocene lake Cabeza de Vaca. The orogeny of the southern extension of the Rocky Mountains was likely the source of the recovered divergence, as the geographic break is congruent with the location of this mountain range. However, if molecular clock rates are in fact too slow, then this genetic break may be the product of the ancient lake if the eastern subspecies, *R. l. tessellatus*, invaded the newly created habitat that filled the northern Chihuahuan Desert as the lake evaporated with the advent of interglacial conditions. Interpretation of this data however, is limited by the paucity of samples analyzed; the apparent divergence may likely be due to inadequate sampling of both the study region as well as from the entire range of the organism. It is possible that the divergence observed between the Arizona and New Mexico/Texas samples is within the normal distribution of mitochondrial variation for this gene region in *Rhinocheilus*, especially considering that another sample (MF 9056) has a sequence divergence that ranges from 2.00 – 2.47% between all other samples within the New Mexico/Texas clade. Additionally, the maximum sequence divergence between *Rhinocheilus* samples (4.94%) falls within previously reported intraspecies divergence rates for colubrid snakes (Rodriguez-Robles and de Jesus-Escobar 1999).

Conclusions. The results presented here depict a history of northern Chihuahuan Desert fauna that have largely been influenced by Miocene and Pliocene phenomena. Climatically, these time periods were relatively stable in comparison to the glacial cycles of the Pleistocene, however, they were periods of intense mountain building in southwestern North America, therefore, geologic, rather than climatic activities may best explain the deep divergences recovered here. The hypothesis that can best explain these

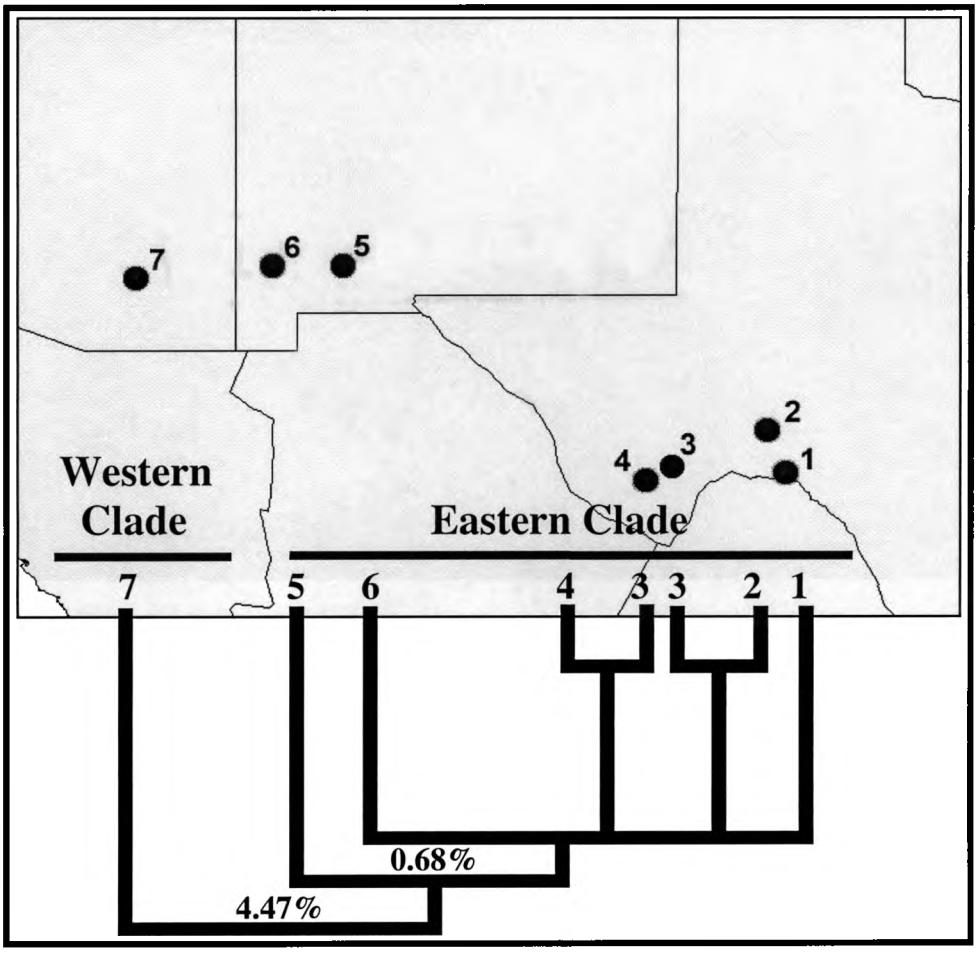

Fig. 28. Geographic depiction of phylogenetic relationships of *R. lecontei* sampled in the current study. Percentages given above branches represent uncorrected sequence divergence between clades.

results is that presented by Riddle (1995) and others, who suggest that observed variation in taxa from this region is due to the Miocene-Pliocene split of the Sonoran and Chihuahuan desert regions. While my results suffer from impoverished sampling, possibly obscuring the true relationships within these taxa, all taxa studied here support at least a Pliocene era vicariance between these two desert biota.

While these results do not indicate the influence of lake Cabeza de Vaca on these taxa, the ancient lake may have been a further barrier to dispersal across northeastern

Mexico and New Mexico, creating a second, more recent vicariant, making the biogeographic activity in this area more complex than previously thought. Selection of alternate taxa may well have resulted in the discovery of genetic breaks that coincide temporally and geographically with the location of the lake, and additional sampling of the taxa presented here may resolve the shallower divergences expected from Cabeza de Vaca, however, our results show no sign of its influence.

I am in agreement with Guerra (1998) that taxonomic revision of *P. cornutum* may be warranted if further research can verify the apparent divergence of eastern and western populations. More widespread sampling, use of both nuclear and mitochondrial gene regions, and use of morphological characters would strengthen subsequent phylogenetic analyses. The large East-West break recovered in *U. ornatus* similarly warrants further investigation, as it suggests that populations east and west of the Rocky Mountains represent unique species that have followed distinct trajectories for several million years, thus the species may require taxonomic revision. Because no known behavioral or sexual selection studies exist that use reciprocal transplants from individuals east and west of the Rocky Mountains, such research would be interesting and might clarify whether these populations can at least potentially interbreed. The relatively small divergence between *R. lecontei* samples, while too deep to have been caused by Cabeza de Vaca, nevertheless coincide with the current morphology-based subspecies classifications of *R. lecontei,* further justifying such classification on a molecular level.

Clearly, further higher density sampling of these taxa is necessary to capture the degree of genetic variation present across the species' entire ranges, however, here I

conclude the starting point for future research and with continued additional sample locales the current data set can be significantly enhanced.

The historical importance of Cabeza de Vaca as a force shaping animal populations in the northern Chihuahuan Desert continues to remain relatively elusive. While I continue to sample taxa in this area for evidence of historical divergence, I anticipate several potential problems. Some species or taxon pairs may be too recent to demonstrate a pattern of divergence (e.g., Zink 1996). Insufficient geographic sampling may also blur any differentiation (Burton 1998), as could recent gene flow (Bowen et al. 1994, Baer 1998). Cabeza de Vaca may not have existed long enough to cause differentiation, but even if differentiation is found in the area, the degree of divergence must correlate with what would be expected given the time frame of the lake's existence. For example, if the divergence is too shallow to have been caused by the ancient lake, it could be attributed to the effects of the last Pleistocene glacial-interglacial cycle and the subsequent formation of the Chihuahuan Desert, or, if the divergence is too deep, it may be due to a barrier that predated the formation of the lake, such as the mountain ranges that held the prehistoric lake. Population modeling may be necessary to disentangle the effects of all of these potential barriers on the population structure of the taxa under study; such modeling *a priori* adds more rigor to the experimental design (Knowles and Maddison 2002, Bermingham and Moritz 1998, Templeton 1998).

Additionally, it may be found that the DNA region used in this study evolves too fast or too slow in the species under study, ideally, more than one DNA region should be analyzed for phylogenies of and below the species level (Hewitt 2001). Additionally, karyotyping of individuals from each population might uncover genomic differences

between populations not discernable by mtDNA sequence data analysis that could be useful for reconstructing the evolutionary paths of these taxa (e.g. Surget-Groba et al. 2001.)

Regardless of the difficulties with interpretation of the data or with assignation of the lake as the ultimate cause of multiple phylogenetic divergences, approaches such as the one presented here, in which consensus across cladograms is sought in order to address recent and regional evolutionary forces, should be encouraged (Bermingham and Moritz 1998, Hewitt 2001), rather than be limited to larger scale or higher order relationships (Humphries and Parenti 1989). Empirical and regional biogeographic studies may also help to separate the relative roles of vicariance and dispersal in shaping the distributions, and thus evolutionary trajectories, of organisms (Zink et al. 2000). While this study design compromised the total number of samples per taxon for an increased number of taxa, our analyses nevertheless recovered substantial genetic breaks in all taxa that are unlikely to be purely artifactual; it is doubtful that such deep breaks will disappear with increased numbers of samples and sample sites. Thus, our approach, in which a 'geo-genetic scan ' of multiple taxa is implemented, may in fact be a particularly useful way to uncover formerly unrecognized population variation, which may be important for discovering new areas of endemism and for identification of areas in which the genetic diversity of conservation unit is sought.

Despite the lack of attention Cabeza de Vaca has received as an evolutionary force in the southwestern United States (Axtell, pers. comm. to J.R. 2003), it is not due to a lack of circumstantial evidence. In addition to the examples previously mentioned, many other taxa demonstrate either range boundaries or subspecific differentiation at the

area in which the lake occurred (see Axtell 1974 for partial list, consult Stebbins 1985, Conant and Collins 1998, and Burt and Grossenheider 1976 for further examples). Additionally, a recent phylogeographic study of the canyon treefrog, (*Hyla arenicolor*) (Barber 1999) and a molecular phylogeny of the short-horned lizard (*Phrynosoma douglassi*) (Zamudio et al. 1997) demonstrate populational and subspecific differentiation, respectively, that, upon reexamination, may be found to be attributable to the presence of the lake.

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APPENDIX 1.

Required Permits. The following permits are required for collection and handling of animal specimens and tissue samples:

A. IACUC # 04-3D2AAE71

B. State of Texas hunting license (Doc. # 439300001179)

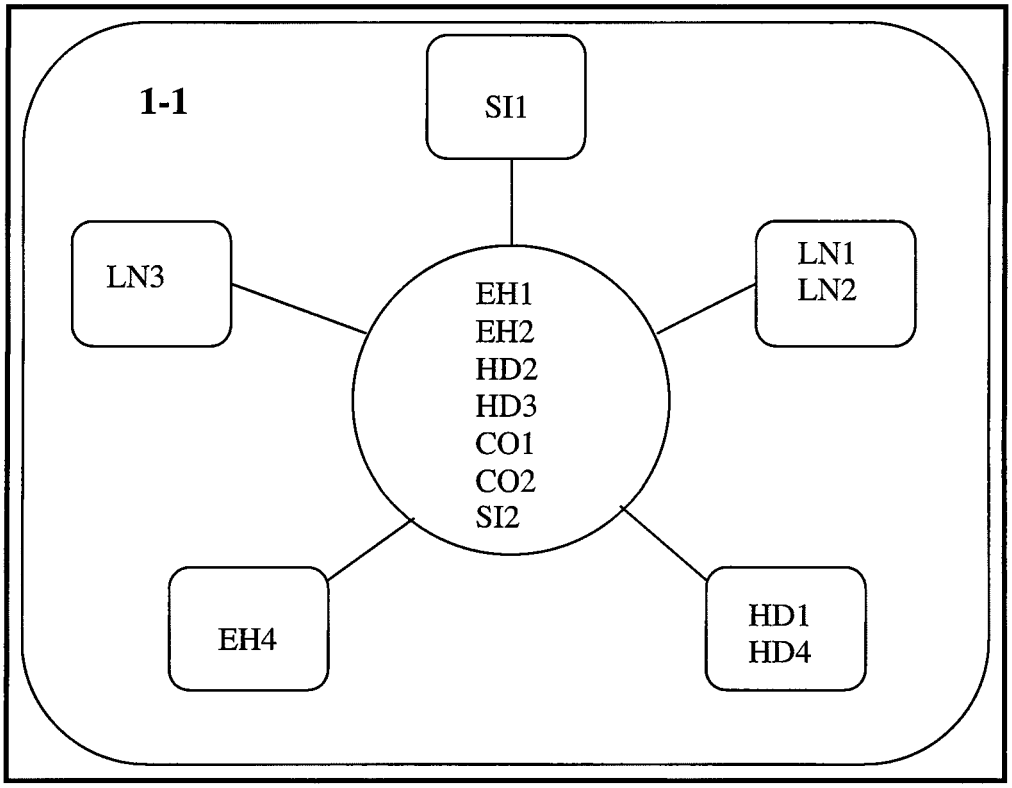
C. Texas Parks and Wildlife Scientific Permit (# SPR-0102-191)

D. New Mexico Game and Fish Scientific Permit (#3236)

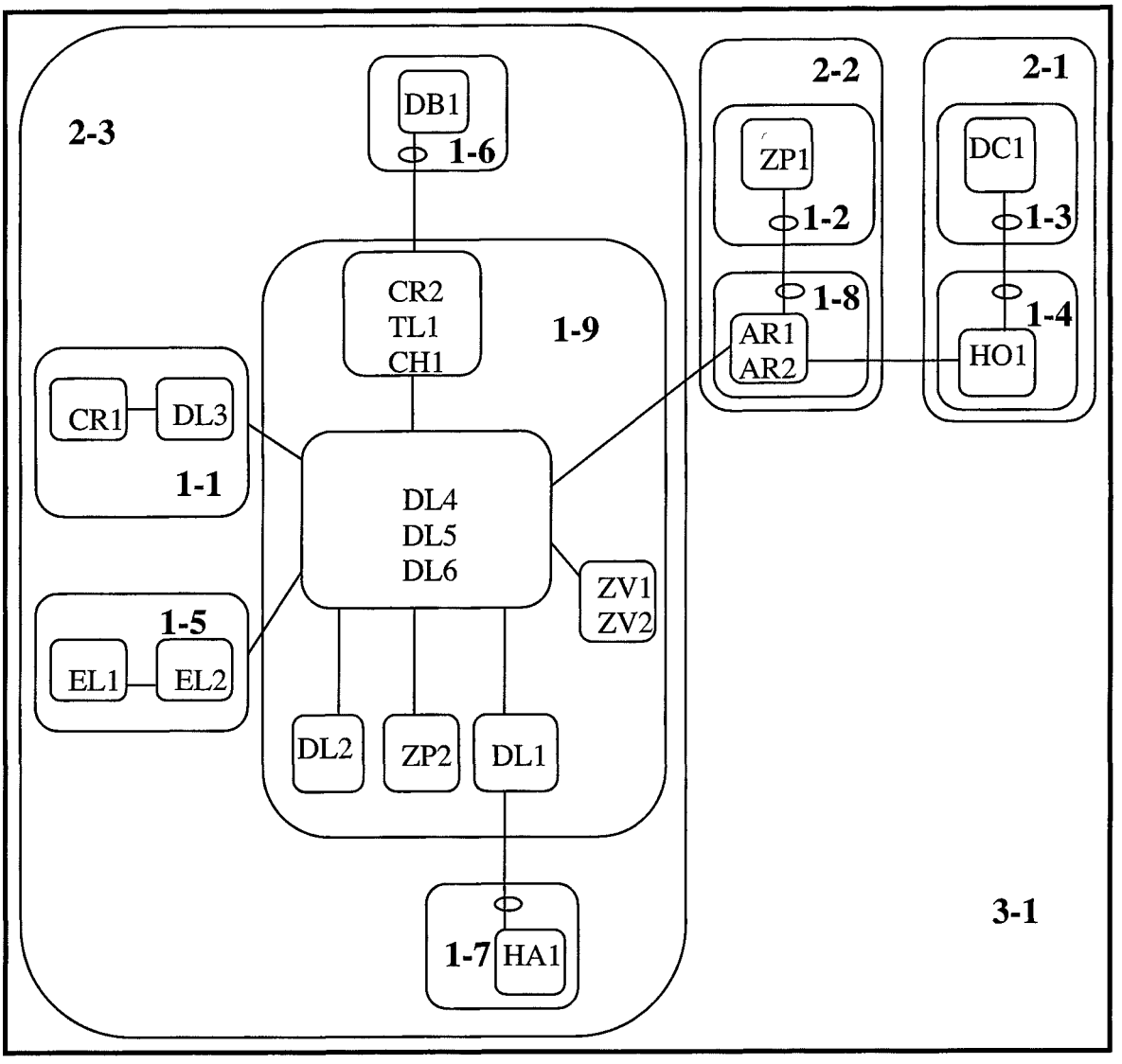
APPENDIX 2.

Nested cladograms of *Phrynosoma cornutum* mitochondrial ND4 sequence data, including 31 samples obtained from Guerra (1998). Statistical parsimony as implemented by TCS resulted in two unconnected networks. Each line represents a single mutation, and a small blue ovals represent haplotypes not present in the samples. Letters refer to individuals listed in Appendix 3.

Haplotype Network 1.



Haplotype Network 2



APPENDIX 3.

Locality data for *Phrynosoma cornutum* samples used in Nested Clade Analysis. Mitochondrial sequence data for the first 31 samples in this list were obtained from Guerra's (1998) analysis. Sequence data for the bottom six samples came from the current study and are referenced by MF catalog numbers in parentheses following county and state information. ID letter and number refer to figures in Appendix 2.

Taxa Name	County and State	ID	Longitude/Latitude
P. cornutum HD1	Hidalgo Co. NM	H1	31.9164 N, 108.7117 W
P. cornutum HD2	Hidalgo Co. NM	H2	31.9164 N, 108.7117 W
P. cornutum HD3	Hidalgo Co. NM	H2	31.9164 N, 108.7117 W
P. cornutum HD4	Hidalgo Co. NM	H3	31.9164 N, 108.7117 W
P. cornutum CO1	Cochise Co. AZ	H4	31.8908 N, 109.7369 W
P. cornutum LN1	Luna Co. NM	H5	32.1807 N, 107.7486 W
P. cornutum LN2	Luna Co. NM	H6	32.1807 N, 107.7486 W
P. cornutum U1	El Paso & Hudspeth Co. TX	H7	31.4521 N, 105.3742 W
P. cornutum EH1	El Paso & Hudspeth Co. TX	H2	31.4521 N, 105.3742 W
P. cornutum EH2	El Paso & Hudspeth Co. TX	H2	31.4521 N, 105.3742 W
P. cornutum EH3	El Paso & Hudspeth Co. TX	H8	31.4521 N, 105.3742 W
P. cornutum DL1	Dimmit & LaSalle Co. TX	H10	28.3442 N, 99.1003 W
P. cornutum ZV1	Zavala Co. TX	H11	28.8643 N, 99.7596 W
P. cornutum ZV2	Zavala Co. TX	H12	28.8643 N, 99.7596 W
P. cornutum CR1	Carson Co. TX	H13	35.4054 N, 101.3548 W
P. cornutum AR1	Aransas Co. TX	H14	28.0956 N, 96.9879 W
P. cornutum EL1	Eastland Co. TX	H15	32.3310 N, 98.8277 W
P. cornutum HO1	Howard Co. TX	H16	32.3091 N, 101.4390 W
P. cornutum AR2	Aransas Co. TX	H17	28.0956 N, 96.9879 W
P. cornutum CR2	Carson Co. TX	H18	35.4054 N, 101.3548 W
P. cornutum TL1	Terrell Co. TX	H19	30.2260 N, 102.0669 W
P. cornutum EL2	Eastland Co. TX	H20	32.3310 N, 98.8277 W
P. cornutum HA1	Hall Co. TX	H21	34.5213 N, 100.6925 W
P. cornutum DL2	Dimmit & LaSalle Co. TX	H22	28.3442 N, 99.1003 W
P. cornutum DC1	Dickens Co. TX	H23	33.6201 N, 100.7790 W
P. cornutum ZP1	Zapata Co. TX	H24	26.9999 N, 99.1830 W
P. cornutum ZP2	Zapata Co. TX	H25	26.9999 N, 99.1830 W
P. cornutum DL3	Dimmit & LaSalle Co. TX	H26	28.3442 N, 99.1003 W

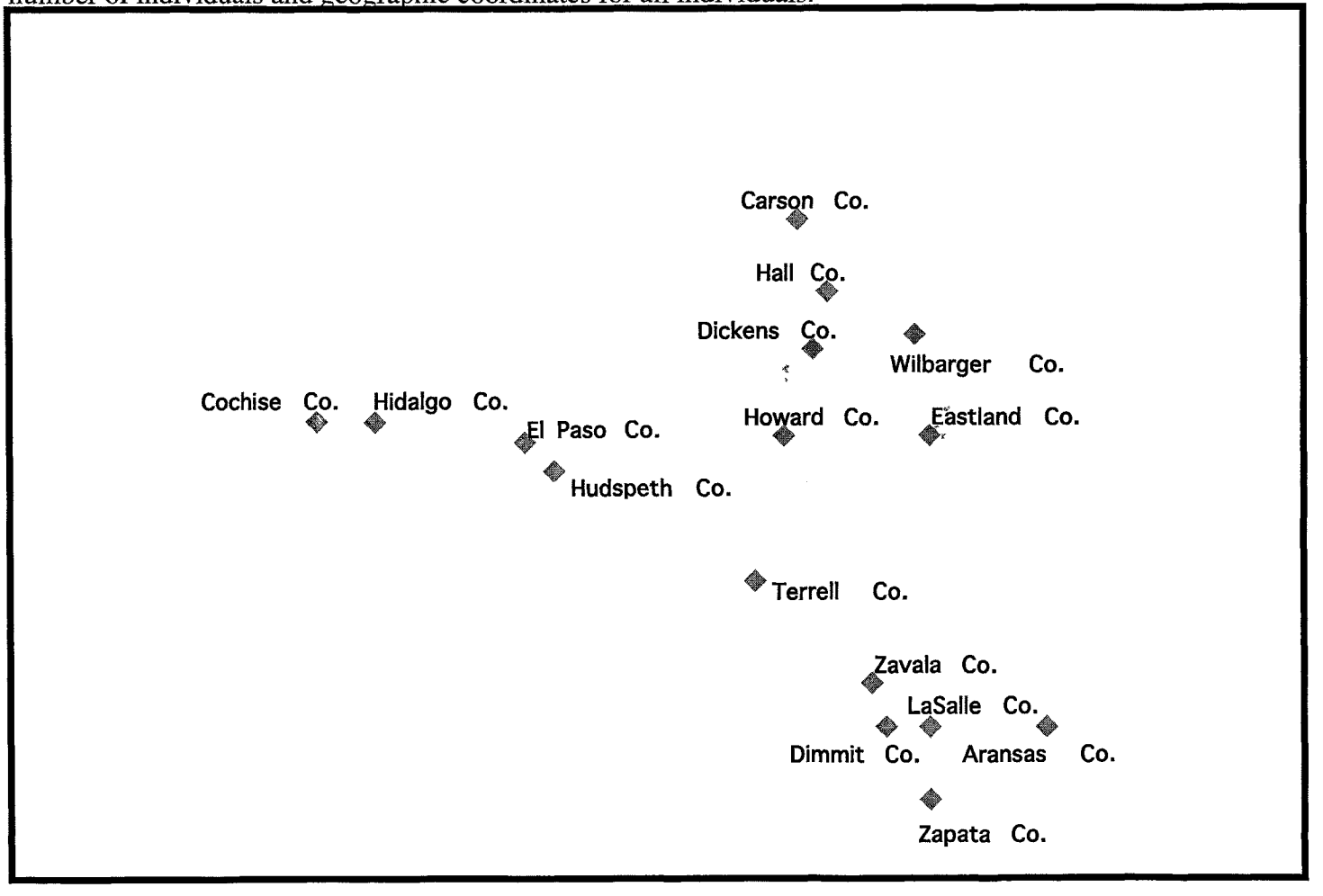
P. cornutum DL4	Dimmit & LaSalle Co. TX	H27	28.3442 N, 99.1003 W
P. cornutum DL5	Dimmit & LaSalle Co. TX	H27	28.3442 N, 99.1003 W
P. cornutum DL6	Dimmit & LaSalle Co. TX	H27	28.3442 N, 99.1003 W
P. cornutum SI1	Sierra Co. NM (MF9622)	H28	33.7377 N, 107.5825 W
P. cornutum DB1	DeBaca Co. NM (MF6128)	H29	34.0194 N, 104.5375 W
P. cornutum SI2	Sierra Co. NM (MF9621)	H2	33.7377 N, 107.5825 W
P. cornutum.LN3	Luna Co. NM (MF9623)	H30	32.5955 N, 108.0161 W
P. cornutum CO2	Cochise Co. AZ (MF7453)	H2	31.8383 N, 109.7033 W
P. cornutum CH1	Chaves Co. NM (MF6102)	H18	33.5002 N, 104.5369 W

APPENDIX 4.

Nested cladistic analysis for *Phrynosoma cornutum* using combined data set. RGF = restricted gene flow; IBD = isolation by distance.

Clade	Chi^2 statistic	Probability	Inference chain	Inferred pattern
3-1	39.6863	0.0030	1-2-11-17-4-No	RGF with IBD

APPENDIX 5. Specimen localities from Guerra (1998) used for nested clade analysis. Appendix 3 provides number of individuals and geographic coordinates for all individuals.



VITA

Jonas was born in East Patchogue, Long Island, New York, in 1975. He grew up in Fort Worth, Texas and California. He moved back to Texas to attend the University of North Texas, where he received his bachelor's degree in 1998. In 2000, he began graduate study at Texas State University-San Marcos. While working toward his Master's Degree, he was employed as an office manager, a delivery driver, an administrative assistant, and was a supervisor at the Austin Zoo. He was also employed as an instructional assistant for Organismal, Herpetology, and Genetics laboratories. During this period he also hosted a radio show on KTSW. He is currently employed by the Oakland Zoo.

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