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01-08-2007

Dear Arizona Game and Fish Department,

Enclosed/Attached is the "Assessment of Genetic Diversity in the Western Shovel-nosed Snake (*Chionactis occipitalis*), with Special Emphasis on the Subspecific Status of the Tucson Shovel-nosed Snake (*C. o. klauberi).*"

These data are released as part of our agreement with you.

If you have any questions, please contact Dustin Wood, Dr. Amy Vandergast or Dr. Robert Fisher of our San Diego Field Station at 619-225-6458. Thank you very much.

Sincerely,

for Karen J. Phillips

Dr. Steven E. Schwarzbach
Center Director
USGS, Western Ecological Research Center
619-278-9490

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Assessment of Genetic Diversity in the Western Shovel-nosed Snake (*Chionactis occipitalis*), with Special Emphasis on the Subspecific Status of the Tucson Shovel-nosed Snake (*C. o. klauberi*)

By Dustin A. Wood, Amy G. Vandergast, and Robert N. Fisher

U.S. Department of the Interior
U.S. Geological Survey

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Prepared for: Arizona Game and Fish Department

San Diego Field Station
Western Ecological Research Center
U.S. Geological Research Center
4165 Spruance Road, Suite 200
San Diego CA, 92101

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Cover photo of the Tucson Shovel-nosed Snake (*C. o. klauberi*) taken by Thomas Brennan.

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Assessment of Genetic Diversity in the Western Shovel-nosed Snake (*Chionactis occipitalis*), with Special Emphasis on the Subspecific Status of the Tucson Shovel-nosed Snake (*C. o. klauberi*)

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Abstract

We examined the phylogeography, population structure, and subspecies taxonomy of the western shovel-nosed snake (*Chionactis occipitalis*) across its geographic range with genetic analysis of approximately 1100 bases of ND1 mitochondrial DNA sequence. A phylogeny was reconstructed from 81 snakes using maximum parsimony and Bayesian methods, and nested cladistic phylogeographical analysis was used to help discriminate between evolutionary processes operating at the population level. The phylogeny reveals significant geographical structuring of haplotypes and two distinct regional lineages (West Mojave and Sonoran/East Mojave). Diversification between these lineages appears to have developed as a result of vicariance. In addition, patterns of isolation by distance, suggesting reduced gene flow, occur throughout regions of each lineage and have contributed to the population structure among collection localities.

While our analysis revealed significant geographical structuring of haplotypes none of the currently defined subspecies form an exclusive/ monophyletic group by themselves. Instead, the simplest conclusion based on the current available data suggests two distinct subspecies, which would be formed by combining western populations of *C. o. occipitalis* with *C. o. talpina,* and eastern populations of *C. o. occipitalis* with *C. o. annulata* + *C. o. klauberi*. In addition, mtDNA data suggest

specimens currently recognized as *C. o. klauberi* are embedded in a larger geographic clade that resulted from recent range expansion from western populations, and these data are concordant with the west-to-east clinal variation exhibited in morphology.

Introduction

The geographic distribution of genetic variation within species can provide valuable information on evolutionary and ecological processes, such as gene flow (migration and successful mating) and vicariance, that have shaped genetic diversity throughout a species' history (Avise 1994; Templeton 1998). Gathering this information can also inform conservation decisions because effective management efforts often first require defining distinct population segments or evolutionarily significant units, which are often based on genetic and/or morphological characters (Stanford Environmental Law Society 2001). While much debate has centered around diagnosing units below the species level, it has become increasingly clear that examination of multiple characters (independent lines of evidence) and the processes that influence the diversity of those characters (or lack thereof) is necessary to accurately delineate units for conservation. For example, many studies based on single lines of evidence, whether genetic sequence data or morphology, have been shown to be problematic once additional characters have been examined (Janzen *et al*. 2002; Shaw 2002; Babik *et al.* 2005). At the present time no universally accepted criteria exist for diagnosing subspecies; however, other units, such as evolutionary significant units (ESU), have received more attention (Crandall *et al.* 2000; Moritz 1994). In principle, an ESU is one or more population units with a distinct long-term evolutionary history (potentially exhibiting adaptive divergence) that is separate from other population units (Ryder 1986). Thus, ESUs are the primary source of historical genetic diversity within a species that merit special consideration in conservation efforts. However, operational genetic criteria for recognizing ESUs have differed. Moritz (1994) defined ESUs as groups of populations that are "reciprocally

monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci". In contrast, Crandall *et al*. (2000) established criteria for ESU designation based on genetic, ecological, recent, and historical categories and recommended management actions based on evidence for 8 separate cases.

We began examination of genetic variation based on mitochondrial DNA (mtDNA) to test hypotheses (=subspecies) concerning the evolutionary history of the western shovel-nosed snake (*Chionactis occipitalis*) and gain insights into processes driving the distribution of genetic diversity within this species. We worked under the assumption that if current morphologically based subspecies are valid taxonomic entities, then these groupings should imply some indication of evolutionary history. Thus, it seemed reasonable to expect that these diagnoses exhibit concordant exclusive or near-exclusive patterns based on mtDNA markers. However, discordance between subspecies (based primarily on color patterns) and mtDNA lineages seems to be a consistent pattern in snakes as well as other taxa (e.g., Rodriguez-Robles *et al*. 1999; Burbrink *et al*. 2000; Janzen *et al*. 2002; Leache & Reeder 2002; Zink *et al*. 2000; Zink *et al*. 2003). If genetic data confirm concordant patterns across subspecies boundaries then multiple lines of evidence can be used to support subspecies evolutionary history. However, if discord between mtDNA and subspecies designations is revealed then further data may be needed to investigate the cause of incongruence, and existing subspecies designations may be called into question.

The western shovel-nosed snake (*C. occipitalis*) is a small colubrid snake inhabiting the arid and semi-arid valley floors of the Mojave and Sonoran deserts. Morphological assessments of taxonomy currently recognize four subspecies: the Mojave shovel-nosed snake (*C. o. occipitalis*), Colorado Desert shovel-nosed snake (*C. o. annulata*), Nevada shovel-nosed snake (*C. o. talpina*), and the Tucson shovel-nosed snake (*C. o. klauberi*). The subspecies are distinguished partly by ventral scale counts and number of dark bands encircling the body, but the most striking variation is in pattern and coloration of

secondary bands (Stickel 1941; Klauber 1951). *C. o. klauberi* has the most restricted range of all the subspecies, known historically from disjunct desert valleys of the Sonoran Desert in south-central Arizona (Fig. 1). There is recent concern that *C. o. klauberi* has experienced a significant decline in population size and range over the past 25 years due to substantial destruction of its habitat through agricultural and urban development, and existing populations are further threatened by continuing urban expansion of Tucson and Phoenix metropolitan areas (Rosen 2003). These factors have prompted a petition to list the subspecies as endangered under the U. S. Endangered Species Act (Center for Biological Diversity 2004). However, the validity of *C. o. klauberi* is obfuscated by the fact that it forms a purported zone of intergradation with *C. o. annulata* across central Arizona. *C. o annulata* occurs within the Lower Colorado Valley portions of the Sonoran desert in southeastern California and extends eastward where it forms a broad zone of contact with *C. o. klauberi* across Maricopa and southwestern Pima Counties, Arizona (Klauber 1951). These two subspecies differ in morphology, both in secondary band color and degree of maculation (which are not mutually exclusive characters); however, specimens taken from within the purported intergrade zone are often difficult to diagnose. The nature of this geographic variation is of interest for evolutionary and conservation reasons. If all *C. o. klauberi* possess *C. o. annulata* mitochondrial haplotypes as the result of either introgression or recent shared history (i.e. haplotypes from each subspecies are not exclusive with respect to each other), then all collection sites of *C. o. klauberi* including locations within the intergrade zone may be nested within a larger ESU, applying the criteria of Moritz (1994). However, if haplotypes from collection locations on opposite sides of the intergrade zone form exclusive or near-exclusive groups (i.e. haplotypes for the most part are not shared between the subspecies) then genetic evidence will support designation of *C. o. klauberi* populations as a separate unit.

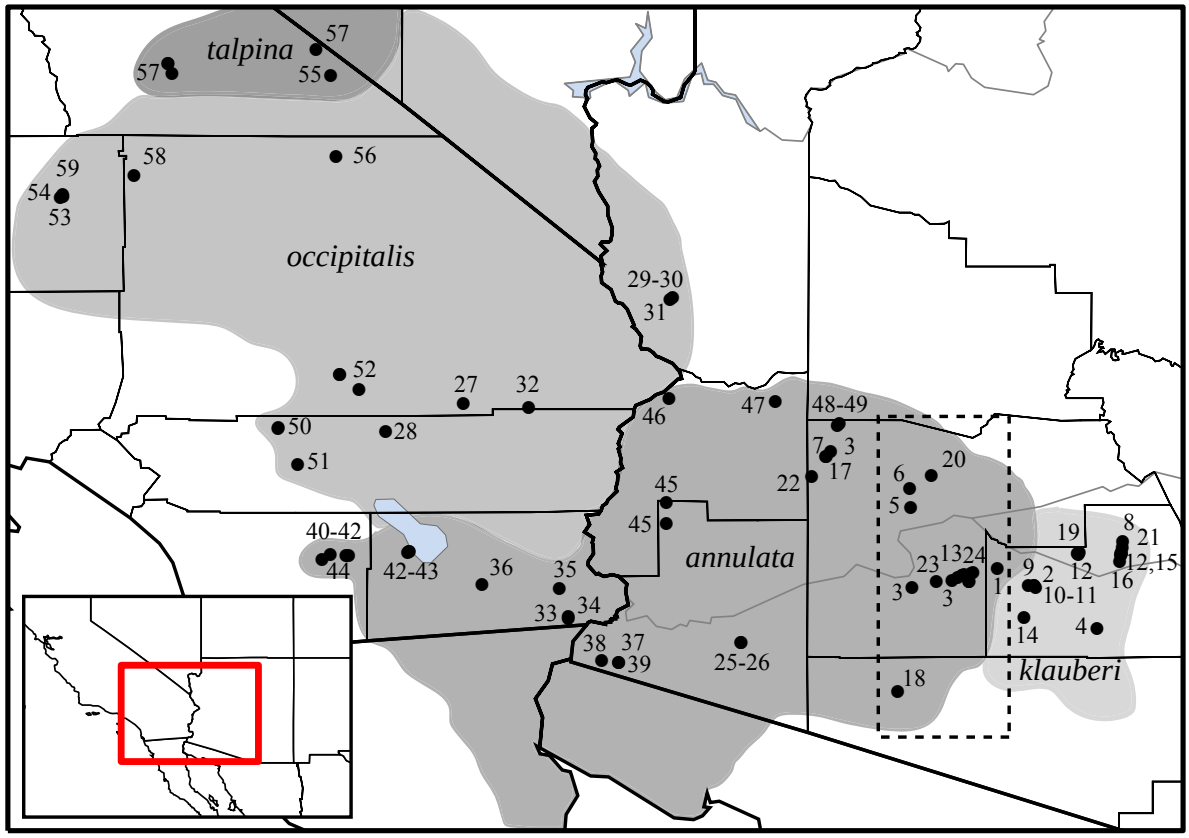

Figure 1. Generalized morphological subspecies ranges of *Chionactis occipitalis*. Shaded areas represent each subspecies range based on Stickel (1941) and Klauber (1951). Black points correspond to collection locations examined in this study and numbers correspond to mtDNA haplotypes observed at each collection location. The dashed box outlines the approximate morphological intergrade zone observed between *C. o. annulata* and *C. o. klauberi*.

Our goal was to examine the genetic structure among subspecies of *C. occipitalis,* with particular focus on individuals within the known range of the *C. o. klauberi.* An understanding of the distribution of genetic variation within *C. occipitalis* may aid in future management/planning decisions, such as determining levels of protection for remaining populations and determining best source populations for recolonization or augmentation if such management actions are deemed necessary. Specifically, we address the following: 1) Do the current subspecies designations represent genetically

distinct lineages? 2) Are collection sites of *C. occipitalis* found in south-central Arizona (currently designated as *C. o. klauberi*) genetically differentiated from collection sites throughout the rest of the species range? 3) Lastly, acquiring knowledge of dispersal patterns via genetic data can be an important first step to effective preservation and management, especially when information on individual movement and population connectivity is only poorly understood. As such, we will attempt to address where genetic connectivity exists.

Materials and Methods

Sampling and tissue acquisition

Tissue samples were obtained from 81 specimens of *Chionactis occipitalis* from 73 collection localities throughout California and Arizona (Fig. 1; Appendix 1). Sampled localities adequately represent the distributional range of the species and include all formally recognized subspecies. We were unable to obtain samples from Nevada or Mexico. The exclusion of these samples, however, should have little effect on our ability to identify major clades and test subspecies hypotheses within *Chionactis* since these regions constitute less than 10% of the species range.

Generation of sequence data

A portion of the mitochondrial NADH dehydrogenase subunit I (ND1) gene was used to estimate population structure and infer phylogenetic relationships within *C. occipitalis*. The ND1 gene is a relatively rapidly-evolving gene and has been useful for resolving relationships both within species and among closely related species for a variety of taxa (e.g. Hedin 1997; Leache & Reeder 2002; Wood 2002)

Tissues of liver and/or tail tips were stored in 95% ethanol until DNA was isolated following standard guanidine extraction methods described in Hillis *et al*. (1996) or commercial tissue extraction

kits (DNEasy kits, Qiagen, Inc.). The polymerase chain reaction (PCR) was used to amplify a ~ 1100 base pair (bp) fragment of mtDNA that included most of the ND1 gene, as well as a small portion of the 16S rRNA gene. The primers used for PCR were 16dR (5'CTACGTGATCTGAGTTCAGACCGGAG-3'; Leache & Reeder 2002) and tIle-R (5'TCTCRGGCACAYTTCCATTGTGGT-3'; Wood 2002). PCR products were purified with ULTRACLEAN™ PCR Clean-up Kit (MoBio Laboratories, Inc.), subjected to direct DNA cycle-sequencing using the BigDye Cycle Sequencing Kit (PE Applied Biosystems), and run on an ABI 3100 capillary system. Primers used for sequencing were the same as those used for PCR amplification, and Sequencher™ 4.5 was used to edit and link DNA sequence fragments. Alignments were not problematic due to conserved amino acid codon positions and low levels of genetic divergence among the samples. However, to aid in the identification of possibly ambiguously aligned insertions/deletions positions within the 16S segment, the sequences were aligned using various opening gap costs (=6, 9, 12) implemented by Clustal X (Thompson *et al*. 1997). Nucleotide positions that were aligned differently under any of the gap opening costs were considered ambiguous and were excluded from analyses (Gatesy *et al*. 1993).

Determining genealogical and geographical relationships among haplotypes

Phylogenies have become essential tools for understanding patterns of lineage diversification at the population level (Avise 2000). As such, three types of phylogenetic analysis were conducted: including Maximum Parsimony (MP), Bayesian inference, and Nested Cladistic Phylogeographical Analysis (NCPA). Analyses were used to define lineage relationships among collection locations, test for genetic evidence for subspecies boundaries, and to provide a phylogenetic context for our samples from south-central Arizona. An unweighted maximum parsimony analysis was implemented in PAUP* 4.0b10 (Swofford 2002) using the heuristic search option with 5000 random addition sequence replicates, tree-bisection-reconnection (TBR) branch swapping, and gaps coded as missing data. Confidence in inferred clades from the maximum parsimony analysis was assessed using nonparametric

bootstrapping (Felsenstein 1985). Nodes supported by bootstrap values of ≥ 70% were considered to be strongly supported (Hillis & Bull 1993).

Bayesian methods of phylogeny reconstruction were also used because they can incorporate important aspects of molecular evolution (e.g., complex nucleotide substitution models) that are difficult to implement in parsimony analyses (Larget & Simon 1999). In addition, Bayesian inference has the ability to analyze the data in a statistical framework producing best sets of trees (i.e. 95% credible set of trees) with an approximation of the posterior probability distributions of all parameters, such as tree topology, branch lengths, and model parameter estimates (Rannala & Yang 1996). Bayesian analyses were conducted using the program MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001). An appropriate model of sequence evolution was determined using the likelihood ratio test (LRT) implemented with MrModeltest (Nylander, 2002). Two simultaneous, independent analyses were run to help determine when convergence had been achieved (i.e. when average likelihood scores, $-\ln L$, from the two runs differed by less than a few tenths of a point). Each analysis consisted of 5.0×10^6 generations with the first 500,000 generations discarded as burn-in (i.e. the number of generations at which convergence was achieved). Clades with posterior probabilities (*Pp*) of ≥ 0.95 were considered strongly supported (Wilcox *et al*. 2002; Alfaro *et al*. 2003). Following the outgroup method, *C. palarostris*, the closest relative of *C. occipitalis* (Klauber 1951; Mahrdt *et al*. 2001), was used to infer the root of the phylogenetic tree for both MP and Bayesian analyses.

NCPA was used to test for significant associations between genetic variation and geography, and to distinguish among alternative potential causes of such geographical associations (Templeton 2004). Using traditional population genetic estimates (such as *F*st estimates) it is often difficult to distinguish between current processes and historical events that influence population genetic structure (Templeton 1998). However, NCPA uses statistical techniques to analyze both the genealogical relationships and spatial distribution of genetic variation which allows the researcher to separate the effects of current

population structure from past events, such as geographic range changes (i.e. range expansion) and fragmentation events (i.e. vicariance), that occurred in the history of the species (Templeton *et al*. 1995). To implement NCPA, we used the Templeton *et al*. (1992) haplotype network estimation, as implemented in the TCS v1.21 software of Clement *et al.* (2000), to resolve intraspecific relationships for all haplotypes recovered. This method estimates the maximum number of mutational steps among haplotypes as a result of single substitutions with a 95% statistical confidence (described in Templeton *et al*. 1992). Multiple haplotype networks were obtained that were comparable to the clades inferred from Bayesian and MP analyses. The networks could not be joined because divergences between them exceeded the 95% confidence limits of statistical parsimony. Therefore, overall relationships between the networks were inferred using the Bayesian tree and grouped as sister clades at equal nesting levels. Ambiguities within networks were resolved using published guidelines and arguments (Crandall & Templeton 1993; Templeton & Sing 1993; Castelloe & Templeton 1994), and converted into a series of 'nesting clades' according to published 'rules' (Templeton *et al*. 1987; Templeton & Sing 1993). The total network was input into the software program GEODIS v2.5 (Posada *et al*. 2000) together with geographic sampling information, and the NCPA method was then used to infer the underlying population processes for each clade that demonstrated a significant geographical association. This is accomplished by examining two main distance measures generated from the population data, the clade distance (D_C) and the nested clade distance (D_N). The clade distance measures the geographic range (or spread) of a particular clade or haplotype (i.e. the larger the D_C value, the more widespread the haplotype or clade). The nested clade distance measures how a particular clade is geographically distributed relative to its older, but presumbably closest, evolutionary sister clade (i.e. the larger the D_N value, the more geographically distant it is from it's sister clade). The distinction is also made between interior and tip (I-T) clades. An interior clade is one that has two or more mutational connections, whereas tip clades only have a single connection (i.e. clades that are not interior nodes in the haplotype

network). Testing for significantly small or large D_C and D_N distances values in each nested clade was then accomplished through 10,000 random permutations (in GEODIS). If a significant departure from simulated randomness was observed in the distance measures, then the null hypothesis of no statistical association between haplotype distributions and geography (i.e. panmixia) could be rejected. The statistical results were then used to make population structure inferences following the most recent inference key of Templeton (2004) and the computer program AUTOINFER v1.0 (Zhang *et al*. 2006). As an example, fragmentation events tend to limit the geographical range of a clade, which results in significantly small clade distances (D_C) for both tip and interior clades. In addition, a significant restriction in clade distances at higher clade levels should continue to occur (Templeton et al. 1995). This pattern originates because of the tendency to accumulate fixed mutational differences after a fragmentation event, and should coincide with larger than average branch lengths in the network. Similarly, specific patterns for D_C and D_N are expected for other population processes (see Templeton *et al*. 1995)

Assessing patterns of genetic isolation by distance

To test for spatially limited genetic connectivity (i.e. isolation by distance) among collection localities, estimates of average pairwise sequence divergence between all collection locality pairs, corrected using the Kimura (1980) 2 parameter model of evolution, were calculated using ARLEQUIN v3.0 (Excoffier *et al*. 2005) and plotted against the geographic distance. We used a nonparametric Mantel test, implemented in the web-based program Isolation By Distance, Web Service (Jensen et al. 2005), to determine correlations between genetic and geographic distance matrices, with statistical significance assessed with 10,000 randomizations of the genetic distance matrix.

We also used genetic landscape analyses to obtain a graphical representation of genetic divergence patterns (after taking geographic distance into account) across the geographic landscape analyzed in this study. First, we performed an 'interpolation of landscape shape analysis' using the

program Alleles In Space (Miller 2005). This analysis generated a connectivity network among all sample locations based on pairwise geographic distances. The genetic distances between pairs of collection locations were then plotted at the geographic midpoints between collection locations. Next, we took this output from Alleles In Space and performed an inverse distance weighted interpolation of genetic distances in ArcGIS 9.1 to obtain a graphical representation of genetic distance patterns that could be overlaid onto our sampling map. Thus, the weighted interpolation of genetic distance between collection locations was visualized as a color gradient from red to blue, where red represents highest genetic differentiation and blue represents lowest genetic differentiation. We performed analyses using residual genetic distances, derived from the linear regression of genetic vs. geographical distances, to account for correlation between genetic and geographical distances that were present in our data set. Therefore, for both analyses the genetic distance between pairs of collection locations were interpolated into a surface image that represents the level of genetic differentiation between locations after taking geographic distance into account. This ensured that areas of high genetic differentiation were not merely interpolated due to the fact that one or a few collection areas were geographically isolated (Miller et al. 2006).

Assessing concordance between subspecies designations and phylogenetic structure

We used the mtDNA hypothesis to define lineage relationships among haplotypes of *C. occipitalis*. A Bayesian approach was then employed using the Bayes factor to test alternative subspecies hypotheses. The Bayes factor measures the amount by which one's opinion is changed after viewing the data and can be interpreted as the relative success of two hypotheses at predicting the data (Kass & Raftery 1995; Newton & Raftery 1994). Subspecific designations for each unique haplotype were determined by using the best estimates of subspecies ranges from Hallowell (1854), Stickel (1941), and Klauber (1951). If voucher specimens were available, a taxonomic key (Klauber 1951) was used to corroborate subspecific designations defined by geography (Appendix 1). Each subspecies was

constrained as monophyletic during a Bayesian analysis and the resulting harmonic mean (sampled from the posterior) was compared against the harmonic mean derived from the original unconstrained Bayesian analysis in which haplotypes were treated independently of one another. Haplotypes designated as "intergrades" were not constrained, allowing them to "float" in the analyses. The harmonic means for both analyses were calculated using the *sump* command in MRBAYES. The Bayes factor, calculated by dividing the harmonic mean of the unconstrained analysis by the harmonic mean of the constrained analysis, was then evaluated using the table provided by Raftery (1996) from which the criterion of 2ln Bayes Factor of ≥10 was treated as very strong evidence for the monophyletic constraint hypothesis.

Results and Discussion

ND1 Sequence Variation

We obtained sequences for 81 *Chionactis occipitalis* and one sequence from *C. palarostris* for outgroup purposes. A total of 1098 base pairs of mtDNA (158 bp 16S rRNA and 940 bp ND1) were unambiguously aligned and used for subsequent analyses. Among these sites, 205 were variable among *Chionactis* sequences and 157 were parsimony informative. For the *C. occipitalis* examined, 59 unique haplotypes were identified (Appendix 1). Uncorrected sequence divergence among *C. occipitalis* haplotypes range from 0.09% to 6.68% (mean 3.78%). Sequence divergence estimates between *C. occipitalis* and its nearest relative (*C. palarostris*) range from 5.62% – 8.22% (mean 6.72%).

Phylogeny of ND1 mtDNA haplotypes

MP and Bayesian analyses of unique haplotypes result in concordant topologies. MP analyses resulted in 16 trees, each with 487 steps in length (consensus not shown). For Bayesian analyses, hierarchical model testing indicated that the best fit model of sequence evolution was the general time

reversible model (GTR + I + Γ; Rodreguez *et al.* 1990). Under this model, the two independent Bayesian analyses converged on similar average log-likelihood values (-ln*L* =3857.49 and 3857.45). Majority rule (50%) consensus tree of Bayesian analyses is shown in Figure 2. The most obvious feature of our tree is the division of haplotypes into two widely distributed lineages. The first lineage (hereafter the West Mojave) includes collection localities primarily in the western Mojave Desert of California within Riverside, San Bernardino, Kern, and Inyo Counties. The second lineage (hereafter the Sonoran/East Mojave) includes collection localities distributed across the Sonoran Desert of California and Arizona, as well as a portion of the eastern Mojave Desert. The Sonoran/East Mojave lineage is more widespread, extending across all Arizona collection sites and into collection sites of eastern California (San Diego, Imperial, Riverside, and San Bernardino Counties). The two clades apparently abut one another in the Coachella Valley region of California. However, given our limited sampling within the Coachella Valley we are unable to pinpoint the exact location of secondary contact between the lineages.

The two lineages differ in degree of resolution and nodal support (Fig. 2). Two subclades were distinguished in the West Mojave lineage. One of these (haplotypes 50-51) occupies a small area restricted to the northern portion of the Coachella Valley in Riverside County, California. The range of the other subclade (haps 52-59) is more geographically widespread extending from the Transverse Ranges, northward across the Mojave Desert. However, the relationship between the two subclades is not strongly supported ($Pp < 0.5$). Bayesian trees within the 95% credible set reveal haplotypes 50-51 to be either nested within the West Mojave lineage and basal to other haplotypes in this lineage (shown in Fig. 2), or paraphyletic with respect to all other *C. occipitalis* haplotypes. In contrast, the Sonoran/East Mojave lineage is well supported and contains two sister clades. A well-supported subclade (haps 1-32) contains two geographically discontinuous units: (1) haplotypes 1-26 form a unit of recently diverged haplotypes, as evidenced by the shorter branch lengths that extend across central Arizona (Pima, Pinal,

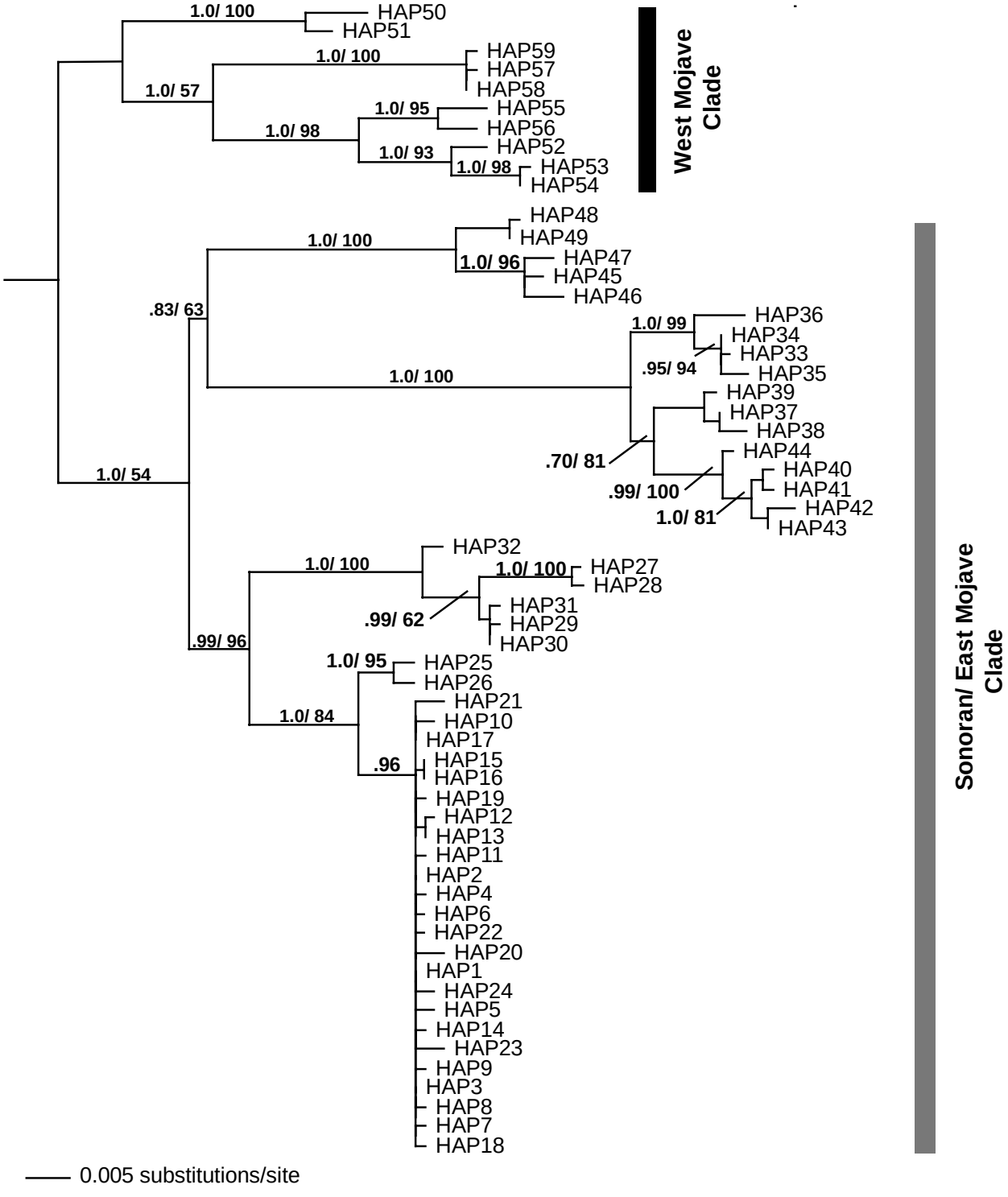

Figure 2. The 50% majority rule consensus tree from the Bayesian analysis of the mtDNA data. List of haplotypes and their geographic locations are found in Appendix 1. The numbers on branches indicate Bayesian posterior probabilities (*Pp*) and MP bootstrap percentage values (respectively). Clades with *Pp* $\geq$ 0.95 and MP values $\geq$ 70% were considered strongly supported.

Maricopa, and Yuma Counties), (2) haplotypes 27-32 extend along a northeastern axis from Riverside County, California into Mohave County, Arizona. The second subclade (haps 33-49) within the Sonoran/East Mojave lineage contains haplotypes extending along the Colorado River Valley of California, southern Arizona, and a geographically disjunct group to the north in Arizona (Yuma, La Paz, and Maricopa Counties). However, the inferred relationships among haplotypes within this group are not well supported (*Pp* = 0.83; MP bootstrap = 63%).

Nested Cladistic Phylogeograpical Analysis

Haplotypes were connected by TCS using a 95% parsimony limit that imposed a maximum of 14 mutational steps between connections. This resulted in seven separate haplotype networks (Fig. 3 & 4). The Sonoran/East Mojave networks included 49 unique haplotypes from 59 localities (Fig. 3). The West Mojave networks included 10 unique haplotypes from 14 localities (Fig. 4). All networks were entirely concordant with our phylogenetic analyses based on MP and Bayesian inference (Fig. 2).

The hierarchical nesting procedures across all networks resulted in seven nesting levels; levels 4 – 6 are shown in Figure 5. The geographical distributions of major clades and inferences of population history are plotted on Figure 6. There were 10 significant associations between haplotype clades and geographic distribution on all clade levels for the Sonoran/East Mojave Lineage (Clade 6-1). However, because the West Mojave lineage (Clade 6-2) included fewer collection sites and samples were generally spaced farther apart, significant associations between haplotype clades and geographic distribution were inferred only at higher nesting levels. Table 1 summarizes the results and inferences about population structure and history following the inference key provided in Templeton (1998). The oldest inferred event was either past fragmentation or high restricted gene flow with isolation by distance (IbD) between clade 6-1 (Sonoran/East Mojave lineage) and clade 6-2 (West Mojave lineage). We were unable to distinguish between the two inferences with NCPA because of inadequate geographic sampling between these clades. Given the close geographic proximity of these two clades

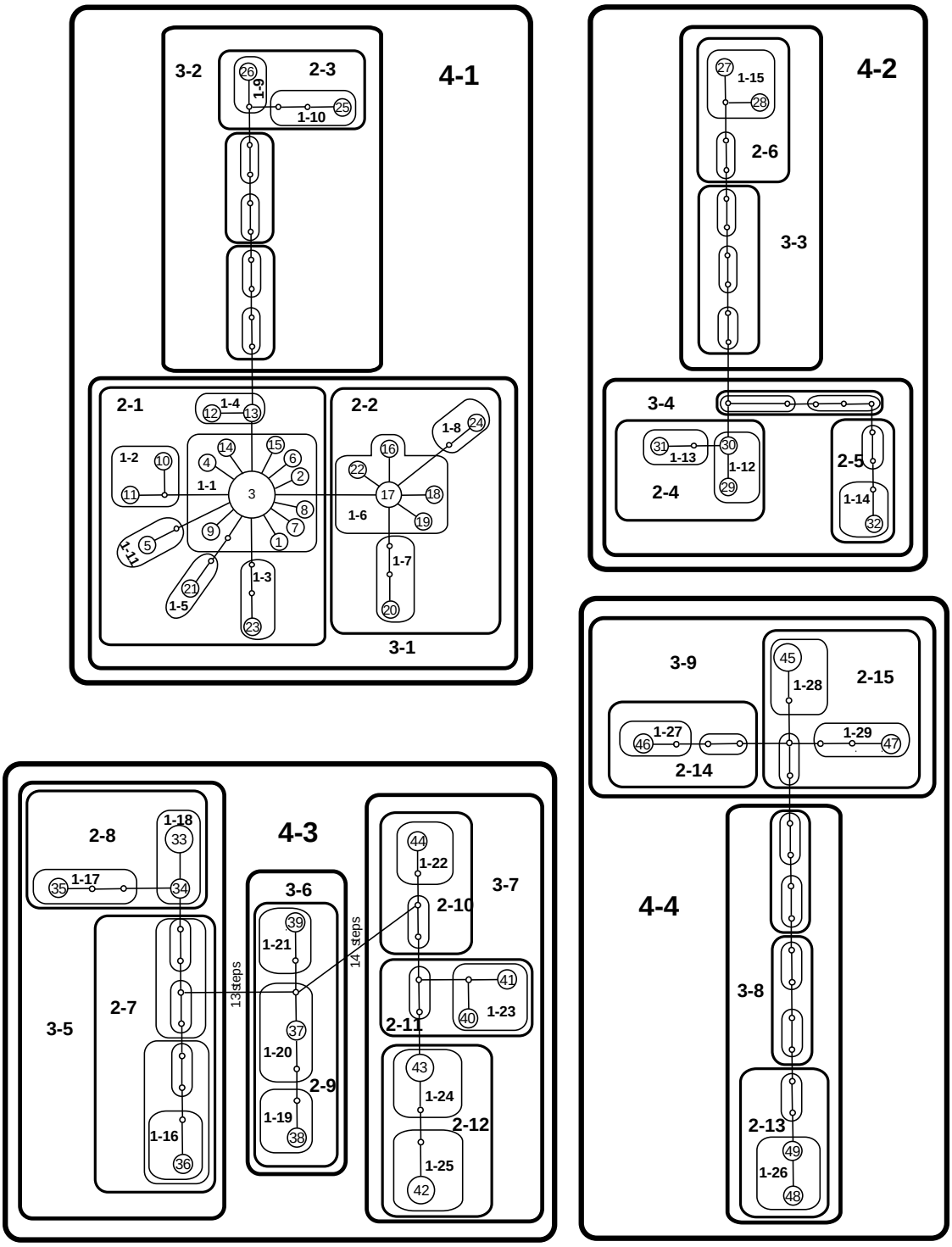

Figure 3. Sonoran/East Mojave haplotype networks estimated in TCS with 1, 2, 3, and 4 step nesting groups shown. Haplotypes numbers are the same as those designated in Figure 2 and Appendix I. Each line represents a single mutational step connecting two haplotypes. Small open circles indicate haplotypes states that are necessary intermediates but were not present in the sample.

within the Coachella Valley and the level of sequence divergence between them (Table 2) past fragmentation, rather than IbD, seems more likely. Thus, we hypothesize that these haplotype lineages differentiated as a result of isolation in independent refugia. Likely mechanisms of vicariance are provided by the fact that the two lineages abut one another near the Transverse Range and that an elevational gradient of approximately 1000 meters exists between the Coachella Valley and areas to the north. However, further sampling in the Coachella Valley region and eastern San Bernardino County will likely illuminate the extent of separation between Clade 6-1 and 6-2.

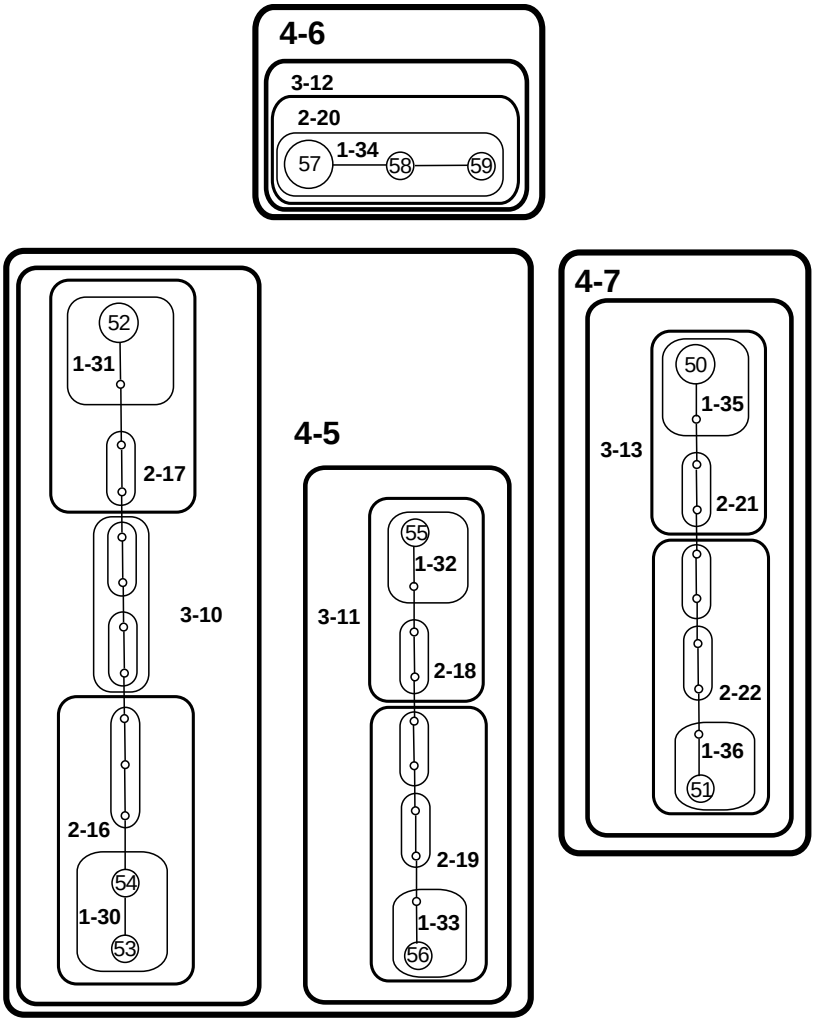

Figure 4. West Mojave haplotype networks estimated in TCS with 1, 2, 3, and 4 step nesting groups shown. Schematics are the same as in Figure 3.

Within clade 6-1 (Sonoran/East Mojave lineage), NCA inferences indicate range expansion and restricted gene flow with IbD were important historical processes, but there was insufficient resolution to discriminate between these processes at highest clade levels. At lower clade levels restricted gene flow with IbD was inferred (clades 4-1, 3-7, 2-2, 2-1, and 1-6; Table 1). Due to the lack of evidence for interior/tip status within clades 5-1 and 5-2, no inferences were possible.

We infer from the available patterns of genetic variation that past fragmentation, followed by range expansion in both lineages (Clades 6-1 & 6-2), has occurred in *C. occipitalis*. In addition, patterns of IbD, suggesting reduced gene flow, occur throughout regions of each lineage (Fig. 6; Table 1). Since a temporal polarity is inherent in the nesting design (i.e. on average the higher the clade-level, the older the clade), we infer that these processes were active early in the formation of the lineages and were likely determinants of the genetic differentiation across collection localities. More recently, restricted gene flow and patterns of IbD continue to contribute to the population structure among collection localities.

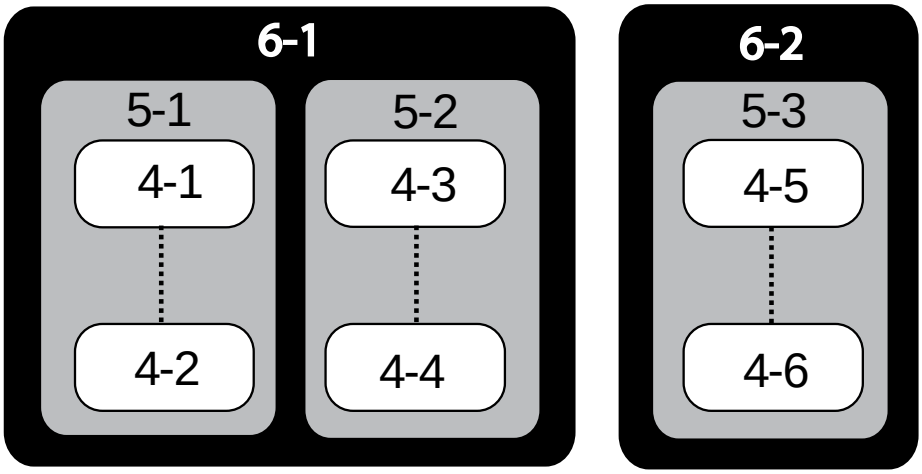

Figure 5. The total network represents connections above the 95% parsimony cut-off. Schematics are the same as in Figure 3. Network 6-1 consists of haplotypes from *C. o. annulata*, *C. o. klauberi*, and eastern locations of *C. o. occipitalis*. Network 6-2 consists of haplotypes from *C. o. talpina* and western locations of *C. o. occipitali*s.

Table 1. Demographic inferences from NCPA for the West Mohave and Sonoran/East Mojave Clades. The nested design is given in Figures 3, 4, 5, and 6. Following the number of any given clade are the clade (Dc) and the nested clade (Dn) distances. Also, for clades containing both tip and interior clades, the average difference between interior vs. tip clades is given in the row labeled I-T. Interior clades are denoted with an "(I)" after the clade number. An "S" superscript indicates the distance measure was significantly small at the 5% level, and an "L" superscript indicates the distance measure was significantly large. The chain of inference refers to the sequence of questions in the inference key and is followed by the biological inference generated by the pattern.

Clade	Nested Clades	D_C	D_N	Chain of inference	Inference
West Mojave Clades					
4-5	3-10(I)	105	114		
	3-11	28^S	95		
	I-T	76^L	19	1-2-3-4-No	**Restricted Gene Flow with IbD**
5-3	4-5(I)	108	111^L		
	4-6	57^S	80^S		
	I-T	51^L	32^L	1-2-3-4-No	**Restricted Gene Flow with IbD**
Sonoran + East Mojave Clades					
1-6	Hap16	0	88		
	Hap17(I)	0	105		
	Hap18	0	108		
	Hap19	0.6^S	64^S		
	Hap22	0	104		
	I-T	-0.3^S	22	1-2-3-4-No	**Restricted Gene Flow with IbD**
2-1	1-1(I)	55	56		
	1-2	0^S	24^S		
	1-3	0	47		
	1-4	26	47		
	1-5	0.2^S	64		
	1-11	0	69		
	I-T	46^S	8	1-2-3-4-No	**Restricted Gene Flow with IbD**

Table 1. Demographic inferences from NCPA for the West Mohave and Sonoran/East Mojave Clades. —Continued

Clade	Nested Clades	D_C	D_N	Chain of inference	Inference
2-2	1-6(I)	89[L]	89[L]		
	1-7	0	51		
	1-8	2.7	22[S]		
	I-T	87[S]	57[S]	1-2-3-4-No	**Restricted Gene Flow with IbD**
3-1	2-1(I)	54	55		
	2-2	73[L]	73		
	I-T	-18[S]	-18	1-2-11-12-No	**Contiguous Range Expansion**
3-7	2-10(I)	0	22		
	2-11	7	19		
	2-12	13[S]	20		
	I-T	-11	2	1-2-3-4-No	**Restricted Gene Flow with IbD**
4-1	3-1(I)	59[S]	59[S]		
	3-2	0[S]	159[L]		
	I-T	59	-99[S]	1-2-3-4-No	**Restricted Gene Flow with IbD**
4-3	3-5	22[S]	51		
	3-6(I)	5[S]	103[L]		
	3-7	21[S]	59		
	I-T	-16	47[L]	1-2-3-5-6-13-14-21-No	**Past Expansion followed by Fragmentation**
5-1	I-T status cannot be determined				**Inconclusive Outcome**
5-2	I-T status cannot be determined				**Inconclusive Outcome**
6-1	5-1	97[S]	130[S]		
	5-2(I)	101[S]	174[L]	1-2-3-5-No	**Insufficient resolution to discriminate btw**
	I-T	4	44[L]		**Range Expansion and Restricted Gene Flow**
Total Network					
7-1	6-1	117[S]	116[S]		
	6-2(I)	206[L]	294[L]	1-2-3-4-9-10-Yes	**Sampling scheme inadequate to discriminate**
	I-T	88[L]	117[L]		**between Fragmentation and IbD**

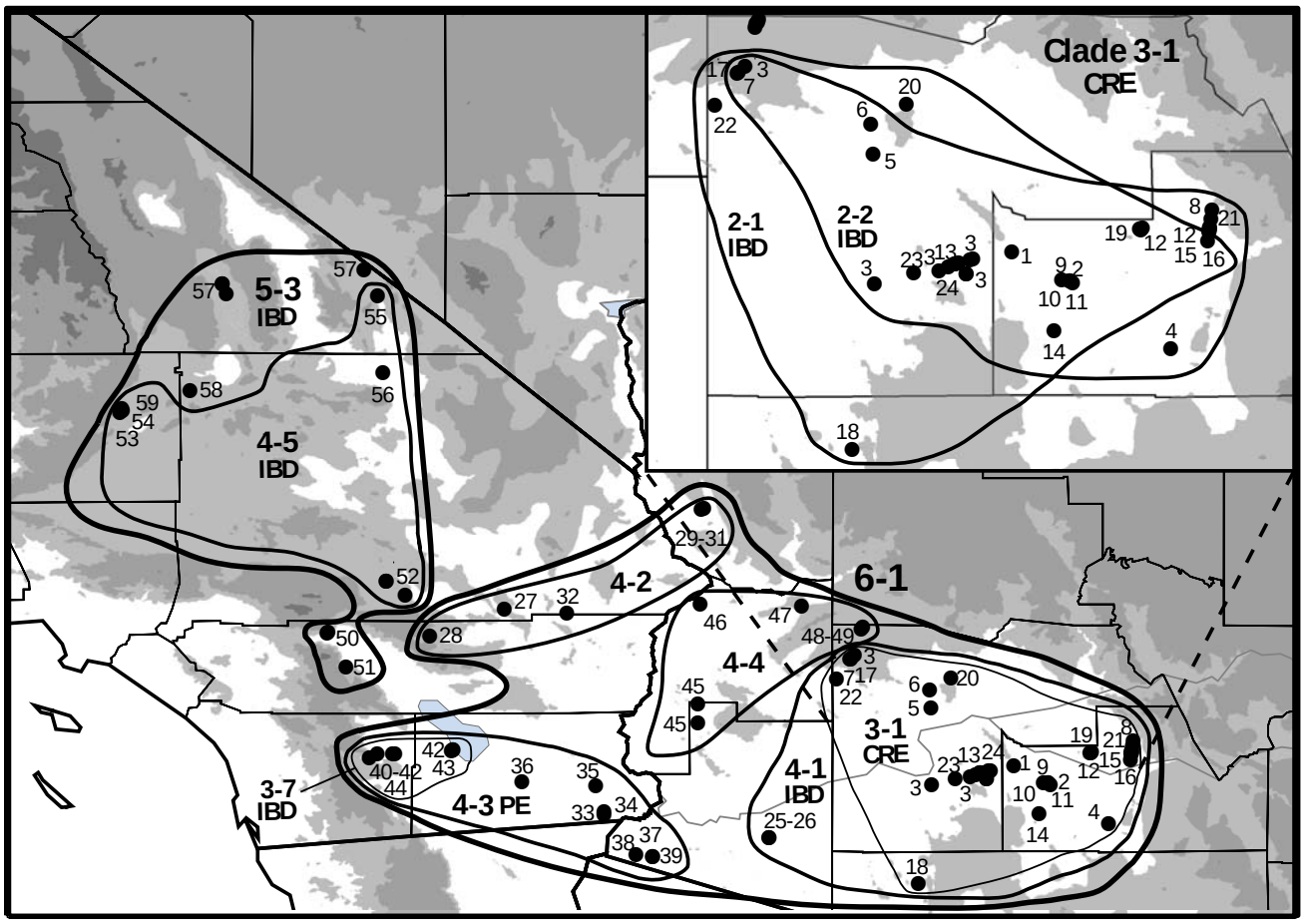

Figure 6. Geographic distribution of selected clades used in NPCA and the biological inference generated by the significant patterns of genetic and geographic association (IBD, isolation by distance; RE, range expansion; CRE, contiguous range expansion). Shading indicates elevation with light grey between 600 and 1000m, grey between 1000 and 2000m, and dark grey above 2000m.

Patterns of gene flow and isolation by distance

To further evaluate patterns of spatially limited gene flow, we performed two separate sets of analyses. Mantel tests were conducted to assess whether genetic differentiation among haplotypes were correlated with geographic distance. In addition, we used genetic landscape analyses to obtain a graphical

representation of genetic differentiation across the landscape to reveal the likely determinates of genetic differentiation patterns. Mantel tests for IbD were performed on all collection localities, within both the West Mojave and Sonoran/East Mojave lineages, and on clade 4-1, a clade containing all *C. o. klauberi* collection sites. Results from our IbD analyses produced qualitatively similar results as our NCPA analyses. IbD analyses revealed a significant positive relationship between genetic differentiation and geographic distance in: all collection localities ($r = 0.626$, $P = 0.001$), Sonoran/East Mojave lineage ($r = 0.693$, $P = 0.0001$), West Mojave lineage ($r = 0.429$, $P = 0.002$), and clade 4-1 ($r = 0.386$, $P = 0.005$). However, the significant relationship within clade 4-1 appeared to be driven by the large sampling gap between the Mohawk Dune collection site (haplotypes 25-26) and all other sites to the east that include *C. o. klauberi* localities. When Mohawk Dune samples were excluded, the results were non-significant ($r = 0.077$, $P = 0.281$) consistent with our NCPA and suggesting contiguous range expansion across collection sites of central-eastern Arizona (clade 3-1; Fig. 6).

Observed patterns of genetic differentiation are probably driven by the limited dispersal abilities of *C. occipitalis* and the patchiness of suitable habitat across the species range. For example, steep topographic gradients (e.g. mountains, changes in elevation) may isolate populations, leading to increased genetic differentiation along the greater elevation gradients of the Mojave Desert regions than among the relatively low elevations and broader valleys of the Sonoran Desert (areas where range expansions were inferred; Fig. 6). This pattern is evident from our Genetic Landscape Interpolation analyses, where higher genetic differentiation seemed to be associated with comparisons between collection localities separated by topographic features (mountain ranges > 600 m; Fig. 7) suggesting that topographic relief (not necessarily distance) plays an important role in restricting gene flow across locations. In contrast, high genetic similarity across large geographic distances were found between collection locations with less topographic relief in the south and east (i.e. within clades 4-1, 4-3, Fig. 6; Fig. 7).

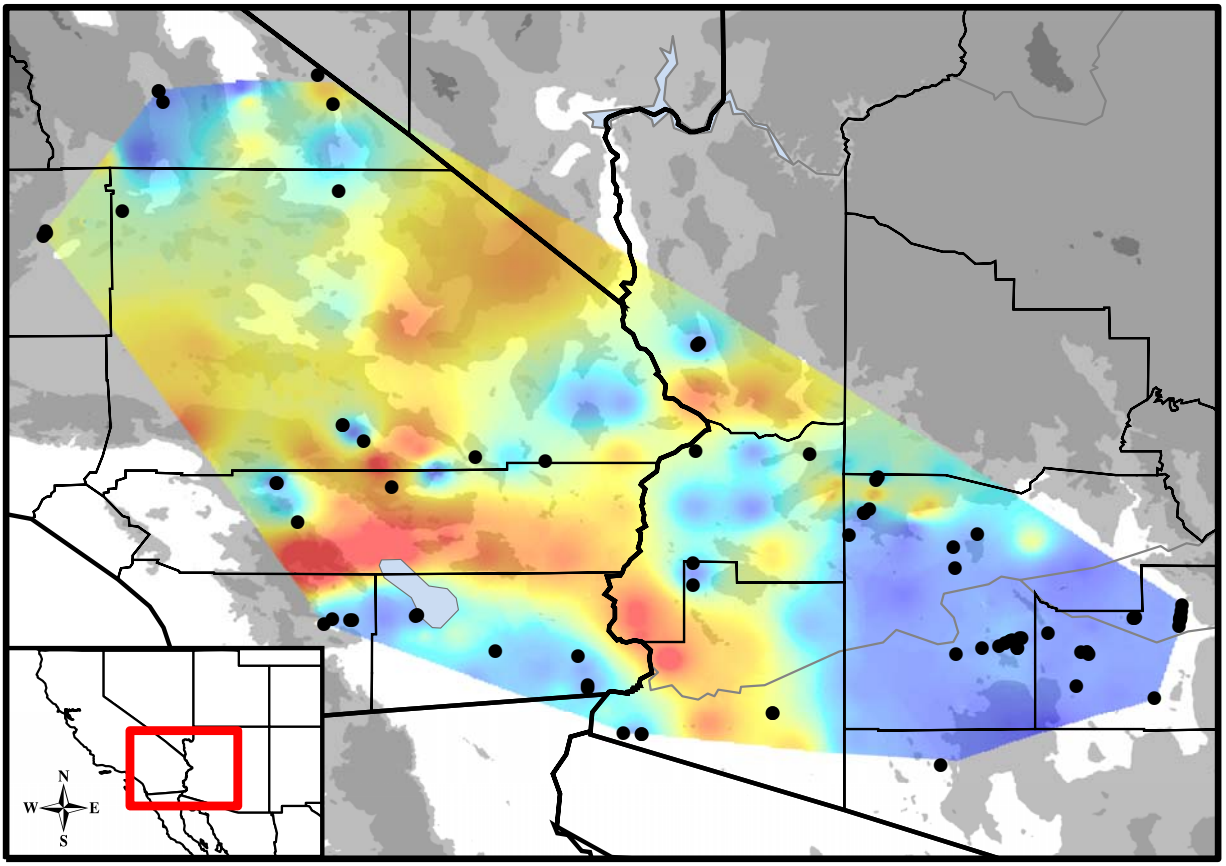

Figure 7. Results of the Genetic Landscape Shape interpolation analysis revealing large genetic differentiation as 'hot spots' between collection locations (black dots), where red indicates highest (hot) genetic differentiation and blue represents low (cool) genetic differentiation between collection locations. Shading indicates elevation with light grey between 600 and 1000m, dark grey between 1100 and 2000m, and black above 2000m. The prominent red 'arc' in the southwest corresponds with the Transverse Ranges (to the west) and the Chocolate Mountains (to the east).

Patterns of genealogical incongruence in C. occipitalis *subspecies*

To further assess confidence in the mtDNA subspecies relationships, an alternative hypothesis was evaluated in a Bayesian framework, where each subspecies was constrained to be monophyletic and 'intergrades' were allowed to float in the analysis. The Bayes factor from the comparison of hypotheses equaled 1.98 indicating that the monophyletic subspecies constraint hypothesis did not predict the data

as well as our preferred hypothesis (i.e. unconstrained analysis), and that this alternative is a poorer explanation for the evolution of the ND1 mtDNA data (2ln Bayes factor < 2 = barely worth mentioning). Mapping subspecies designations on to the preferred tree reveals three cases in which recovered clades are inconsistent with the morphological subspecies (Fig. 8). These cases involve the following subspecies pairs: *C. o. talpina* within *C. o. occipitalis*; *C. o. klauberi* within *C. o. annulata*; and *C. o. occipitalis* within *C. o. annulata*. The inconsistencies among morphologically based subspecies and mtDNA relationships may reflect retention of ancestral mtDNA polymorphism since divergence, ongoing gene flow across subspecies boundaries, or inadequate morphological taxonomy (i.e. members of the subspecies pairs are actually synonymous). In most cases, given the current limits of our genetic data and the absence of independent analysis (e.g. nuclear gene analysis) we are not able to distinguish among these alternatives (Funk & Omland 2003). Nonetheless, some patterns are discernable.

Table 2. Pairwise comparisons of average uncorrected sequence divergence within (along diagonal) and among (below diagonal) the West Mojave and Sonoran/ East Mojave lineages.

Clade	West Mojave	Sonoran/East Mojave
West Mojave	0.032	—
Sonoran/ East Mojave	0.054	0.031

In the first two subspecies pairs, members of *C. o. talpina* and *C. o. klauberi* are found dispersed in relatively shallow tip clades of *C. o. occipitalis* and *C. o. annulata*, respectively. Two alternative explanations are possible. The first is that the mtDNA marker may not be sufficiently variable to detect differentiation at the subspecies level because *C. occipitalis* has diverged range-wide relatively recently. We feel this is not likely since relatively high sequence variation was detected across clades recovered

in our phylogenetic analyses (average sequence divergences > 5.0%; Table 2), and because patterns of strong genetic differentiation were exhibited across the range of this species. Therefore, we lean towards a synonymy hypothesis (i.e. *C. o. talpina* synonomous with *C. o. occipitalis* and *C. o. klauberi* synonomous with *C. o. annulata*) and suggest that gene flow (albeit potentially restricted) is ongoing or has been until very recently between these putative subspecies pairs. We find it interesting that both *C. o. talpina* and *C. o. klauberi* are found at extreme northern and eastern portions (respectively) of *C. occipitalis'* range and that color pattern characteristics between these subspecies are similar (i.e. secondary dark maculation across dorsal/lateral interspaces). Thus, it may be possible that localized environmental forces at the limits of *C. occipitals'* range are influencing color patterns and that the population structure identified in this study (restricted gene flow) may be helping to drive these localized forces in a directional sense, creating locally adapted morphological phenotypes or ecomorphs (King & Lawson 1995; Wiens *et al*. 1999; Leache & Reeder 2002).

The inconsistencies observed across *C. o. occipitalis* haplotypes are less clear, where a *C. o. occipitalis* clade of eastern Mojave haplotypes is nested within a largely *C. o. annulata* clade (Fig. 8). Again the three alternatives mentioned above are possible. Interestingly, Klauber (1951) noted that eastern populations of *C. o. occipitalis* ranging from Clark County, Nevada to San Bernardino and Riverside counties in California "show a tendency toward annulata" in having lower body band counts and a higher frequency of ventral markings. He also noted that populations of *C. o. occipitalis* "most widely differentiated from *C. o. annulata*", as well as the other subspecies, inhabit the extreme western part of the Mojave Desert. These patterns are consistent with our mtDNA and provide some support for an incomplete lineage sorting hypothesis.

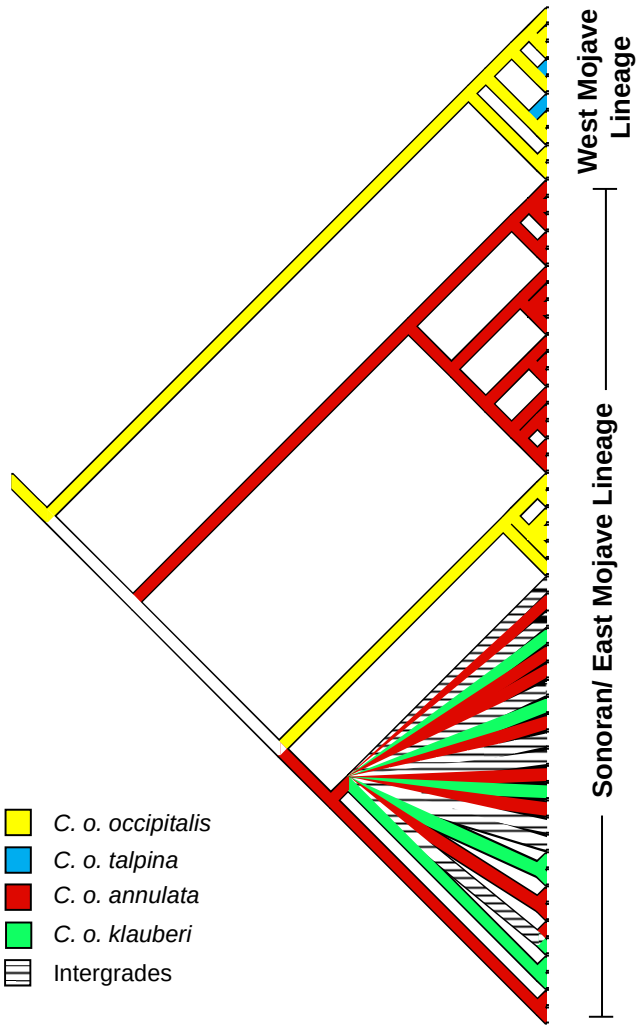

Figure 8. Morphological subspecies designations mapped on the Bayesian tree. This illustrates the discordance between mtDNA groups and subspecies based on scutulation and color pattern.

Conclusions

A few general conclusions can be drawn from our mtDNA analysis of *C. occipitalis*. First, phylogenetic analysis of the mtDNA data revealed significant geographical structuring of haplotypes and two distinct regional lineages. One consisted of a combined western Mojave Desert *C. o. occipitalis* + *C. o. talpina* group and the other a combined eastern Mojave Desert *C. o. occipitalis* + *C. o. annulata* + *C. o. klauberi* group. In most analyses these groups were monophyletic or nearly so; however, none of

the subspecies were completely monophyletic within these distinct clades. The same 'non-exclusive' pattern was evident in the haplotype networks. In addition, results from both NCPA and IBD analyses indicated a history of range expansion and restricted gene flow with IbD for most clades, at multiple clade levels, which indicates some gene flow within the major groups. Thus, the simplest and most consistent conclusion based on the current available data suggests two distinct subspecies or ESUs, which would be formed by combining western populations of *C. o. occipitalis* with *C. o. talpina*, and eastern populations of *C. o. occipitalis* with *C. o. annulata* and *C. o. klauberi*.

Second, past vicariance appears to have led to diversification between the two regional lineages. Possible mechanisms for complete isolation are indicated by the fact that the two lineages abut one another near the Transverse Range and that an elevational gradient of approximately 1000 meters separates the Coachella Valley from areas to the north of this range. In addition, given that IbD was observed within these lineages, we suggest that limited dispersal abilities and the mosaic of appropriate habitat that exists across *C. occipitalis'* range probably play a major role in the observed population structure.

Finally, the preponderance of evidence (mtDNA and clinal variation in morphology) indicate that the subspecies *C. o. klauberi* is not a valid taxonomic unit. The mtDNA data clearly show specimens identified as *C. o. klauberi* carry sequences very similar to specimens within both the intergradation zone and pure *C. o. annulata* populations (i.e. Mohawk Dunes). Molecular data thus suggest *C. o. klauberi* specimens are part of a larger geographic clade that resulted from recent range expansion from western populations. These data are consistent with morphological patterns of west/east clinal variation as observed in the taxa.

Conservation Implications

We conclude, based on our data and the available morphological data, that *C. o. klauberi* is not a clearly defined subspecies. Our mtDNA data do not support the treatment of collection locations east

and west of the purported morphological intergradation zone as separate units for conservation and management purposes. Rather, based on mtDNA, *C. o. klauberi* seems to represent a morphological endpoint of clinal variation without concordant phylogenetic distinction. If we employ the criteria of Moritz (1994), the "subspecies" *C. o. klauberi* would not qualify as an ESU due to the lack of reciprocal monophyly/exclusivity. Using the less stringent criteria for ESU designation of Crandall *et al*. (2000), the "subspecies" *C. o. klauberi* might show, at most, recent genetic inexchangeability due to anthropogenic effects (e.g. habitat fragmentation and loss). As such, it would fall under Case 8 with a management recommendation to "treat as a single population" (eastern *C. o. occipitalis* + *C. o. annulata* + *C. o. klauberi*) and "restore [population structure] to historical condition" (Crandall *et al*. 2000). It is clear from our mtDNA data that in most cases, geographically proximate populations are more genetically similar to each other than to more distant populations, some even sharing the same haplotypes. Therefore, if translocations are proposed for reintroduction of *C. occipitalis* where populations have declined dramatically (e.g. Avra Valley populations in Arizona), then we would recommend harvesting from the most geographically proximate populations, although we would emphasize the need for further investigation prior to undertaking interpopulation translocations (Dodd & Seigel 1991).

Our data also allow preliminary insight into the geographical structure of genetic variation of *C. occipitalis* within Arizona. Currently, three subspecies have been thought to occur in Arizona: *C. o. annulata* in the southwest, *C. o. occipitalis* in the northwest, and *C. o. klauberi* in the east, suggesting at least three populations or ESUs in Arizona might be considered in management and recovery plans. In contrast, our phylogenetic analysis of sampled mtDNA haplotypes reveals a single ESU or exclusive group occurs within Arizona. However, at least four areas of unique genetic variation in western Arizona should be considered within this exclusive unit (Clade 4-1, 4-2, 4-3, 4-4; Fig. 6), two of which converge northwest of Phoenix near the border between Maricopa and La Paz Counties (4-1 and 4-4;

Fig. 6). Thus, conservation activities in this portion of the state should focus on planning measures that would best preserve the network of connectivity and population structure in order to maintain/restore historical levels of gene flow.

Lastly, genetic patterns revealed across the Coachella Valley warrant some discussion. The Coachella Valley consists of low-elevation valleys and playas, surrounded by mesas consisting of sand dunes; however, recent human activities have impacted this region. The two main ESUs or population segments for *C. occipitalis*, revealed through our phylogenetic analysis, are found throughout the Coachella Valley. However, some of the oldest or most ancestral haplotypes (50-51) are represented in the northern portion of the valley. A multiple species conservation plan (Coachella Valley Multiple Species Habitat Conservation Plan, 2006) has been developed for the entire Coachella Valley and surrounding mountains; however, management practices of covered species are largely based on conserving ecological processes of aeolian sand dune systems, with priority given to active dune versus stabilized dune systems. Therefore, if *C. occipitalis* habitat overlaps with that of species covered by the MSHCP then the species will likely be protected under the conservation plan. However, if *C. occipitalis* is more likely associated with stabilized dune systems then further protection may be required, especially in the northern portion of the valley.

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Appendix

Appendix 1. Haplotype number, clade affiliation, collection locality, subspecies, and tissue number of samples used in this study. Subspecies were assigned based on geographic location and when vouchers were available a dichotomous key (Klauber 1951) was also used to assign subspecies.

Hap #	Clade	Collection Location	Subspecies by geography	Subspecies by key	Tissue #
1	Sonoran/East Mojave	Arizona: Maricopa, 8.0 mi (by road) E Mobile	intergrade		B-6750
2	Sonoran/East Mojave	Arizona: Pinal, Val Vista Rd, 1.3 mi W of Hwy 387, N of Casa Grande	klauberi	annulata	ATH 714
3	Sonoran/East Mojave	Arizona: Maricopa, Eagle Eye Rd, N of Salome Rd, 10 mi S of Aguila	annulata	annulata	ATH 708
3	Sonoran/East Mojave	Arizona: Maricopa, 5.6 mi (by road) WSW Mobile	intergrade		B-6660
3	Sonoran/East Mojave	Arizona: Maricopa, 1.2 mi (by road) W Mobile	intergrade		B-6738
3	Sonoran/East Mojave	Arizona: Maricopa, 5.3 mi (by road) W Mobile	intergrade		B-6752
3	Sonoran/East Mojave	Arizona: Maricopa, 7.2 mi (by Hwy 238) WSW Mobile	intergrade		B-6857
3	Sonoran/East Mojave	Arizona: Maricopa, Hwy 238, 22.7 mi (by road) W of Hwy 347	intergrade		TRJ 833
3	Sonoran/East Mojave	Arizona: Maricopa, Hwy 238, 17.7 mi (by road) W jct Hwy 347	intergrade		TRJ 839
3	Sonoran/East Mojave	Arizona: Maricopa, Hwy 238, 1.5 mi (by road) E of Hwy 85 jct at Gila Bend	intergrade	klauberi	TRJ 926
4	Sonoran/East Mojave	Arizona: Pinal, Picacho Hwy, 0.2 mi S Nutt Rd, 0.8 mi N Harmon Rd	klauberi		B-6981
5	Sonoran/East Mojave	Arizona: Maricopa, Sun Valley Parkway, S of McDowell	intergrade	annulata	ATH 713
6	Sonoran/East Mojave	Arizona: Maricopa, Sun Valley Parkway, 9.9 mi N of I10	intergrade	annulata	ATH 710
7	Sonoran/East Mojave	Arizona: Maricopa, Eagle Eye Rd, 0.75 mi S of Pump Mine Wash	annulata		ASU 34684
8	Sonoran/East Mojave	Arizona: Pinal, Hwy 79, 4.4 mi S of RR Xing	klauberi		ASU 34622
8	Sonoran/East Mojave	Arizona: Pinal, Hwy 79, 1.4 mi S of RR Xing	klauberi		ASU 34623
8	Sonoran/East Mojave	Arizona: Pinal, Florence	klauberi		RLB 6829
9	Sonoran/East Mojave	Arizona: Pinal, Val Vista Rd, 1.7 mi NE of Maricopa - Casa Grande Hwy	klauberi	annulata	ATH 715
10	Sonoran/East Mojave	Arizona: Pinal, Casa Grande Hwy, 1.2 mi E of Val Vista Rd	klauberi	annulata	ATH 717
11	Sonoran/East Mojave	Arizona: Pinal, Casa Grande Hwy, 0.2 mi E of Montgomery Rd	klauberi	klauberi	ATH 718

Appendix 1. Haplotype number, clade affiliation, collection locality, subspecies, and tissue number of samples used in this study. Subspecies were assigned based on geographic location and when vouchers were available a dichotomous key (Klauber 1951) was also used to assign subspecies.—Continued.

Hap #	Clade	Collection Location	Subspecies by geography	Subspecies by key	Tissue #
12	Sonoran/East Mojave	Arizona: Pinal, West Phillips Rd, S of Hunt Hwy near the Santan Mountains	klauberi	annulata	ATH 723
12	Sonoran/East Mojave	Arizona: Pinal, Hwy 79, 5.3 mi S of Florence Jct	klauberi	klauberi	TCB 182
13	Sonoran/East Mojave	Arizona: Maricopa, Hwy 238 W of Mobile E of Maricopa Mtns	intergrade	annulata	ATH 707
14	Sonoran/East Mojave	Arizona: Pinal, ca 5.0 mi (by road) NNW Vaiva Vo; 0.1 mi N Tohono Boundary	klauberi		B-6794
15	Sonoran/East Mojave	Arizona: Pinal, Hwy 79: ca 6 mi S of Florence Jct	klauberi		ASU 35076
16	Sonoran/East Mojave	Arizona: Pinal, Hwy 79, 9.4 mi S of Florence Jct	klauberi	klauberi	TCB 181
17	Sonoran/East Mojave	Arizona: Maricopa , Eagle Eye Rd, 0.3 mi S of Pump Mine Wash	annulata		ASU 34683
18	Sonoran/East Mojave	Arizona: Pima, Hwy 85 S of Ajo	annulata	annulata	RDB 0074
19	Sonoran/East Mojave	Arizona: Pinal, San Tan Mtns W Phillips Rd, S of Hunt Hwy	klauberi	klauberi	ATH 709
19	Sonoran/East Mojave	Arizona: Pinal, San Tan Mtns W Phillips Rd, S of Hunt Hwy	klauberi	annulata	ATH 711
19	Sonoran/East Mojave	Arizona: Pinal, San Tan Mtns intersection of Royce & Judd Rds	klauberi		ATH 716
20	Sonoran/East Mojave	Arizona: Maricopa, Sun Valley Parkway, W of 219th Ave	intergrade	annulata	ATH 712
21	Sonoran/East Mojave	Arizona: Pinal, Hwy 79, 6.5 mi S of Florence Jct	klauberi		ASU 35071
21	Sonoran/East Mojave	Arizona: Pinal, Hwy 79, 6.8 mi S of Florence Jct	klauberi	klauberi	TCB 180
22	Sonoran/East Mojave	Arizona: Maricopa, Eagle Eye Rd, N of Salome Rd	annulata	annulata	ATH 722
23	Sonoran/East Mojave	Arizona: Maricopa, 16.1 mi (by road) WSW Mobile	intergrade		B-6661
24	Sonoran/East Mojave	Arizona: Maricopa, Hwy 238, 15.0 mi (by road) W jct AZ hwy 347	intergrade		TRJ 840
25	Sonoran/East Mojave	Arizona: Yuma, Mohawk Dunes	annulata		UTA R 54424
26	Sonoran/East Mojave	Arizona: Yuma, Mohawk Dunes	annulata		UTA R 54425

Appendix 1. Haplotype number, clade affiliation, collection locality, subspecies, and tissue number of samples used in this study. Subspecies were assigned based on geographic location and when vouchers were available a dichotomous key (Klauber 1951) was also used to assign subspecies.—Continued.

Hap #	Clade	Collection Location	Subspecies by geography	Subspecies by key	Tissue #
27	Sonoran/East Mojave	California: San Bernardino, Hwy 62 E of 29 Palms, near Sheep Hole Mtns	occipitalis		JMM 109
28	Sonoran/East Mojave	California: Riverside, Joshua Tree	occipitalis		JOS 1106
29	Sonoran/East Mojave	Arizona: Mohave, S of Yucca on I40 Frontage Rd, 6.6 mi S of Yucca	occipitalis		TCB 195
30	Sonoran/East Mojave	Arizona: Mohave, S of Yucca on I40 Frontage Rd, 7.2 mi S of Yucca	occipitalis		TCB 194
31	Sonoran/East Mojave	Arizona: Mohave, S of Yucca on I40 Frontage Rd, 7.4 mi S of Yucca	occipitalis		TCB 196
32	Sonoran/East Mojave	California: San Bernardino, Hwy 62, 32 mi W of Vidal Jct	occipitalis		CAS 223594
33	Sonoran/East Mojave	California: Imperial, Algadones Dunes; ca 1/8 mi S of Ogilby exit off I-8	annulata		ASU 34682
34	Sonoran/East Mojave	California: Imperial, Cargo Muchacho Mtns, Hwy 34, 1.1mi (by road) N of I-8	annulata		JMM 111
35	Sonoran/East Mojave	California: Imperial, Ogilby Rd, 15.0 mi (by road) N of I-8	annulata		KWS 157
36	Sonoran/East Mojave	California: Imperial, Hwy 78, 21.9 mi (by road) W jct Ogilby Rd	annulata		KWS 143
37	Sonoran/East Mojave	Arizona: Yuma, Barry M Goldwater Airforce Base, 6 mi SE of 19th ST, Yuma AZ	annulata		JMM 1
39	Sonoran/East Mojave	Arizona: Yuma, Barry M Goldwater Airforce Base, 6 mi SE of 19th ST, Yuma AZ	annulata		JMM 2
38	Sonoran/East Mojave	Arizona: Yuma, Yuma Dunes, vicinity of County Rd 21 and Avenue A	annulata		ASU 35960
40	Sonoran/East Mojave	California: San Diego, Hwy 78, 0.4 mi (by road) W jct Yaqui Pass Rd	annulata		KWS 169
41	Sonoran/East Mojave	California: San Diego, Hwy 78, 0.7 rd mi E jct Borrego Springs Rd	annulata		KWS 49
42	Sonoran/East Mojave	California: San Diego, Hwy 78, 1.6 rd mi E jct Borrego Springs Rd	annulata		KWS 39
42	Sonoran/East Mojave	California: Imperial, Salton Sea Array 10	annulata		SASSP2-65
43	Sonoran/East Mojave	California: Imperial, Salton Sea Array 12	annulata		SASSP3-8

Appendix 1. Haplotype number, clade affiliation, collection locality, subspecies, and tissue number of samples used in this study. Subspecies were assigned based on geographic location and when vouchers were available a dichotomous key (Klauber 1951) was also used to assign subspecies.—Continued.

Hap #	Clade	Collection Location	Subspecies by geography	Subspecies by key	Tissue #
43	Sonoran/East Mojave	California: Imperial, Salton Sea Array 11	annulata		SASSP12-14
44	Sonoran/East Mojave	California: San Diego, Borrego Springs Rd, 3.5 mi (by road) NW jct Hwy 78	annulata		KWS 53
45	Sonoran/East Mojave	Arizona: Yuma, Hwy 95 S of Quartzite	annulata	annulata	ATH 719
45	Sonoran/East Mojave	Arizona: Yuma, Hwy 95 S of Quartzite, 1.8 mi S of Palm Canyon Rd	annulata	annulata	ATH 720
46	Sonoran/East Mojave	Arizona: La Paz, E of Parker on Shea Rd (Osborne Well Rd)	annulata		ASU 35072
47	Sonoran/East Mojave	Arizona: La Paz, Alamo Dam Rd, 30.8 mi N of Hwy 60, S of Alamo Lake	annulata	annulata	ATH 706
48	Sonoran/East Mojave	Arizona: Yavapai, Hwy 71, 10.0 mi SW jct Hwy 93	annulata	annulata	TRJ 936
49	Sonoran/East Mojave	Arizona: Yavapai, Hwy 71, 15.3 mi SW jct Hwy 93	annulata	annulata	TRJ 937
50	West Mojave	California: Riverside, Mesa Array 10	occipitalis		MES 571
50	West Mojave	California: Riverside, Mesa Array 3	occipitalis		MES 295
51	West Mojave	California: Riverside, Palm Canyon	occipitalis		CJH3-127-86
52	West Mojave	California: San Bernardino, Twentynine Palms, outside of	occipitalis		BLM 179
52	West Mojave	California: San Bernardino, Marine Corp Air Command Center, Twentynine Palms	occipitalis		MCC 385
52	West Mojave	California: San Bernardino, Marine Corp Air Command Center, Twentynine Palms	occipitalis		MCC 209
53	West Mojave	California: Kern, Dove Springs	occipitalis		SDSNH 72189
54	West Mojave	California: Kern, Dove Springs	occipitalis		SDSNH 72192
55	West Mojave	California: Inyo, Hwy 127, 12.4mi S of Death Valley jct	talpina		JMM 62
56	West Mojave	California: San Bernardino, Dumont Dunes, Hwy 127	occipitalis		CSB3-56-1
57	West Mojave	California: Inyo, Panamint Valley Rd, 7.2mi S of jct Hwy 190	talpina		JMM 113
57	West Mojave	California: Inyo, Panamint Valley Rd, 7.2mi S of jct Hwy 190	talpina		JMM 114
57	West Mojave	California: Inyo, 12.6mi S jct Hwy 190 on Panamint Valley Rd	talpina		JMM 78
57	West Mojave	California: San Diego, Hwy 78, 3.5 rd mi W Ocotillo Wells Ranger Station	talpina		KWS 50

Appendix 1. Haplotype number, clade affiliation, collection locality, subspecies, and tissue number of samples used in this study. Subspecies were assigned based on geographic location and when vouchers were available a dichotomous key (Klauber 1951) was also used to assign subspecies.—Continued.

Hap #	Clade	Collection Location	Subspecies by geography	Subspecies by key	Tissue #
58	West Mojave	California: San Bernardino, Trona Rd, 8.3 mi S jct Hwy 178	occipitalis		JMM 80
59	West Mojave	California: Kern, Dove Springs	occipitalis		SDSNH 72190