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LOMA LINDA UNIVERSITY

Graduate School

Phylogeography of the Night Lizard,
Xantusia henshawi, from Southern California

by

Robert Edward Lovich

A Thesis in Partial Fulfillment
of the Requirements for the Degree Master of
Science in Biology

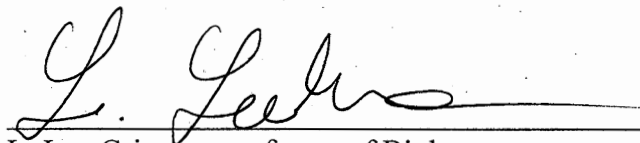
December 1999

Each person whose signature appears below certifies that this thesis in their opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.



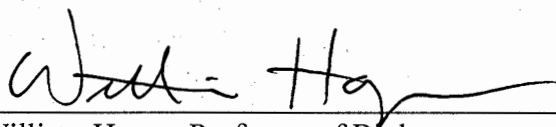
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ABSTRACT OF THE THESIS

Phylogeography of the Night Lizard,

Xantusia henshawi, from Southern California

by

Robert Edward Lovich

Master of Science, Graduate Program in Biology

Loma Linda University, December 1999

Dr. Ronald Carter, Chairperson

The granite night lizard, *Xantusia henshawi*, is a highly specialized crevice dwelling lizard found in the Peninsular Ranges of southern California, United States and northern Baja California, México. Generally, little morphological variation is seen in this species over a variety of habitats throughout its range, from elevations near sea level to approximately 2000 meters, and from Mediterranean habitats near the Pacific coast to arid desert habitat in the Colorado Desert of California. One exception is the variation seen in the subspecies *X. h. gracilis*, which differs from *X. h. henshawi* in morphology, allozymes, ecology, and natural history.

In this study, intraspecific variation within *Xantusia henshawi* was analyzed by constructing a genealogy using mitochondrial DNA (mtDNA) sequence data. A 379 base pair region of mtDNA (cytochrome *b*) was amplified and sequenced for lizards representing 13 localities across their known range in southern California. The resulting gene tree was compared to the known geologic history of the area comprising the range of this species. Three (3) haploclades were found in the maximum parsimony (MP)

analyses that were separated geographically from one another by two major geologic fault zones. It is believed that the unstable nature of habitat surrounding these faults has led to the unique evolution of the three haploclades. Using the evolutionary species concept, results of this study in combination with those of previous studies warrant the elevation of the subspecies *Xantusia henshawi gracilis* to full species, *Xantusia gracilis*. This study has assisted in identifying unknown barriers to gene flow that have contributed to the evolution of these species.

INTRODUCTION

The night lizard family Xantusiidae ranges disjunctly throughout the southwestern United States southward through parts of México and Central America. It is also represented on Cuba in the Caribbean Sea, and on the Channel Islands of California (Fig. 1). The family is comprised of highly specialized lizards with narrow microhabitat requirements (Bezy 1988). The night lizard, *Xantusia henshawi*, is known for its saxicolous, crevice dwelling lifestyle. It contains two recognized subspecies, *X. h. henshawi* and *X. h. gracilis*. The granite night lizard, *X. h. henshawi*, is restricted to metavolcanic and granodiorite boulder outcrops along the Peninsular Ranges and coastal mountains of southern California and northern Baja California, México (Lee 1976). The sandstone night lizard, *X. h. gracilis*, is restricted to a unique sandstone formation known as the Truckhaven Rocks, located in Anza Borrego Desert State Park, San Diego county, California (Grismer and Galvan 1986; Fig. 2).

The granite night lizard (Fig. 3), *Xantusia henshawi henshawi*, is a small lizard ranging between 56-62 mm adult snout-vent length (SVL). It is dorsoventrally compressed, allowing it to reside within the narrow confines of granite exfoliations, and has a marked diel color phase change. *Xantusia. h. henshawi* is found from the north slope of the San Jacinto Mountains, California southward to the south slope of the Sierra San Pedro Mártir, Baja California, México, and from the coastal to desert slopes of the Peninsular Ranges. It occurs from altitudes of 130 to 2225 m (Lee 1976). The microhabitat affinity of this subspecies is believed to be a limiting factor in its distribution (Lee 1975). Lee (1975) found this species to be sedentary during the summer months, often confined to particular boulder outcrops or crevices; and rarely found away

from rocks. The two greatest distances moved by individual lizards were reported as 67 and 219 feet respectively (Lee 1975). The majority (88%) of recaptures were made on the same boulder where lizards were first captured (Lee 1975). Recent pitfall trapping efforts in areas inhabited by *X. h. henshawi* habitat yielded only 21 captures out of 30,000 total reptile and amphibian captures (R. Fisher, pers. comm. 9/9/99). Low capture numbers are further evidence of the sedentary nature of *X. h. henshawi*.

The sandstone night lizard (Fig. 3), *Xantusia henshawi gracilis*, ranges from 50-70 mm adult SVL. These lizards are also dorsoventrally flattened, although they are less saxicolous than *X. h. henshawi* (Grismer and Galvan 1986). When compared to *X. h. henshawi*, their limbs are not as splayed, and they have a shorter interfore- and interhind-limb widths (Grismer and Galvan 1986). The diel color phase change is reduced to absent in these lizards, and the resulting coloration is similar to, but lighter than, the light (evening) phase of *X. h. henshawi* (Grismer and Galvan 1986). Reduction of a diel color phase change and the resulting lighter coloration renders these lizards more cryptic on the lighter tones of the sandstone substrate they inhabit (Grismer and Galvan 1986).

Xantusia henshawi gracilis is restricted to a 3 square kilometer sandstone rock formation known as the Truckhaven Rocks, Miocene (~24 million years ago (MYA)), lying at the southern end of the Santa Rosa Mountains, San Diego County, California (Grismer and Galvan 1986). These lizards use exfoliations and crevices resulting from erosion of the sandstone formation, but they also exploit a wider range of microhabitats including small mammal burrows on hardened siltstone (Grismer and Galvan 1986).

A protein electrophoretic study by Bezy and Sites (1987) supports Grismer and Galvan (1986) in considering *X. h. henshawi* and *X. h. gracilis* as sister taxa, given that

four out of 22 presumptive gene loci were unique and fixed, and that a genetic distance of 0.188 was found between *X. h. henshawi* and *X. h. gracilis*. Grismer and Galvan (1986) found *X. h. gracilis* to have at least seven unique diagnostic morphological characters and indicated that it differed from *X. h. henshawi* in several aspects of its life history. Sites et al. (1986) found *X. h. gracilis* to express a protein (lactate dehydrogenase B₄ subunit) not present in *X. h. henshawi*.

Mitochondrial DNA data have been used successfully in the field of molecular systematics for analyzing both intra- and interspecific relationships (Avice et al. 1987; Hillis et al. 1996). Results from studies of this type can be useful in determining mtDNA haplotypes present in a species or population, and to construct a gene tree to infer phylogenetic relationships among populations. Since this type of DNA is maternally inherited in most species, and is non-recombinant, all parts of the molecule share a pattern of common descent (Hillis et al. 1996). Only half of the history of the organism under study is indicated in mtDNA analyses since the paternal history of the molecule is not necessarily identical.

This study will use mitochondrial DNA (mtDNA) sequence data to analyze the phylogeographic relationships within and between the populations of the two subspecies of *Xantusia henshawi*. The goal of this study is to analyze mtDNA variation over the range of *X. henshawi* in southern California. Given the circumscribed distribution of *X. h. gracilis*, it is hypothesized here to have evolved out of *X. h. henshawi* (Grismer and Galvan 1986). If this hypothesis is correct, these data should indicate to which population of *X. h. henshawi* *X. h. gracilis* is most closely related. Following this, a historical biogeographical scenario will be provided that is consistent with the inferred

phylogeny and the historical environmental events that may have led to the evolution of *X. h. gracilis*.

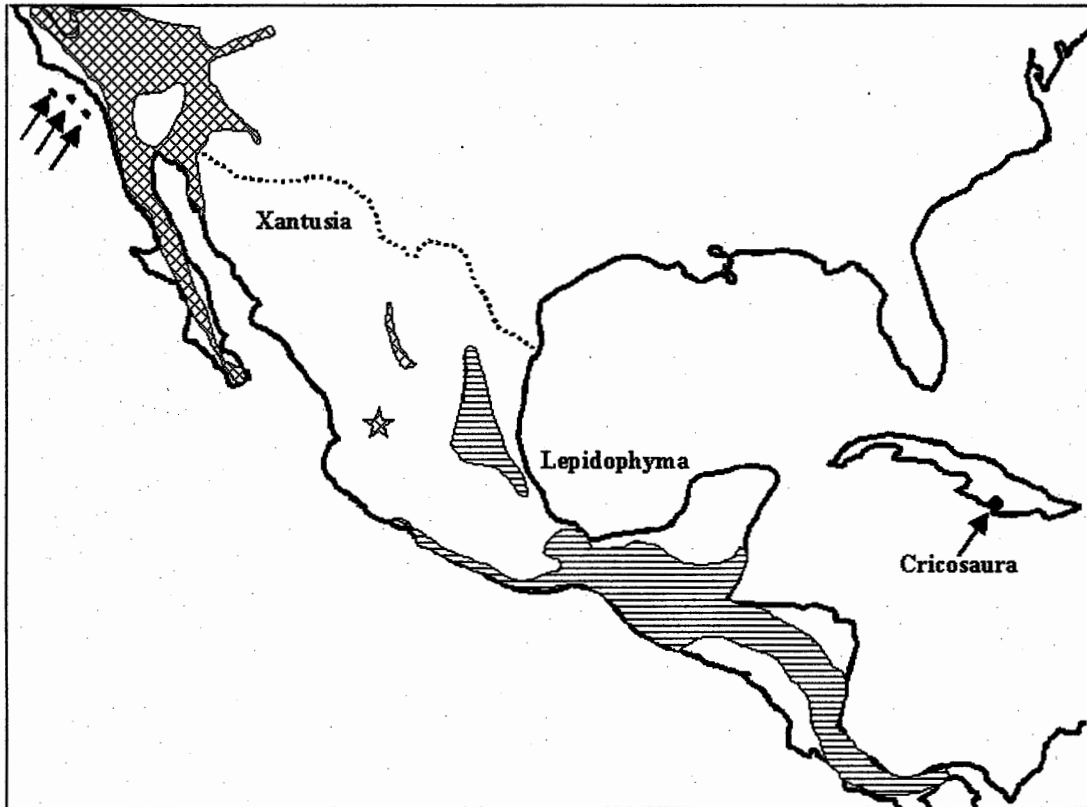


Figure 1. Distribution map of the three extant genera comprising the family Xantusiidae (sensu Bezy 1988). Arrows indicate three Channel Islands where *Xantusia riversiana* is found. The star indicates the location of the recently described *Xantusia sanchezi* (Bezy and Flores-Villela 1999). The solid dot indicates the single known location for *Cricosaura smithii*.

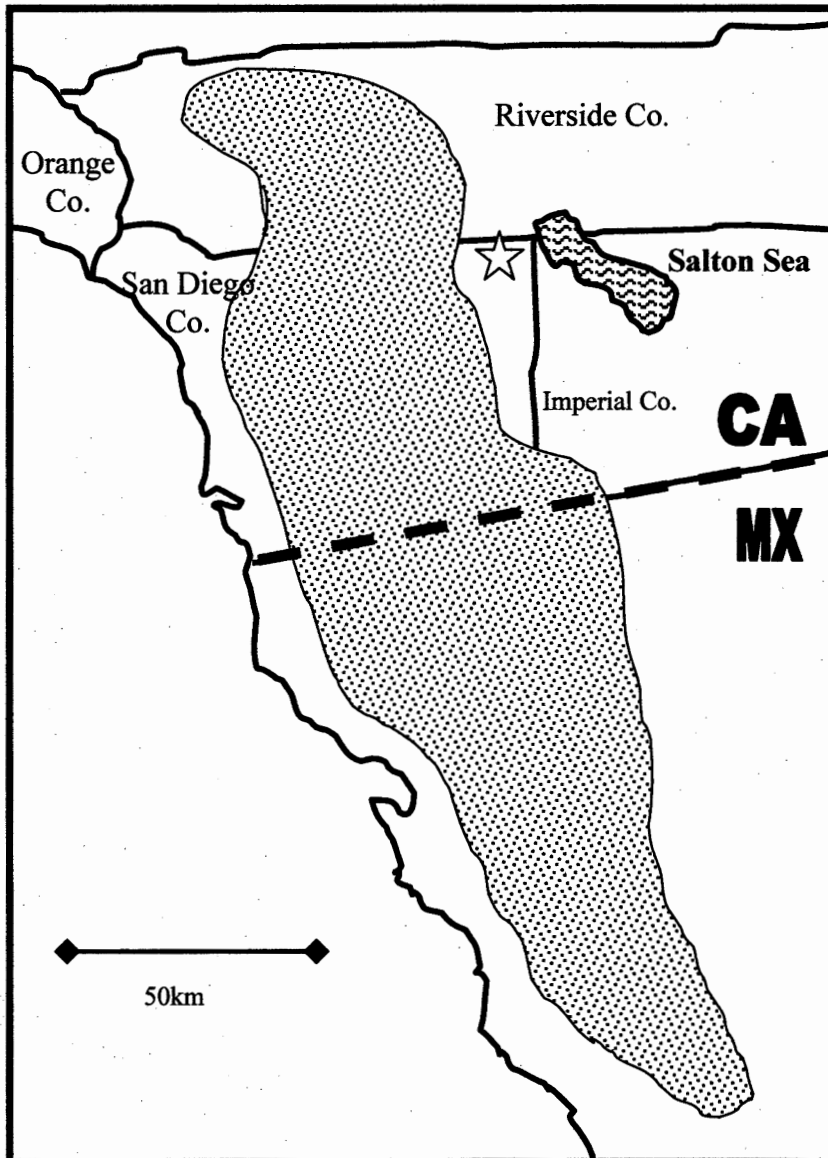


Figure 2. Approximate range map for *Xantusia henshawi henshawi* and *X. h. gracilis*. Solid star indicates the single locality for *X. h. gracilis*.



Figure 3. *Xantusia henshawi henshawi* (top) and *X. h. gracilis* (bottom)

MATERIALS AND METHODS

Sampling

The sampling design used in this study focused on gathering samples from across the known range of *Xantusia henshawi* within California. Specimens were collected with a dual purpose: 1) to maximize geographic coverage over the range of *X. henshawi* to represent environmental and geographic extremes, and 2) to minimize the geographic sampling gap between *X. h. henshawi* and *X. h. gracilis*. All specimens were collected between 1998-1999. A total of 14 *X. h. henshawi* and 4 *X. h. gracilis* samples were collected, representing 13 localities (Fig. 4). All specimens were collected under a permit issued by the California Department of Fish and Game (#801051-02). Various methods were employed for the capture of lizards, which included nighttime lantern walking and non-invasive daytime searching beneath boulders and rock flakes. In addition, the DNA sequence of a single individual of *X. h. henshawi* from Banning, California was retrieved from GenBank for use in this study.

Tissue samples and voucher specimens are to be deposited in the collection of the San Diego Natural History Museum (SDNHM) and Los Angeles County Museum of Natural History (LACM). Specimens used and collection localities are listed in Appendix I. Thirteen populations and five outgroup species (*Cricosaura typica*, *Lepidophyma smithi*, *Xantusia vigilis*, *Xantusia riversiana* and *Xantusia bolsonae*) were used in this analysis (See appendix I).

Laboratory Protocol

DNA was obtained from muscle or liver tissues. Total genomic DNA was isolated by methods outlined in Palumbi et al. (1991). A single segment of mitochondrial DNA(mtDNA) was amplified using the polymerase chain reaction (PCR; Mullis 1986, Gyllensten and Erlich 1988) and a pair of primers. The region sequenced totaled 379 bases of the cytochrome *b* gene. Primer sequences are as follows: GLUDG-L (=L14704, Palumbi 1996) (5'-TGA CTT GAA RAACCA YCG TTG-3'); and H15149 (Kocher et al. 1989) (5'-AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A-3'). PCR conditions consisted of 35 thermal cycles: one minute denaturation at 94°C, one minute annealing at 50°C, and 90 seconds extension at 72°C. PCR products were electrophoresed on a 3.5% polyacrylamide gel and stained with ethidium bromide solution to visualize and verify product amplification and band size. Following visualization of the PCR product, samples were purified using GeneClean before sequencing. Products were sequenced with Applied Biosystems, Inc. Fluorescent Automated Sequencers 373 and 377 at the Sequencing Facility for Loma Linda University, and at the California State University, Northridge DNA Sequencing Facility.

Sequences were edited for correct peak calls and aligned using Sequencher 3.0 (Gene Codes Corporation 1995). Aligned sequences were exported using Sequencher 3.0 as a NEXUS file, and formatted for phylogenetic analysis using MacClade 3.01 (Maddison and Maddison 1992). Maximum parsimony (MP) analysis was executed using the heuristic search algorithm in PAUP* (Version 4.0b2a, Swofford 1999), using a random addition sequence. Formatting in MacClade 3.01 included codon position assignment, and transition versus transversion weighting using Sankoff stepmatrices

(Sankoff and Rousseau 1975). Base pair composition, pair-wise variation, codon position variation, and total variation calculations were executed using PAUP*.

Tree Reconstruction

All phylogenetic analyses were done using PAUP* (Version 4.0b2a, Swofford 1999). DNA sequence data was first analyzed using unweighted (=equally) parsimony analysis on only phylogenetically informative characters. Weighted parsimony analyses were conducted using a transition to transversion ratio of four to one. It has been shown that transversion weighting should be based on the data at hand and not on empirical observations from outside sources (Reeder 1995). Transversion to transition weights were chosen by calculating the transition to transversion ratio using likelihood analysis. A value of 3.65 was derived for the data set including ingroup and outgroup taxa, and 4.61 for only ingroup taxa. A weighting value of four was selected as a whole number between the two calculations. Homoplasy levels were evaluated using the consistency index (Kluge and Farris 1969) and the retention index (Farris 1989, 1990). Since uninformative characters affect the consistency index, only informative characters were used to determine this value.

Outgroup Selection

Outgroup choice has been shown to affect tree rooting positions in phylogenetic analyses (e.g. Messenger and McGuire 1998, Hollingsworth 1999). Outgroups used in this analysis were selected using the proposed phylogeny of the Xantusiidae by Hedges et al. (1991). Single representatives for each outgroup genus, and three species from within

the genus *Xantusia* were chosen. *Cricosaura typica*, *Lepidophyma smithii*, *X. riversiana*, *X. vigilis* and *X. bolsonae* were used in this analysis (Appendix I). Four *Xantusia vigilis* were collected for this study from Phelan, California, one of which was used to generate outgroup sequence data for this study. The remaining outgroup sequence data was obtained from GenBank from the submissions of Hedges and Bezy (1993). All parsimony analyses were subjected to a constrained outgroup topology following Hedges et al. (1991; Fig. 5).

Confidence and Signal

Nonparametric bootstrap analysis was used to evaluate confidence within phylogenetic trees (Felsenstein 1985) using 1000 bootstrap replicates with the fast-heuristic search option in PAUP*. Only bootstrap values of over 50% are reported and support of 70% or greater is believed to represent a strongly supported clade corresponding to a 95% confidence interval (Hillis and Bull 1993).

The ingroup data set was examined for phylogenetic signal using the g_1 statistic (Hillis and Huelsenbeck 1992) to ensure that the most parsimonious trees were not generated from random data. The g_1 statistic measures the skewness of the distribution of random trees (10,000 random trees in this analysis) to discern phylogenetic information relative to random noise. Critical values for random data were derived by extrapolating values from table 2 of Hillis and Huelsenbeck (1992).

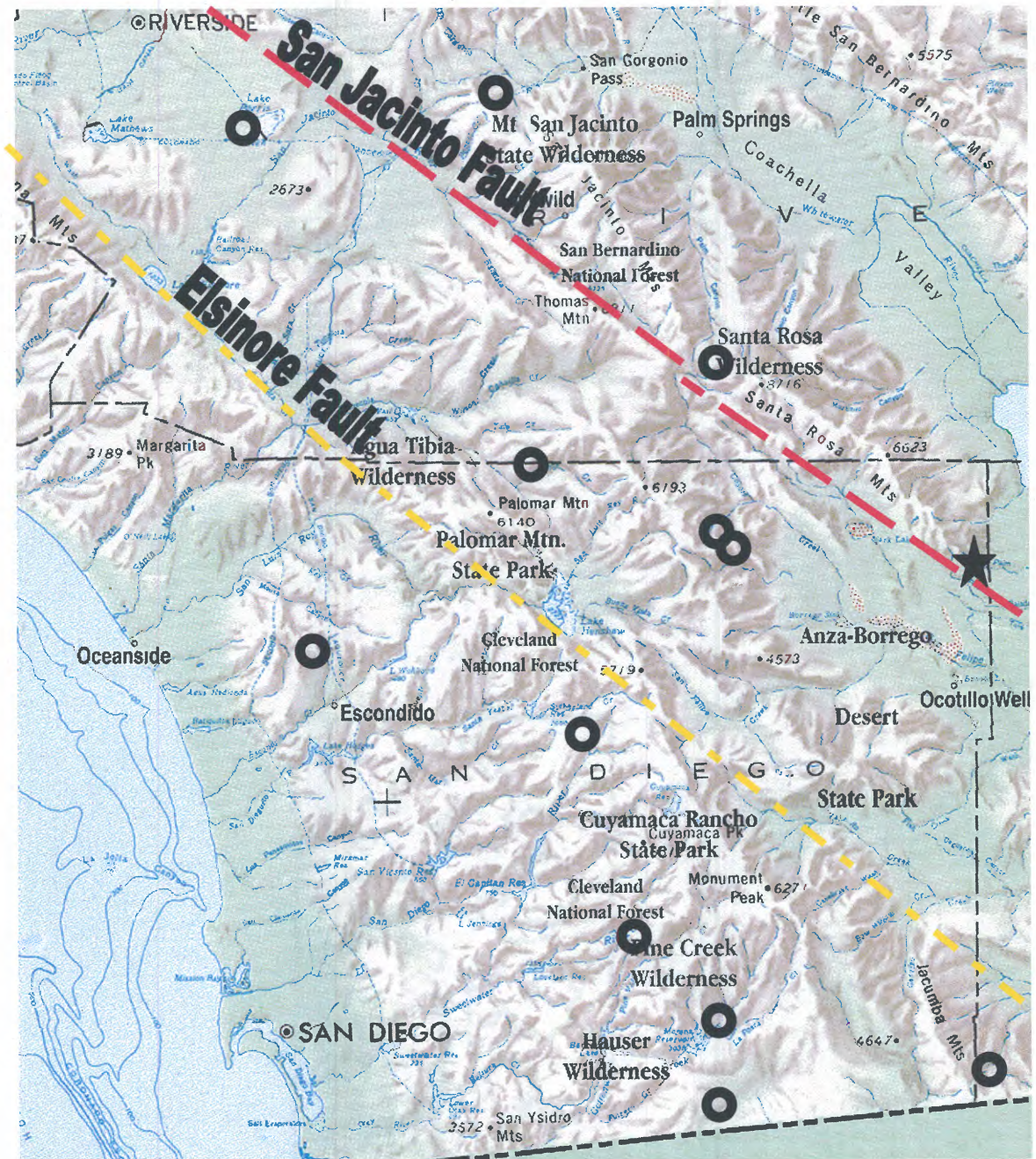


Figure 4. Map showing collecting localities *Xantusia henshawi henshawi* (circles) and *X. h. gracilis* (star). Dashed lines indicate the approximate location of the San Jacinto and Elsinore Fault Zones.

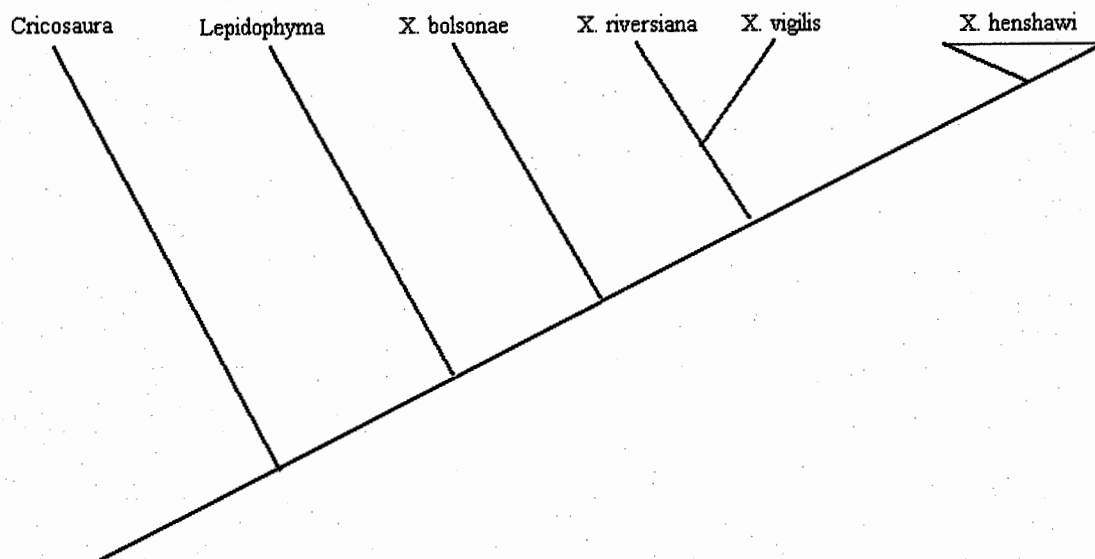


Figure 5. Constrained outgroup topology of xantusiid genera after Hedges et al. (1991).

RESULTS

Phylogenetic Signal

The g_1 analysis indicates that the data set used in this study contains phylogenetic signal (as opposed to random data) based on significantly left-skewed g_1 values at $P < 0.01$ (Hillis and Huelsenbeck 1992). The lengths of 10,000 randomly sampled trees resulted in a g_1 value of -0.59 for ingroup taxa alone. The critical g_1 value for 10 four-state characters (i.e. DNA characters) from 15 or more taxa is -0.16 . ($P = 0.05$; Hillis and Huelsenbeck 1992). Hillis and Huelsenbeck (1992) do not give g_1 statistic values for the 19 ingroup taxa used in this study. The more conservative figure of 15 ingroup taxa was used to estimate phylogenetic signal. Therefore the g_1 value indicates that the data set is significantly more structured than random data.

DNA Sequences Data

The data consist of 379 aligned nucleotide positions from the cytochrome *b* gene. This aligned sequence does not include primer nucleotide positions. The total aligned sequence includes 30.3% adenine (A), 25.9% cytosine (C), 13.4% guanine (G), and 30.4% thymine (T). The data set contains a total of 116 informative characters, of which a minimum of 71 characters are informative within the ingroup. Outgroup relationships were not analyzed in this study, and are excluded here. Of the 71 informative characters within the ingroup, 12 (17%) are at first codon positions, 8 (11%) are at second codon positions, and 51 (72%) are at third codon positions. Pairwise sequence divergence ranged from 0.0 – 17.0%.

Unweighted Maximum Parsimony Analysis

The unweighted parsimony analysis, using all variable characters, produced 50 trees with 361 steps for all variable characters, or 286 steps for only informative characters. The most parsimonious trees have a consistency index of 0.58, and a retention index of 0.78. Results from the strict consensus tree indicate a trichotomy of three well-supported haploclades (A, B, and C) within *Xantusia henshawi* (Fig. 6). Some additional substructuring within these haploclades is well supported (Fig. 6).

A 92% bootstrap value and 16 nucleotide changes support haploclade A (node 6). This haploclade contains four individuals of *Xantusia henshawi gracilis*. It is supported by a 100% bootstrap value and nine nucleotide changes (node 8). Thirty of the 50 most parsimonious trees generated indicate that *X. h. gracilis* is most closely related to the Ranchita and Culp Valley group of individuals. Ten of 50 trees support an unresolved polytomy between *X. h. gracilis*, the Ranchita and Culp Valley group, and individuals from Lake Perris and Chihuahua Valley. The remaining 10 trees support the sister taxa relationship between either the Chihuahua Valley and/or Lake Perris individuals and *X. h. gracilis*. A well-supported sister group relationship exists between individuals from Ranchita and Culp Valley (node 7), supported by a 100% bootstrap value and eight unambiguous nucleotide changes.

A 100% bootstrap value and 15 nucleotide changes support haploclade B (node 4). Individuals from Lake Morena, Campo, Ocotillo, Escondido, and Pine Valley form a group supported by an 86% bootstrap value and one nucleotide change (node 5). A 92% bootstrap value and 12 nucleotide changes support haploclade C (node 2). Within

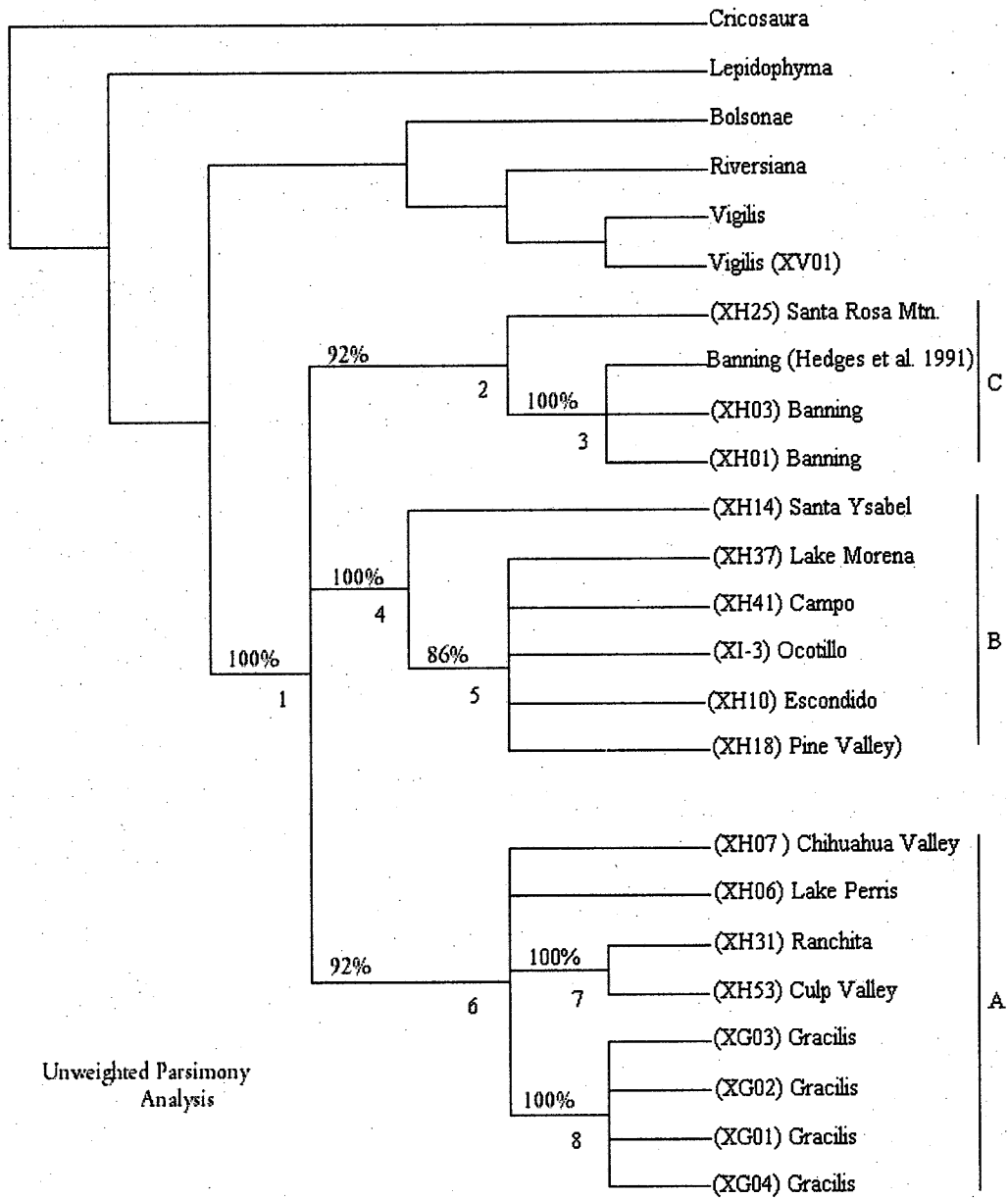


Figure 6. Strict consensus tree from unweighted maximum parsimony analysis. Letters A, B, and C on the right refer to major haploclades recognized in this study.

this haploclade, the three individuals from Banning (node 3) form a well-supported group (100% bootstrap value) with eleven nucleotide changes.

Weighted Parsimony Analysis

The weighted parsimony analysis, using all variable characters, produced 2 trees with 689 steps for all variable characters, or 511 steps using only informative characters. The most parsimonious trees have a consistency index of 0.72, and a retention index of 0.78. The strict consensus tree in the weighted analysis is congruent with the strict consensus tree in the unweighted analysis. The weighted strict consensus tree (Fig. 7) contains the same three well-supported haploclades (A, B, and C). The weighted analysis produced a tree with increased resolution within the nodes of haploclades A, B, and C (Fig. 7). However, all of the increased resolution shown in the weighted analysis is supported by bootstrap values of less than 70% (Fig. 7 Nodes A-F). Nodes in common to both the weighted and unweighted analyses are referred to by the same designations (i.e. 1-9) as seen in figure 6, and additional resolution is designated by A-F (Fig. 7).

Results from the weighted analysis are chosen over the unweighted because of its increased consistency index (C.I.) value, increased resolution, weighting based on the data at hand is preferable to empirical observations from outside sources (Reeder 1995), and estimates of phylogenetic relationships are improved using a weighting scheme based on data at hand (Voelker and Edwards 1998).

Haploclade A is well supported by 13 nucleotide changes and an 88% bootstrap value (Fig. 7). Similar to the unweighted analysis, *Xantusia henshawi gracilis* is well supported by seven nucleotide changes and a 100% bootstrap value (node 8). The

individuals from Ranchita and Culp Valley are supported by eight nucleotide changes and a 99% bootstrap value (node 7). In the weighted analysis, a sister group relationship between Lake Perris and Chihuahua Valley is formed (node A), who share nine nucleotide changes (bootstrap value of 69%). Both of the trees generated in the weighted analysis, as well as the strict consensus tree (Fig. 7), place *X. h. gracilis* as the sister to the group formed (node B) containing the individuals from Chihuahua Valley, Lake Perris, Ranchita, and Culp Valley. However, this group is supported by a bootstrap value of only 69%.

Haploclade C is identical to the results from the unweighted analysis and contains individuals from Banning and the Santa Rosa Mountains (node 2). A 100% bootstrap value and fourteen nucleotide changes support this haploclade. Within this haploclade the three Banning samples form a sister group (node 3) relationship supported by a 100% bootstrap value and twelve nucleotide changes.

Haploclade B is congruent with the results from the unweighted analysis, but the strict consensus tree contains added resolution. This haploclade is supported by a 98% bootstrap value and 10 nucleotide changes. Santa Ysabel is the most basal individual and contains 10 nucleotide changes. As in the unweighted analysis all other members form a group supported by three nucleotide changes and a 78% bootstrap value.

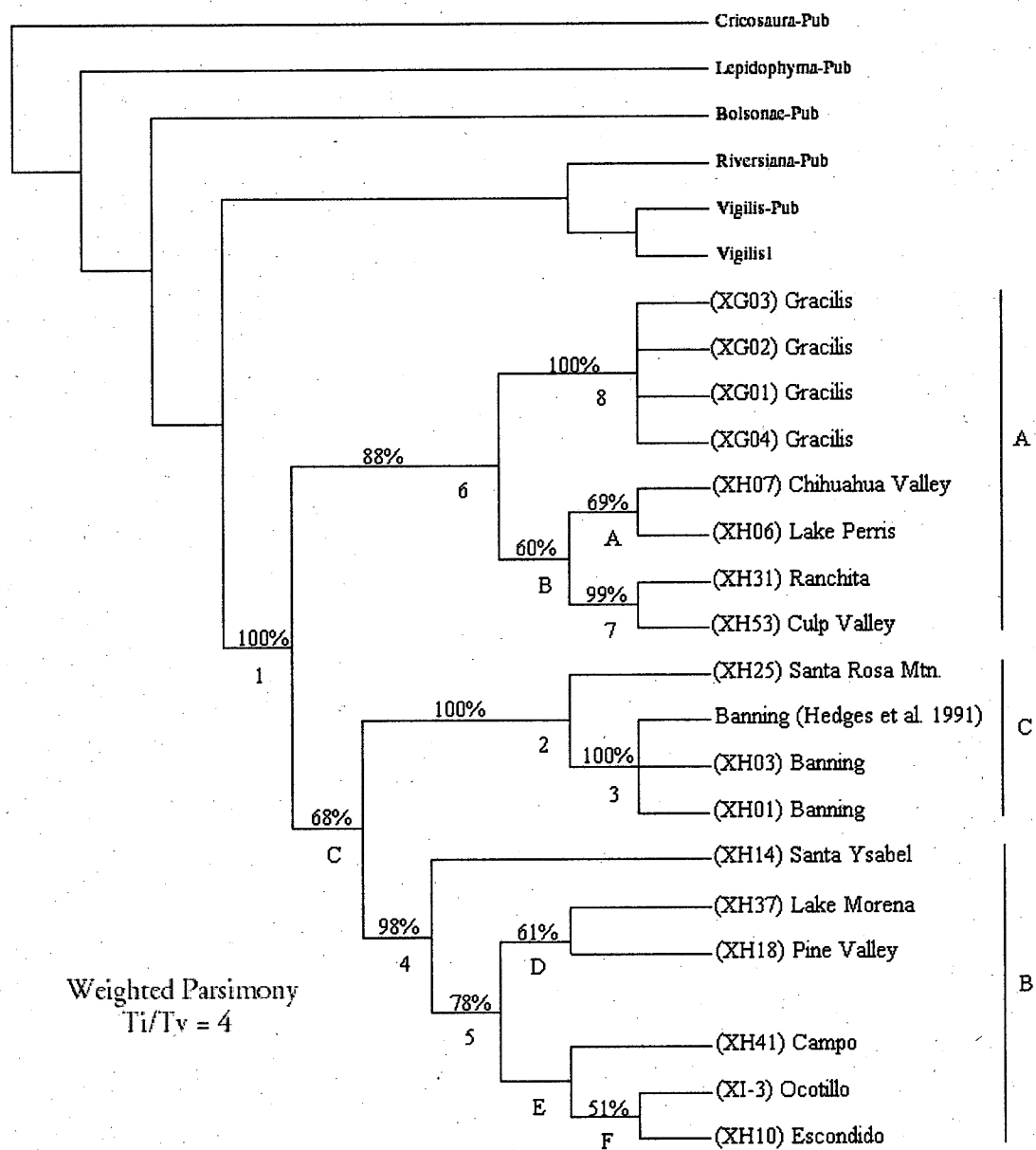


Figure 7. Strict consensus tree from weighted ($Ti/Tv = 4.0$) maximum parsimony analysis.

DISCUSSION

Sandstone Night Lizard

The gene tree relationships from the unweighted and weighted analysis indicate that *Xantusia henshawi gracilis* is an exclusive lineage that evolved from within *Xantusia h. henshawi*. *Xantusia h. henshawi* thus forms a non-exclusive species, in the sense that some individuals are more closely related to *X. h. gracilis* than to other individuals of *X. h. henshawi*. Grismer and Galvan (1986) found that *X. h. henshawi* and *X. h. gracilis* were allopatric (Fig. 8), and that the latter was distinct in several morphological characters (e.g. having a narrower head and body, fewer dorsal scales at mid-body, thinner limbs, greatly enlarged temporal scales, a lack of significant diel color phase change, smaller dorsal markings, and a general lack of ventral markings). They also found differences in several life history attributes (e.g. they inhabit sandstone, are exclusively found within a desert environment, and utilize small burrows in addition to exfoliating sandstone). Sites et al. (1986) studied isozyme expression in the family Xantusiidae and concluded that *X. h. gracilis* was one of only two Xantusiid species (the other being *X. riversiana*) to evolve the A₃B₁ lactate dehydrogenase isozyme. A later study found four fixed unique alleles in *X. h. gracilis* with respect to *X. h. henshawi* (Bezy and Sites 1987). MtDNA data from this study indicate that *X. h. gracilis* differs from the most closely related populations of *X. henshawi* in having nine nucleotide changes in the unweighted analysis (Appendix 2), and seven nucleotide changes in the weighted analysis (Appendix 3).

The allopatry and numerous discrete differences between *X. h. gracilis* and *X. h. henshawi* indicate they are on separate phylogenetic trajectories and should be considered

different species under the evolutionary species concept (sensu Frost and Hillis 1990).

Based on these differences, *X. h. gracilis* is recognized here as a full species.

Xantusia gracilis shares a most recent common ancestor with *X. henshawi* individuals from Culp Valley, Ranchita, Lake Perris and Chihuahua Valley (Haploclade A; Figs. 6 and 7). Grismer and Galvan (1986) found that *Xantusia henshawi* was absent from the southern slopes of the Santa Rosa Mountains, the geographically most proximal habitat to the Truckhaven Rocks. In this analysis, a single individual from the northern slopes of the Santa Rosa Mountains, located 64 km to the northwest of *X. gracilis* was shown to be more closely related to individuals from Banning, a locality further to the north (Haploclade C). These results indicate that the populations of *X. henshawi* which inhabit portions of the Santa Rosa Mountains do not share a close affinity with *X. gracilis* and is in accord with its noted absence from the southern slopes of the Santa Rosa Mountains. In both the unweighted and weighted analyses, *X. gracilis* is most closely related to individuals from 30 km away in Culp Valley to as far as 135 km in Lake Perris. In the unweighted analysis, 30 of the 50 most parsimonious trees support the sister relationship between *X. gracilis* and the Ranchita and Culp Valley group. However, in the weighted analysis, both of the most parsimonious trees support the sister relationship between *X. gracilis* and the remaining individuals within haploclade A. In either scenario, it is likely that *X. gracilis* originated from an ancestral population located somewhere in the vicinity of Culp Valley, the geographically most proximate locality to the Truckhaven Rocks.

Granite Night Lizard

Three haploclades of *Xantusia henshawi* are geographically separated by two major fault zones, the San Jacinto Fault in the north and the Elsinore Fault in the south (Fig. 4). Results of this study suggest that these faults have created barriers to dispersal in the form of fault valleys, canyons and arroyos that have limited the dispersal capability of these already low vagility lizards. Valleys, canyon floors, and arroyos are disturbed too frequently by erosional forces to generate stable boulder outcrops and their respective microhabitats, such that *X. henshawi* is not found in these areas. Valleys, canyon floors, and arroyos generally consist of sedimentary material, alluvium, and cobble (Jennings 1958). The low vagility of these lizards (Lee 1975; Fellers and Drost 1991, Fellers et al. 1998) is attributed to their highly specialized crevice dwelling habits and their preference for stable microhabitats. The dynamic nature of rock formations along fault zones and associated features is probably prohibitive to the dispersal of these lizards.

Haploclade A contains four members of *Xantusia henshawi* who are more closely related to *X. gracilis* than to other members of *X. henshawi*. The close relationship of Culp Valley and Ranchita individuals are well supported in both the weighted and unweighted analyses. The relationships for samples from Lake Perris and Chihuahua Valley are not well supported in the unweighted analysis, and are weakly supported (69% bootstrap value) in the weighted analysis. The maximum geographic distance among these populations is 87 km (e.g. distance between the Lake Perris and Ranchita or Culp Valley populations). A geographic distance of 66 km separates the Chihuahua Valley and Lake Perris individuals. The Ranchita and Culp Valley populations are only 5 km apart and suitable habitat is continuous between them.

Haploclade B in both analyses is comprised of samples from Santa Ysabel, Ocotillo, Campo, Lake Morena, Escondido, and Pine Valley. These samples all represent populations that occur south of the Elsinore Fault. Strong bootstrap support (100%) is seen for this haploclade as a whole, in addition to a number of haploclades within this group. This haploclade roughly approximates the geographic center of range for *Xantusia henshawi* and suitable habitat corridors exist throughout the area of this haploclade. Presence of continuous suitable habitat with no major geographic barriers is believed to have facilitated dispersal. This haploclade spans nearly twice the geographic area as the other haploclades and is not bisected by any major fault zones.

Haploclade C contains individuals from two major geographic regions, the Santa Rosa and San Jacinto Mountains. A distance of approximately 48 km separates these two mountain ranges from one other. Continuous suitable granite habitat is present between them, but significant elevational differences of up to 700 m create large habitat differences over the 48 km. Two of the three Banning individuals (XH01 and XH03) represent the only samples collected from within several hundred meters of one another at the same location, and all three samples from this locality form a very strong relationship in both the weighted and unweighted analyses. The only *Xantusia henshawi* sample taken from published data (Hedges et al. 1991) was originally collected a few kilometers more distant from the other two collection localities in Banning, and is nested with the within the polytomy representing all Banning Samples.

Relationships among the three haploclades (A, B, and C) are not clearly shown by this study. Results of the unweighted parsimony analysis (Fig. 6) indicate an unresolved polytomy representing the three haploclades. Results of the weighted parsimony analysis

indicate that haploclades B and C are sister groups and together form a sister group to haploclade A. This relationship, however, is weakly supported by a 69% bootstrap value.

MtDNA Variation

The high levels of mtDNA sequence variation (raw ingroup variation of 15%) that are found in this study may be associated with the ecological limitations and age of these lizards. The Xantusiidae generally have a delayed reproductive maturity, low reproductive potential, limited dispersal capability (i.e. low vagility), long life span, exhibit low mortality, and lack differential mortality (Lee 1975). Their age is well supported as dating from the Cretaceous (Schatzinger 1980, Sullivan 1982, Hedges et al. 1991). The relatively old age of this lizard group, and the disjunct distribution of preferred microhabitat features may be contributing to the high levels of sequence divergence indicated in this study.

Biogeographic History

Xantusia henshawi and *X. gracilis* are confined to what is referred to as the Peninsular Ranges batholith, which dates to the mid-Cretaceous (Gastil et al. 1981: Fig. 8). Circumscribed distributions of haploclades A, B, and C are indicated on the area cladogram shown in figure 8.. The affinity of these lizards for formations of this age is consistent with molecular data from the findings of Hedges et al. (1991), and the fossil record (Schatzinger 1980, Sullivan 1982), which suggest that *Xantusia* arose sometime after the late Cretaceous. Formation of mountain ranges along fault zones in the southern

California and northern México region began at least 20 mya (Pat Abbott pers. comm. 8/10/99), in the Miocene.

Relationships indicated by this study strongly coincide with the known geologic history of the region. The Peninsular Ranges of southern California consist of several distinctive erosional surfaces such as the Perris Plain and the inland San Diego County region (Norris and Webb 1990). The Perris Plain is bounded by the San Jacinto Fault in the northeast, and the Elsinore Fault in the southwest (Norris and Webb 1990). The Elsinore and San Jacinto faults are strike-slip fault zones characterized for the most part by left-lateral movements. Lands to the south of the San Jacinto Fault are moving to the northwest at a rate of 12 mm per year, and lands to the south of the Elsinore fault are moving to the northwest at a rate of 5 mm per year (Harold Magistrale, pers. comm. 9/1/99). The inland San Diego County erosional surface exists from the Laguna and Cuyamaca Mountains on the eastern side, to the erosional surface that is the marine terraces region occurring from San Onofre southward to the Mexican border and beyond (Norris and Webb 1990). There is still some question as to whether any of the above mentioned terraces pre-date the Pleistocene in their present form (Norris and Webb 1990).

Haploclade A (Figs. 6 and 7) in the parsimony analyses is loosely confined to the Perris Plain, indicating that it is presently unknown whether the Truckhaven Rocks were once part of the Perris Plain. Haploclade B (Figs. 6 and 7) is confined to the erosional surface area of inland San Diego County. Haploclade C (Figs. 6 and 7) is confined to areas northwest of the Perris Plain, in the Santa Rosa and San Jacinto Mountains. The molecular evidence of this study supports the evolution of *Xantusia henshawi* and *X.*

gracilis being directly correlated to the formation of the three distinct erosional surfaces bounded by the respective fault zones of this region (Norris and Webb 1990).

Norell (1989) described *Xantusia downsi** from the late Neogene as a probable sister taxon to *Xantusia*, on the basis of reduction of the tricuspid teeth, small size, and more delicate dentary compared with other members of the “*Paleoxantusia*” grade and other xantusiids. Both *X. vigilis* and *X. henshawi* share the above-mentioned features. Based on the geologic data above, molecular data from this study, and fossil evidence above, it is hypothesized that the evolution of *Xantusia henshawi* and *X. gracilis*, as well as the evolution of the three distinct haploclades shown by this study, had their origin during or after the Neogene, as a result of Peninsular Ranges orogeny, vicariant tectonic events, and increasing aridity in the eastern portion of their range.

Truckhaven Rocks

The formation of the Truckhaven Rocks is likely to have played an important role in the evolution of *Xantusia gracilis*. These lizards are found strictly in conjunction with this geologic formation, indicating an affinity for this lithic feature and its associated microhabitats. The Truckhaven Rocks comprise a small area of only 4 square kilometers and are composed of sand and siltstone from the late Miocene and pre-Cretaceous (Jennings 1958). Weathering has created deep canyons, as much as 30 meters in depth and one meter wide. This location is located on the southern flank of the Santa Rosa Mountains in Anza-Borrego Desert State Park, California and lies on the eastern side of the Clark Fault, which is a strike-slip fault zone and part of the larger San Jacinto Fault Zone (Remeika and Lindsay 1992).

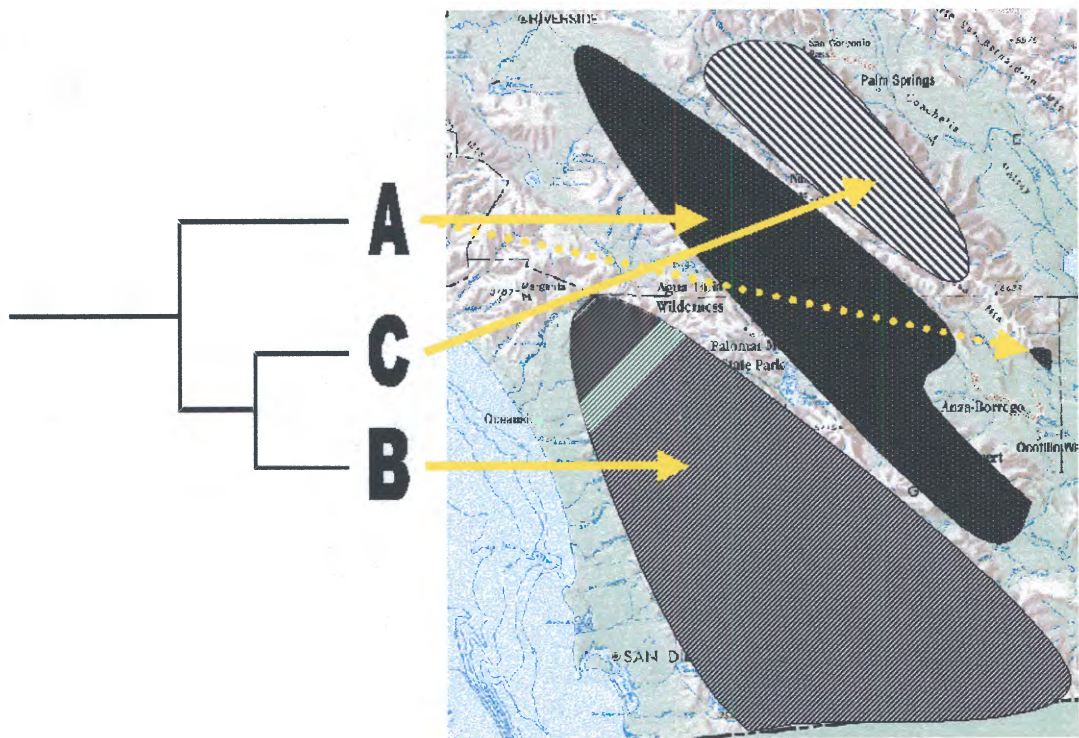


Figure 8. Area cladogram showing the locations of the haploclades indicated in the weighted strict consensus tree (Fig. 7). Dashed arrow shows the location of *Xantusia gracilis*.

A recent hypothesis for the formation of the Truckhaven Rocks is that they are derived from material to the south in the Borrego Mountain region where lithic analogues are found (Remeika pers. comm. 4/15/99). Rocks in this area, and near the Borrego Badlands, are similar to the sand and siltstone formations of Truckhaven Rocks. Under this model, horizontal movements opened Borrego Valley along the San Jacinto Fault Zone and effectively split the proto-Truckhaven Rocks to move the northern portion up against the Santa Rosa Mountains, and the southern portion against the northern end of Borrego Mountain (Remeika pers. comm. 4/15/99).

The results of this study directly support this model since members of *Xantusia gracilis* are most closely related to individuals from Ranchita and Culp Valley. Fossil evidence indicates the presence of Xantusiid lizards in the Vallecito Mountains to the south of the Truckhaven Rocks during the Pliocene (Norell 1989; Fig. 9). These Mountains are adjacent to areas currently occupied by *X. henshawi* on Whale Peak (SDNHM 41003), Granite Mountain (SDNHM 18639), and in the San Felipe Valley area (R. Lovich pers. obs.; Fig. 9). These mountains occupy an area on the desert slope of the Peninsular Mountains, and habitats vary from montane deciduous to the Colorado Desert proper, dependent largely upon the elevation. *Xantusia henshawi* appears to be absent from the lower elevation valleys in this region. It is thought that the presence of substrate features (i.e. granitic boulder outcrops) consistent with those throughout the range of *X. henshawi*, as well as higher, cooler temperatures on upper elevations of the Peninsular Ranges provide suitable conditions for these lizards. A continuous suitable granodiorite substrate feature links the Vallecito Mountains with both Ranchita and Culp Valley (Jennings 1958), crossing the desert floor in the area of Yaqui Pass and San Felipe Creek.

It is believed that post-Pleistocene desertification and associated climatic changes have eliminated xantusiids from suitable substrates between the Truckhaven Rocks and Vallecito Mountains, and from the Vallecito Mountains to suitable upland habitats in the vicinity of the San Felipe Hills. These climatic changes, in conjunction with habitat discontinuities are thought to have isolated populations of *X. gracilis* within the Truckhaven Rocks, and isolated *X. henshawi* on the high peaks of the Vallecito Mountains and the neighboring Peninsular Range.

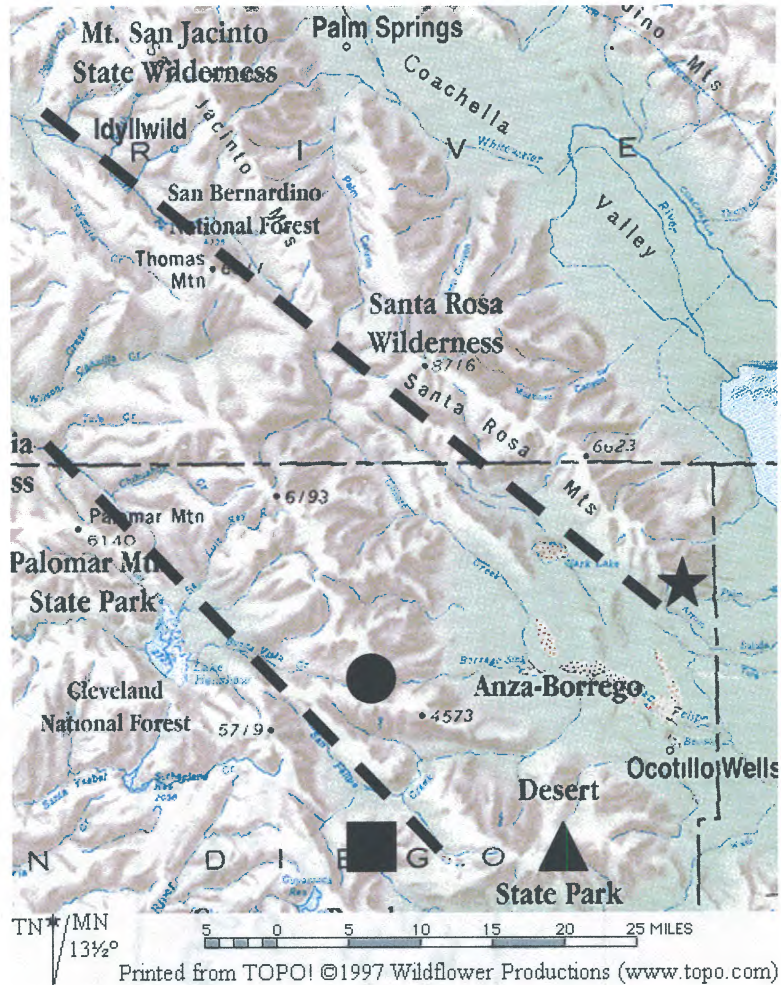


Figure 9. Map of Anza Borrego and adjacent regions showing approximate locations of *Xantusia gracilis* (Truckhaven Rocks) with a star, and *X. henshawi* locations at Whale Peak with a triangle, Granite Mountain with a square, and Ranchita and Culp Valley with a circle. Dashed lines indicate major fault zone approximations.

Conservation Implications

Results from this study indicate high levels of sequence divergence relative to other species (e.g. compared with Frost et al. 1998, Hollingsworth 1999). Conservation of unique haplotypes should be exercised in light of rapid urban development in certain parts of the range of *Xantusia henshawi*. While large areas of suitable habitat remain for *X. henshawi*, coastal and inland habitats are under pressure from urban development, prevalent in the southern California region. Populations from areas between the San Jacinto and Elsinore Faults (Fig. 4) are especially fragmented by urban development. Habitat in this area is largely discontinuous and *X. henshawi* occurs only in "islands" of stable granite features, or monadnocks (e.g. Bernasconi Hills and Lakeview Mountains: Norris and Webb 1990) that are exposed on the surface of respective hilltops. It is not currently known what additional effects urban pressure, habitat loss, or exotic species may be having on these lizards. While disturbance effects were not the focus of this study, the high genetic variation indicates these lizards need to be protected to preserve their natural diversity. Patterns in evolution and variation can be useful in developing a management strategy for these lizards in light of the rapid urban growth (Dimmick et al. 1999, Soltis and Gitzendanner 1999).

The case for conservation of *Xantusia gracilis* is more pressing, since this species is found in only a single, small area within Anza-Borrego Desert State Park. Human access to this area, and its associated impacts, should be reduced, in addition to increased monitoring of the habitat. This extreme isolation puts them at risk of extinction due to catastrophic or stochastic events, potential collecting pressures, introduced or subsidized predators, and habitat loss.

Present conservation of the sandstone and granite night lizard does not address the different evolutionary groups indicated in this study. It could be argued that each of the strongly supported groups in this study are evolutionarily distinct units that should be protected, and prioritized into management and conservation actions. Incorporating phylogeny into conservation is arguably more useful in preserving biodiversity than those actions that do not incorporate it (Dimmick et al. 1999, Soltis and Gitzendanner 1999). The biogeographic regions that support these distinct evolutionary lineages should be protected as well, especially since the California floristic province has been targeted as a global biodiversity hotspot (Mittermeier et al. 1998).

CONCLUSIONS

Xantusia gracilis is an exclusive lineage in that all individuals are more closely related to other individuals of *X. gracilis* than to other members of *X. henshawi*. Results of this study indicate that *Xantusia gracilis* is most closely related to individuals of *X. henshawi* from Ranchita and Culp Valley, California.

Xantusia henshawi appears to be a non-exclusive lineage in that some individuals are more closely related to *X. gracilis* than to other individuals of *X. henshawi*. At least three distinct haploclades exist for *X. henshawi* (Fig. 6 and 7). Relationships among these three haploclades are unresolved.

Xantusia gracilis, the sandstone night lizard, is elevated to full species based on mtDNA relationships in this study, allopatry, morphological and life history attributes (Grismer and Galvan 1986), unique isozyme expression (Sites et al. 1986), and unique fixed allelic differences (Bezy and Sites 1987).

There is a strong correlation between the well-supported haploclades and geographic barriers to dispersal, in the form of major fault zones and their respective features. *Xantusia henshawi* and *X. gracilis* are likely to have evolved *in situ*, and their inability to disperse is attributable to the constraints associated with a crevice-dwelling lifestyle. Dispersal is hampered by the unstable nature of boulder outcrops within washes, valleys and arroyos along the San Jacinto and Elsinore Faults, as well as thermal constraints along the Colorado Desert slopes of the Peninsular Mountain Range that they inhabit.

The intraspecific variation and evolutionary history of these specialized lizards indicates a conservative history and strong affinities for particular lithic features. Webb

(1970) and Grismer and Galvan (1986) found little morphological variation between northern and southern populations of *Xantusia henshawi*. The relationships proposed in this study indicate the presence of major subdivisions within *X. henshawi* and therefore mtDNA variation is decoupled from morphological evolution (Wiens and Hollingsworth 2000). Studies such as this are useful in determining population boundaries not readily apparent when using other types of data.

Future Research Needs

Future research should focus on gaining a better understanding of the biology and natural history of *Xantusia gracilis*, since it is poorly known at present. Our understanding of this lizard only began within the last 15 years, and considerable gaps in our knowledge of it remain.

Since *Xantusia gracilis* are related to *X. henshawi* from the vicinity of Culp Valley, additional specimens should be gathered and analyzed from areas such as the Vallecito Mountains, Granite Mountain, and perhaps the Santa Rosa Mountains. Sampling of these regions could help indicate what population of *X. henshawi* that *X. gracilis* is derived from, and how they came to be isolated in the Truckhaven Rocks. Studies of nuclear DNA from samples on either side of major fault zones would be useful in determining whether dispersal and successful mating events are taking place.

Future research on these lizards should include samples from throughout their range in México. Additional haplotypes besides the three indicated in this study may occur in more southerly portions of the range of *Xantusia henshawi*. Shared evolutionary patterns may exist within other parts of the range of *X. henshawi* along historical habitat

discontinuities. One example of a potential habitat discontinuity is the Paseo de San Matías, which separates *X. henshawi* in the Sierra San Pedro Mártir from those in the Sierra de Juarez. Other less obvious habitat discontinuities may occur in the form of fault zones and their associated features, or river valleys and canyons. Indeed, vicariant isolation events have been indicated as having an impact on lizard groups, including river valleys (Frost et al. 1998) and fault regions associated with mountain ranges (Macey et al. 1999a, Macey et al. 1999b).

Sympatric and specialized taxa (e.g. *Phyllodactylus* sp.) with similar life history and ecological constraints as those exhibited by *X. henshawi* and *X. gracilis* may share a common evolutionary pattern resulting from the active nature of this region throughout recent geologic history. Studies of these and other such lizards may indicate evolutionary correlation resulting from shared geologic and habitat constraints.

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APPENDIX I

Material Examined

Specimens and tissues — Collection acronyms: XH-, XV-, or XG- prefix refers to those specimens that are from the collection of Robert E. Lovich.

Xantusia gracilis — UNITED STATES: CALIFORNIA: Riverside Co.: Calcite Mine, Anza Borrego Desert State Park (XG01-XG04).

Xantusia henshawi — UNITED STATES: CALIFORNIA: Riverside Co.: 3 km south of Beaumont (XH01, XH03); 1 km north of Lake Perris (XH06); 8 km from Highway 74 on Santa Rosa Mountain Road (XH25). San Diego Co.: 1 km south of Chihuahua Valley Road (XH07); 2 km North of Ranchita (XH31); Culp Valley Campground, Anza Borrego Desert State Park (XH53); 2 km northwest of intersection I-15 and Highway S-12 (XH10); 1 km west of Highway 78 at Witch Creek Mountain (XH14); 2 km east of Highway 79 at Samagatuma Valley (XH18); 2 km north of Lake Morena (XH37); 3 km southwest of Campo (XH41). Imperial Co.; 1 km south of Mountain Springs Exit, I-15 (XI-3).

Xantusia vigilis — UNITED STATES: CALIFORNIA: San Bernardino Co.: Across street from Phelan High School (XV01).

Published Sequences: *Cricosaura typica*, *Lepidophyma smithi*, *Xantusia bolsonae*, *Xantusia henshawi*, *Xantusia riversiana*, and *Xantusia vigilis*: GenBank (http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=n_r) accession numbers M65117-M65123 respectively.

APPENDIX 2

Results of the Computer program PAUP* 4.0

A list of apomorphies for the unweighted parsimony analysis is presented below. Characters refer to sequence alignment position. Unambiguous nucleotide base changes are indicated with a double arrow; ambiguous changes with a single arrow.

Branch	Character	Steps	CI	Change
Nearest outgroup --> node 1	19	1	0.333	C --> T
	76	1	0.600	C ==> A
	98	1	0.500	C --> T
	103	1	0.667	T --> C
	157	1	1.000	C ==> A
	167	1	1.000	T ==> A
	220	1	0.333	C --> T
	238	1	0.250	C --> T
	253	1	0.200	C --> T
	263	1	0.667	C ==> A
	286	1	0.250	C --> T
	289	1	1.000	C ==> T
	292	1	0.750	C ==> T
	307	1	0.667	A ==> T
	346	1	1.000	C ==> T
	371	1	0.667	A ==> G
	node_1 --> node_6	187	1	0.500
226		1	0.250	C --> T
283		1	0.667	G ==> A
337		1	0.667	A --> G
361		1	0.500	T ==> C
4		1	0.667	A ==> C
7		1	0.500	C ==> T
16		1	0.500	T ==> C
31		1	1.000	C ==> T
40		1	0.667	C --> T
55		1	0.500	C --> T
76		1	0.600	A ==> T
103		1	0.667	C ==> A
136		1	0.500	A ==> G
205		1	0.400	C --> T
209		1	1.000	C ==> T
222		1	0.500	T ==> C
235	1	0.400	A ==> G	
262	1	0.250	C ==> T	
338	1	0.500	C ==> T	
343	1	1.000	A ==> G	

Branch	Character	Steps	CI	Change
node_6 --> Lake Perris	329	1	0.500	A ==> G
	373	1	0.500	C ==> A
node_6 --> Chihuahua	79	1	1.000	C ==> T
	86	1	0.500	C ==> T
	331	1	0.500	C --> T
	19	1	0.333	T ==> C
	76	1	0.600	T ==> G
	115	1	0.600	T ==> C
node_6 --> Culp Valley	79	1	1.000	C ==> T
	86	1	0.500	C ==> T
	331	1	0.500	C --> T
	128	1	0.500	C ==> T
	205	1	0.400	T --> C
	286	1	0.250	T ==> C
	46	1	1.000	A ==> G
	136	1	0.500	G ==> A
	157	1	1.000	A ==> G
	235	1	0.400	G ==> A
	292	1	0.750	T ==> C
	306	1	0.500	C ==> T
	367	1	0.500	T ==> C
	370	1	0.333	T ==> C
node_6 --> Ranchita	79	1	1.000	C ==> T
	86	1	0.500	C ==> T
	331	1	0.500	C --> T
	128	1	0.500	C ==> T
	205	1	0.400	T --> C
	286	1	0.250	T ==> C
	46	1	1.000	A ==> G
	136	1	0.500	G ==> A
	157	1	1.000	A ==> G
	235	1	0.400	G ==> A
	292	1	0.750	T ==> C
	306	1	0.500	C ==> T
	367	1	0.500	T ==> C
	370	1	0.333	T ==> C
node_6 --> Gracilis	7	1	0.500	T ==> C
	79	1	1.000	C ==> T
	86	1	0.500	C ==> T
	331	1	0.500	C --> T
	128	1	0.500	C ==> T
	205	1	0.400	T --> C
	286	1	0.250	T ==> C
	4	1	0.667	C ==> A

Branch	Character	Steps	CI	Change
	37	1	1.000	C ==> T
	67	1	1.000	A ==> G
	92	1	0.500	C ==> T
	113	1	0.750	C ==> A
	178	1	0.500	C ==> A
	222	1	0.500	C ==> T
	232	1	0.200	C ==> T
	247	1	0.333	T ==> C
node_1 --> Santa Ysabel	187	1	0.500	C --> T
	226	1	0.250	C --> T
	283	1	0.667	G ==> A
	337	1	0.667	A --> G
	361	1	0.500	T ==> C
	43	1	1.000	A ==> T
	98	1	0.500	T --> C
	238	1	0.250	T --> C
	257	1	0.667	T ==> C
	307	1	0.667	T ==> C
	310	1	0.500	C ==> T
	326	1	1.000	G ==> A
	327	1	1.000	T ==> C
	337	1	0.667	G --> T
	358	1	1.000	C ==> A
node_4 --> node_5	82	1	0.750	A ==> G
node_5 --> Lake_Morena	4	1	0.667	A ==> G
	306	1	0.500	C ==> T
	373	1	0.500	C ==> T
	232	1	0.200	C ==> T
	262	1	0.250	C ==> T
	340	1	0.500	C ==> T
node_5 --> Pine Valley	4	1	0.667	A ==> G
	306	1	0.500	C ==> T
	226	1	0.250	T ==> C
	373	1	0.500	C ==> T
node_5 --> Ocotillo	3	1	0.500	C --> G
	102	1	1.000	T ==> C
node_5 --> Escondido	3	1	0.500	C --> G
	102	1	1.000	T ==> C
	262	1	0.250	C ==> T
node_5 --> Campo	3	1	0.500	C --> G
	102	1	1.000	T ==> C
	262	1	0.250	C ==> T
	3	1	0.500	G --> C
	19	1	0.333	T ==> C

Branch	Character	Steps	CI	Change
	85	1	0.500	A ==> T
	118	1	0.667	A ==> G
	268	1	0.500	C ==> T
node_1 --> node_2	28	1	1.000	A ==> C
	74	1	1.000	T ==> C
	85	1	0.500	A --> C
	92	1	0.500	C ==> T
	142	1	0.500	C ==> T
	156	1	1.000	C ==> T
	177	1	0.500	T --> C
	244	1	0.500	C ==> T
	331	1	0.500	C ==> T
	350	1	1.000	G ==> A
	364	1	0.667	A ==> G
	373	1	0.500	C ==> A
node_2 --> node_3 (Banning)	16	1	0.500	T ==> C
	40	1	0.667	C --> A
	105	1	0.333	T ==> C
	178	1	0.500	C ==> T
	196	1	0.333	T --> C
	205	1	0.400	C ==> T
	220	1	0.333	T --> C
	253	1	0.200	T --> C
	301	1	0.500	A ==> G
	325	1	1.000	T ==> A
	374	1	1.000	C ==> T
node_3 --> Banning3_(03)	269	1	1.000	A ==> T
node_3 --> Banning1_(01)	139	1	0.500	C ==> T
node_2 --> Santa Rosa Mtn.	184	1	0.333	C ==> T
	232	1	0.200	C ==> T
	286	1	0.250	T --> C
	295	1	0.500	A ==> G
	310	1	0.500	C ==> T

APPENDIX 3

Results of the Computer program PAUP* 4.0

A list of apomorphies for the weighted parsimony analysis is presented below. Characters refer to sequence alignment position. Unambiguous nucleotide base changes are indicated with a double arrow; ambiguous changes with a single arrow.

Branch	Character	Steps	CI	Change
Nearest outgroup --> node_1	19	1	0.333	C --> T
	98	1	0.500	C --> T
	101	4	0.556	C --> A
	103	1	0.833	T --> C
	112	1	0.500	C ==> T
	154	1	0.500	T --> C
	157	4	1.000	C ==> A
	166	1	0.500	T --> C
	167	4	1.000	T ==> A
	232	1	0.167	T --> C
	263	4	0.556	C ==> A
	289	1	1.000	C ==> T
	292	1	0.600	C ==> T
	307	4	0.556	A ==> T
	346	1	1.000	C ==> T
	371	1	0.833	A ==> G
node_1 --> node_7	7	1	0.500	C ==> T
	16	1	0.500	T ==> C
	31	1	1.000	C ==> T
	79	1	0.500	C ==> T
	103	4	0.833	C ==> A
	128	1	0.333	C --> T
	136	1	0.500	A --> G
	187	1	0.500	C ==> T
	209	1	1.000	C ==> T
	235	1	0.625	A --> G
	262	1	0.200	C ==> T
	337	1	0.833	A ==> G
	343	1	1.000	A ==> G
node_7 --> node 9 (Gracilis)	37	1	1.000	C ==> T
	67	1	1.000	A ==> G
	92	1	0.714	C ==> T
	113	4	0.462	C ==> A
	178	4	0.500	C ==> A
	232	1	0.167	C --> T
247	1	0.333	T ==> C	

Branch	Character	Steps	CI	Change
node_7 --> node_B	4	4	1.000	A ==> C
	222	1	1.000	T ==> C
node_B --> node_A	128	1	0.333	T --> C
	205	1	0.714	C ==> T
	286	1	0.250	C ==> T
	305	1	1.000	A --> G
	329	1	0.500	A --> G
	331	1	0.714	T --> C
	373	4	0.385	C --> A
node_A --> Chihuahua	19	1	0.333	T ==> C
	76	4	0.545	T ==> G
	115	1	0.750	T ==> C
	178	1	0.500	C ==> T
	236	1	1.000	G ==> A
	250	1	0.333	C ==> T
	253	1	0.200	T ==> C
	256	1	1.000	C ==> T
	263	4	0.556	A ==> C
	298	1	0.333	C ==> T
	305	4	1.000	G ==> C
	306	4	0.500	C ==> G
	308	4	1.000	A ==> T
	309	1	1.000	T ==> C
	310	4	0.500	C ==> A
	311	4	1.000	A ==> T
	314	1	1.000	T ==> C
315	1	1.000	G ==> A	
316	4	1.000	A ==> T	
317	1	1.000	A ==> G	
node_A --> Lake Perris	79	1	0.500	T ==> C
	86	1	0.333	T ==> C
node_B --> node_8	46	1	1.000	A ==> G
	136	1	0.500	G --> A
	157	1	1.000	A ==> G
	235	1	0.625	G --> A
	292	1	0.600	T ==> C
	306	1	0.500	C ==> T
	367	1	0.500	T ==> C
	370	1	0.333	T ==> C
node_8 --> Ranchita	7	1	0.500	T ==> C
node_8 --> Culp_Valley	372	1	1.000	T ==> C
node_1 --> node_C	40	1	1.000	T ==> C
	55	1	1.000	T ==> C
	76	4	0.545	T ==> A

Branch	Character	Steps	CI	Change
	86	1	0.333	T --> C
	286	1	0.250	C --> T
	310	1	0.500	C --> T
	338	1	0.500	T ==> C
node_C --> node_2	28	4	1.000	A ==> C
	74	1	1.000	T ==> C
	85	4	0.312	A ==> C
	92	1	0.714	C ==> T
	142	1	0.500	C ==> T
	156	1	1.000	C ==> T
	177	1	0.500	T ==> C
	226	1	0.250	T ==> C
	244	1	0.500	C ==> T
	283	1	0.714	A --> G
	350	1	1.000	G ==> A
	361	1	0.333	C --> T
	364	1	0.833	A ==> G
	373	4	0.385	C ==> A
node_2 --> node_3	16	1	0.500	T ==> C
	40	4	1.000	C ==> A
	105	1	0.333	T ==> C
	178	1	0.500	C ==> T
	196	1	0.333	T ==> C
	205	1	0.714	C ==> T
	220	1	0.333	T ==> C
	253	1	0.200	T ==> C
	301	1	0.500	A ==> G
	310	1	0.500	T --> C
	325	4	0.667	T ==> A
	374	1	1.000	C ==> T
node_3 --> Banning(03)	269	4	1.000	A ==> T
node_3 --> Banning (01)	139	1	0.500	C ==> T
	264	1	1.000	T ==> C
node_2 --> Santa_Rosa (25)	22	1	1.000	C ==> T
	29	1	1.000	T ==> C
	184	1	0.333	C ==> T
	212	1	1.000	A ==> G
	232	1	0.167	C --> T
	286	1	0.250	T --> C
	295	1	0.500	A ==> G
node_C --> node_4	43	4	1.000	A ==> T
	98	1	0.500	T --> C
	238	1	0.250	T --> C
	257	1	0.833	T ==> C

Branch	Character	Steps	CI	Change
	307	1	0.556	T ==> C
	326	1	1.000	G ==> A
	327	1	1.000	T ==> C
	331	1	0.714	T ==> C
	337	4	0.833	A ==> T
	358	4	1.000	C ==> A
node_4 --> node_5	82	1	0.600	A ==> G
	187	1	0.500	C ==> T
	262	1	0.200	C --> T
node_5 --> node_D	4	1	1.000	A ==> G
	306	1	0.500	C ==> T
	373	1	0.385	C ==> T
node_D --> Lake_Morena	232	1	0.167	C ==> T
	340	1	0.714	C ==> T
node_D --> Pine Valley	226	1	0.250	T ==> C
	262	1	0.200	T --> C
node_5 --> node_E	102	1	1.000	T ==> C
node_E --> Campo	19	1	0.333	T ==> C
	85	4	0.312	A ==> T
	118	1	0.833	A ==> G
	268	1	0.500	C ==> T
node_E --> node_F	3	4	1.000	C ==> G
node_F --> Ocotillo3	46	4	1.000	A ==> T
	47	1	1.000	C ==> T
	97	1	0.333	C ==> T
	115	1	0.750	T ==> C
	128	1	0.333	C ==> T
	167	1	1.000	A ==> G
	201	4	1.000	A ==> T
	262	1	0.200	T --> C
node_F --> Escondido (10)	49	4	0.500	A ==> C
	232	1	0.167	C ==> T
	306	1	0.500	C ==> T
	340	1	0.714	C ==> T
	375	4	1.000	T ==> G
node_4 --> Santa_Ysabel	61	4	1.000	A ==> T
	193	1	0.500	C ==> T
	259	4	0.600	A ==> T