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PHYLOGEOGRAPHY OF THE NIGHT LIZARD, *XANTUSIA HENSHAWI*, IN SOUTHERN CALIFORNIA: EVOLUTION ACROSS FAULT ZONES

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ABSTRACT: I analyzed intraspecific relationships, biogeography, and taxonomy of *Xantusia henshawi* occurring in southern California by constructing a gene tree phylogeny using mitochondrial DNA (mtDNA) sequence data from the cytochrome *b* region. Three well-supported haploclades show high degrees of sequence divergence in contrast to a generally conservative morphology. Fault zones and their associated habitat features in the form of fault valleys, canyons, and arroyos geographically separate the different gene tree haploclades. This suggests that the evolution of *X. henshawi* is tied to the occurrence of stable exfoliating granitic features that are lacking in riparian and flood plain corridors. Sequence data indicate that *X. h. gracilis* evolved from within one of the three haploclades of *X. henshawi* and represents an exclusive lineage. *Xantusia henshawi* remains non-exclusive. Under the evolutionary species concept, results of this study in combination with those of previous studies warrant the elevation of *X. h. gracilis* to full species. This study has assisted in hypothesizing previously unknown barriers to gene flow that have contributed to the evolution of *X. henshawi* and *X. gracilis*.

Key words: *Xantusia henshawi*; Granite night lizard; *Xantusia gracilis*; Sandstone night lizard; Truckhaven Rocks; Fault zone; Granodiorite; Exclusivity

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. Many species in this family are highly specialized and have narrow microhabitat requirements (Bezy, 1988). The granite night lizard, *Xantusia henshawi*, is known for its saxicolous, crevice dwelling lifestyle (Bezy, 1988; Lee, 1973, 1975) and contains two recognized subspecies, *X. h. henshawi* and *X. h. gracilis* (Grismer and Galvan, 1986). The granite night lizard is restricted to metavolcanic and granodiorite boulder outcrops along the Peninsular Ranges and coastal mountains of southern California and northern Baja California, México (Lee, 1975; Fig. 1). 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. 1).

Grismer and Galvan (1986) first described *Xantusia henshawi gracilis* on the basis of at least seven unique diagnostic morphological characters with respect to *X. h. henshawi*, and they indicated that it also differed from *X. h. henshawi* in several aspects of its life history. A protein electrophoretic study by Bezy and Sites (1987) supported the distinctness of *X. h. henshawi* and *X. h. gracilis* as distinct taxa which were found to have fixed allelic differences at seven of 26 presumptive gene loci, and a Nei genetic distance of 0.19. Sites et al. (1986) found *X. h. henshawi* and *X. h. gracilis* to differ in the intensity of expression of two subunits of lactate dehydrogenase.

Mitochondrial DNA (mtDNA) data have been used successfully in the field of molecular systematics for analyzing both intra- and interspecific relationships (Avise, 1994; 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. The use of

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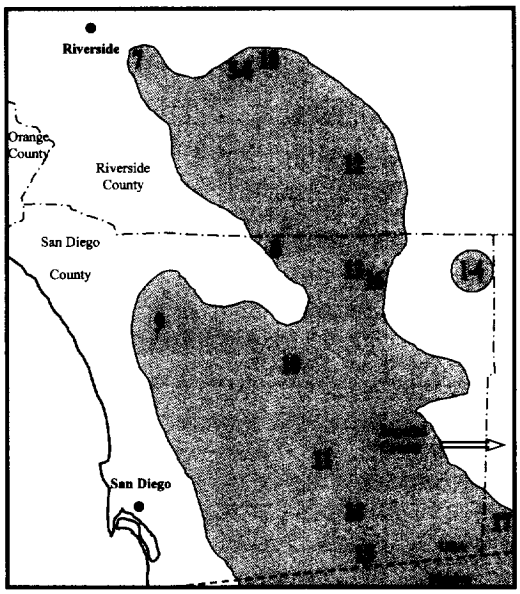

FIG. 1.—Map showing collecting localities for *Xantusia henshawi henshawi* (5–18) and *X. h. gracilis* (1–4). Shaded area represents approximate circumscribed distribution for *Xantusia henshawi* in southern California.

mtDNA in phylogenetic and systematic studies can also be useful in determining the boundaries of genetically divergent groups not previously identified (Lovich and Gibbons, 1997; Moritz, 1994).

This study uses mtDNA sequence data to infer the phylogeographic relationships within and between the populations of the two subspecies of *Xantusia henshawi*. Given the circumscribed distribution of *X. h. gracilis* (Fig. 1), it is hypothesized here to have diverged from *X. h. henshawi* (Grismer and Galvan, 1986). Following this, a historical biogeographical scenario is provided that is consistent with the inferred phylogeny of *X. henshawi* and the historical environmental events that may have led to the evolution of *X. h. gracilis*.

MATERIALS AND METHODS

Specimens were collected from across the known range of *Xantusia henshawi* within California (Table 1). Collecting efforts maximized geographic coverage over the range of this species so as to represent environmental and geographic extremes, and to minimize the geographic sampling gap between *X. h. henshawi* and *X. h. gracilis*. Collection of specimens occurred between 1998 and 1999. Fourteen specimens of *X. h. henshawi* and four of *X. h. gracilis* were used, representing 13 different collecting localities (Fig. 1). All lizards were collected under permits from California Department of Fish and Game and Anza Borrego Desert State Park. Methods to capture the lizards included nighttime lantern walking and non-invasive daytime searching beneath small boulders and rock flakes. In addition to collected samples, the mtDNA sequence of a single individual of *X. h. henshawi* from Banning, California (Hedges et al., 1991) was retrieved from GenBank (accession number M65121) for use in this study. Tissue samples and voucher specimens collected specifically for this study are deposited at the San Diego Natural History Museum (SDNHM) and Anza Borrego Desert State Park (ABDSP).

Outgroup choice has been shown to affect tree rooting positions in phylogenetic analyses (Messenger and McGuire, 1998; Wiens and Hollingsworth, 2000). Outgroups used in this analysis were selected using the proposed phylogeny of the Xantusiidae of Hedges et al. (1991). I chose single representatives for each outgroup genus and three species from within the genus *Xantusia*. I included one individual each of *Cricosaura typica, Lepidophyma smithii, X. riversiana, X. vigilis*, and *X. bolsonae* in sequence data generated by Hedges et al. (1991) (Appendix I). An additional specimen of *Xantusia vigilis* was collected for this study for use as an outgroup sample and sequenced (Appendix I). All parsimony analyses were subjected to a constrained outgroup topology following the maximum parsimony analysis results from Hedges et al. (1991), where *X. riversiana* and *X. vigilis* comprised the first outgroup and *X. bolsonae, Lepidiphyma smithii*, and *Cricosaura typica* comprised the second, third, and fourth outgroups, respectively.

I obtained mtDNA from muscle or liver tissues. Genomic DNA was isolated by methods outlined in Palumbi et al. (1991). A single segment of mtDNA was amplified

TABLE 1.—Eighteen ingroup and six outgroup samples, GenBank accession numbers, identification numbers, and collection localities for all samples used in this study. Museum acronyms follow Leviton et al. (1985). Non-standard acronym ABDSP-SRC refers to Anza Borrego Desert State Park Stout Research Center.

Taxon	ID No.	Genbank accession number, voucher number, and locality
Cricosaura typica	Outgroup	M65118; USNM 306539:Cuba; Santiago de Cuba; From Hedges et al. (1991)
Lepidophyma smithii	Outgroup	M65119; LACM 136359; México: Chiapas; From Hedges et al. (1991)
Xantusia bolsonae	Outgroup	M65120; LACM 138478; México: Durango; From Hedges et al. (1991)
X. riversiana	Outgroup	M65122; LACM 125513; From Hedges et al. (1991)
X. vigilis	Outgroup	M65123; LACM 136813; From Hedges et al. (1991)
X. vigilis	19	AF313372; US: California, San Bernardino Co., Phelan, next to Phelan High School
X. h. gracilis	1	AF313373:ABDSP-SRC 625-2-01-625-2-02; US: California, San Diego Co., ABDSP
X. h. gracilis	2	AF313373:ABDSP-SRC 625-2-01-625-2-02; US: California, San Diego Co., ABDSP
X. h. gracilis	3	AF313373:ABDSP-SRC 625-2-01-625-2-02; US: California, San Diego Co., ABDSP
X. h. gracilis	4	AF313373:ABDSP-SRC 625-2-01-625-2-02; US: California, San Diego Co., ABDSP
X. h. henshawi	5	AF313377; SDSNH 68800; US: California, Riverside Co., 3 km. south of Banning
X. h. henshawi	6	AF313375; SDSNH 68802; US: California, Riverside Co., 3 km. south of Banning
X. h. henshawi	7	AF313386; SDSNH 68805; US: California, Riverside Co., 1 km north of Lake Perris
X. h. henshawi	8	AF313374; SDSNH 68806; US: California, Riverside Co., 1 km south Chihuahua Valley Road
X. h. henshawi	9	AF313384; SDSNH 68809; US: California, Riverside Co., 2 km north of I-15
X. h. henshawi	10	AF313380; SDSNH 68813; US: California, San Diego Co., 1 km west of highway 78
X. h. henshawi	11	AF313379; SDSNH 68817; US: California, San Diego Co., 2 km west of highway 79
X. h. henshawi	12	AF313381; SDSNH 68824; US: California, Riverside Co., Santa Rosa Mountain Road
X. h. henshawi	13	AF313382; SDSNH 68830; US: California, San Diego Co., 2 km north of Ranchita
X. h. henshawi	14	AF313376; SDSNH 68836; US: California, San Diego Co., 2 km north of Lake Morena
X. h. henshawi	15	AF313378; SDSNH 68840; US: California, San Diego Co., 3 km southwest of Campo
X. h. henshawi	16	AF313385; SDSNH 68850; US: California, San Diego Co., Culp Valley Campground
X. h. henshawi	17	AF313383; SDSNH 68859; US: California, Imperial Co., 1 km south Mountain Springs and I-8
X. h. henshawi	18	M65121; US: California, From Hedges et al. (1991)

using the polymerase chain reaction (PCR; Gyllensten and Erlich, 1988; Mullis and Faloona, 1987) 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 RAA CCA 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: 1 min denaturation at 94 C, 1 min annealing at 50 C, and 90 s 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. Compli-

mentary strands were sequenced using the two PCR primers as sequencing primers in separate reactions. Single stranded products were sequenced with Applied Biosystems, Inc. Fluorescent Automated Sequencers 373 and 377 at the Sequencing Facility for Loma Linda University and at 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 were 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*.

All phylogenetic analyses were performed using PAUP* (Version 4.0b2a: Swofford, 1999). DNA sequence data were 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. I evaluated homoplasy levels using the consistency index (Kluge and Farris, 1969) and the retention index (Farris, 1989, 1990). Because uninformative characters affect the consistency index, only informative characters were used to determine this value.

I used nonparametric bootstrap analysis to evaluate confidence within phylogenetic trees (Felsenstein, 1985) using 1000 bootstrap replicates with the fast-heuristic search option in PAUP*. Only bootstrap values of >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).

I examined the ingroup data set for phylogenetic signal using the g_1 statistic (Hillis and Huelsenbeck, 1992). 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).

RESULTS

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. 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% for ingroup taxa (Table 2). Zero percent pair-wise sequence divergence was only seen within populations or among samples from geographically proximate localities (among samples 1–4, and between samples 6 and 18: Table 2).

The g_1 analysis indicates that the data set used contains phylogenetic signal 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

TABLE 2.—Pairwise percent sequence variation for all samples. Column and row numbers refer to identification numbers from Table 1.

	1	2	3	4	16	13	7	8	10	9	14	15	17	11	12	6	5	18
1		0.0	0.0	0.0	5.0	5.0	5.0	10.0	10.0	11.0	10.0	11.0	11.0	11.0	11.0	12.0	12.0	12.0
2			0.0	0.0	5.0	5.0	5.0	10.0	10.0	11.0	10.0	11.0	11.0	11.0	11.0	12.0	12.0	12.0
3				0.0	5.0	5.0	5.0	10.0	10.0	11.0	10.0	11.0	11.0	11.0	11.0	12.0	12.0	12.0
4					5.0	5.0	5.0	10.0	10.0	11.0	10.0	11.0	11.0	11.0	11.0	12.0	12.0	12.0
16						1.0	5.0	9.0	10.0	11.0	10.0	11.0	11.0	10.0	12.0	13.0	13.0	13.0
13							5.0	9.0	10.0	10.0	9.0	10.0	11.0	9.0	12.0	12.0	13.0	13.0
7								7.0	9.0	10.0	8.0	9.0	10.0	8.0	11.0	11.0	11.0	11.0
8									14.0	14.0	13.0	14.0	15.0	13.0	17.0	15.0	15.0	16.0
10										3.0	3.0	3.0	4.0	2.0	9.0	11.0	11.0	11.0
9											2.0	3.0	3.0	3.0	10.0	12.0	13.0	13.0
14												3.0	4.0	1.0	9.0	12.0	12.0	12.0
15													3.0	3.0	10.0	12.0	12.0	12.0
17														3.0	11.0	13.0	13.0	13.0
11															9.0	11.0	11.0	11.0
12																5.0	6.0	5.0
6																	1.0	0.0
5																		1.0
18																		

−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) did not give a g_1 statistic value for the number 19 (i.e., ingroup taxa used in this study), but only for 15 and 20. A more conservative value 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.

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. The strict consensus tree has a trichotomy of three well-supported haploclades (A, B, and C) within *Xantusia henshawi* that contains well supported substructuring (Fig. 2).

A 92% bootstrap value and 16 nucleotide changes support haploclade A (Fig. 2: node 6). Within this haploclade, four individuals of *Xantusia henshawi gracilis* share identical sequences and form an exclusive group 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 subclade, and individuals from Lake Perris and Chihuahua Valley. The remaining 10 trees support the sister taxon 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 (Fig. 2: 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 this haploclade, the three individuals from Banning (node 3) form a well-supported group (100% bootstrap value) with 11 nucleotide changes.

The weighted parsimony analysis, using all variable characters, produced two trees with 689 steps for all variable characters, or 511 steps using only informative char-

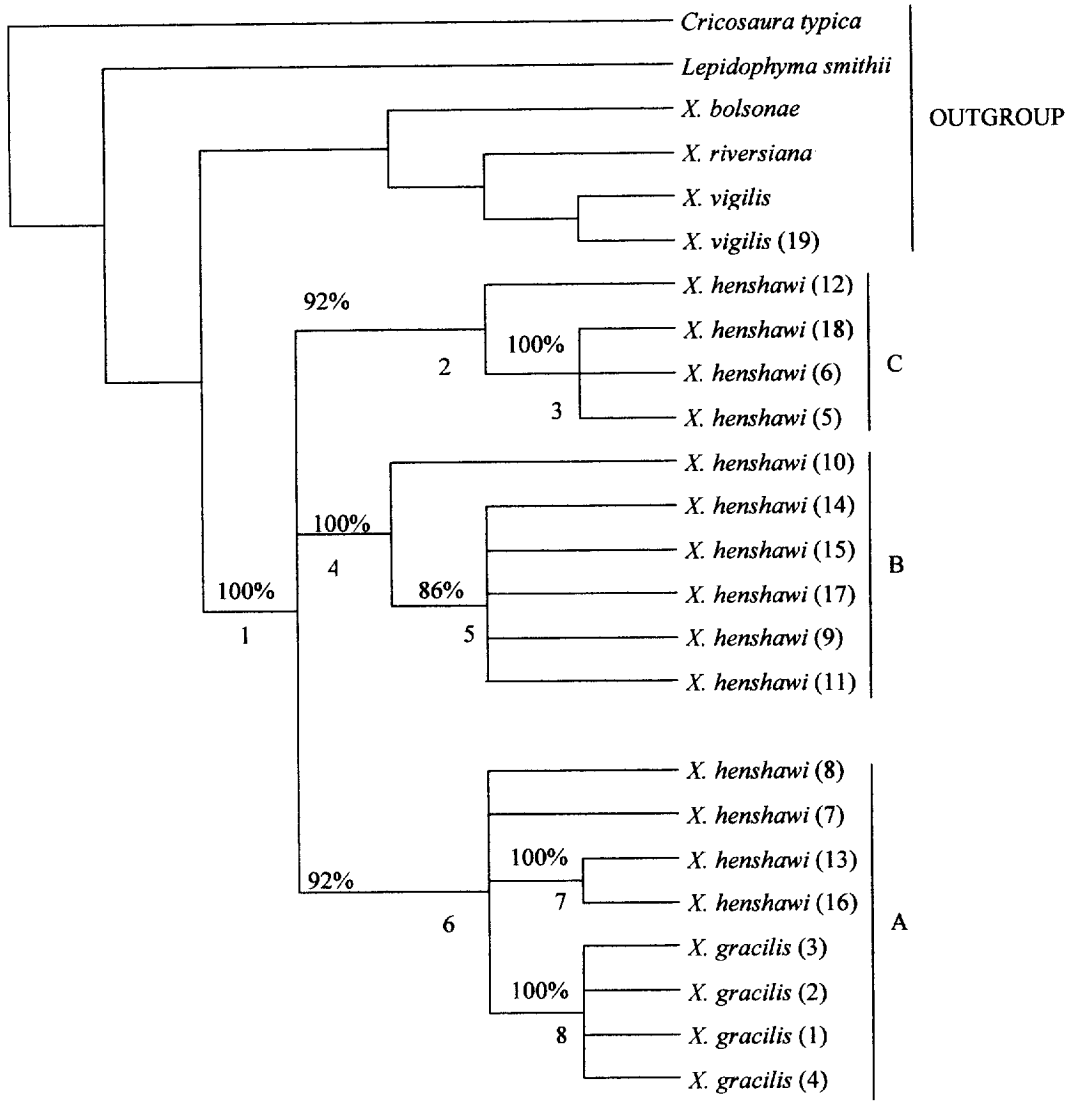

FIG. 2.—Strict consensus tree from unweighed maximum parsimony analysis. C.I. = 0.58, R.I. = 0.78.

acters. 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. 3) contains the same three well-supported haploclades (A, B, and C), and has increased resolution within the nodes of haploclades A, B, and C (Fig. 3) and an increased CI (0.72 versus 0.58). However, all of the increased resolution shown in the weighted analysis is supported by bootstrap values of <70% (Fig. 3: Nodes A–F). While it has been shown that estimates of phylogenetic relationships are improved using a weighting scheme based on data at hand (Reeder, 1995; Voelker and Edwards, 1998), weighting of this data set and its subsequent parsimony analysis did not produce any added resolution with bootstrap support values of 70% or greater. Nodes in common to both the weighted and unweighted analyses are referred to by

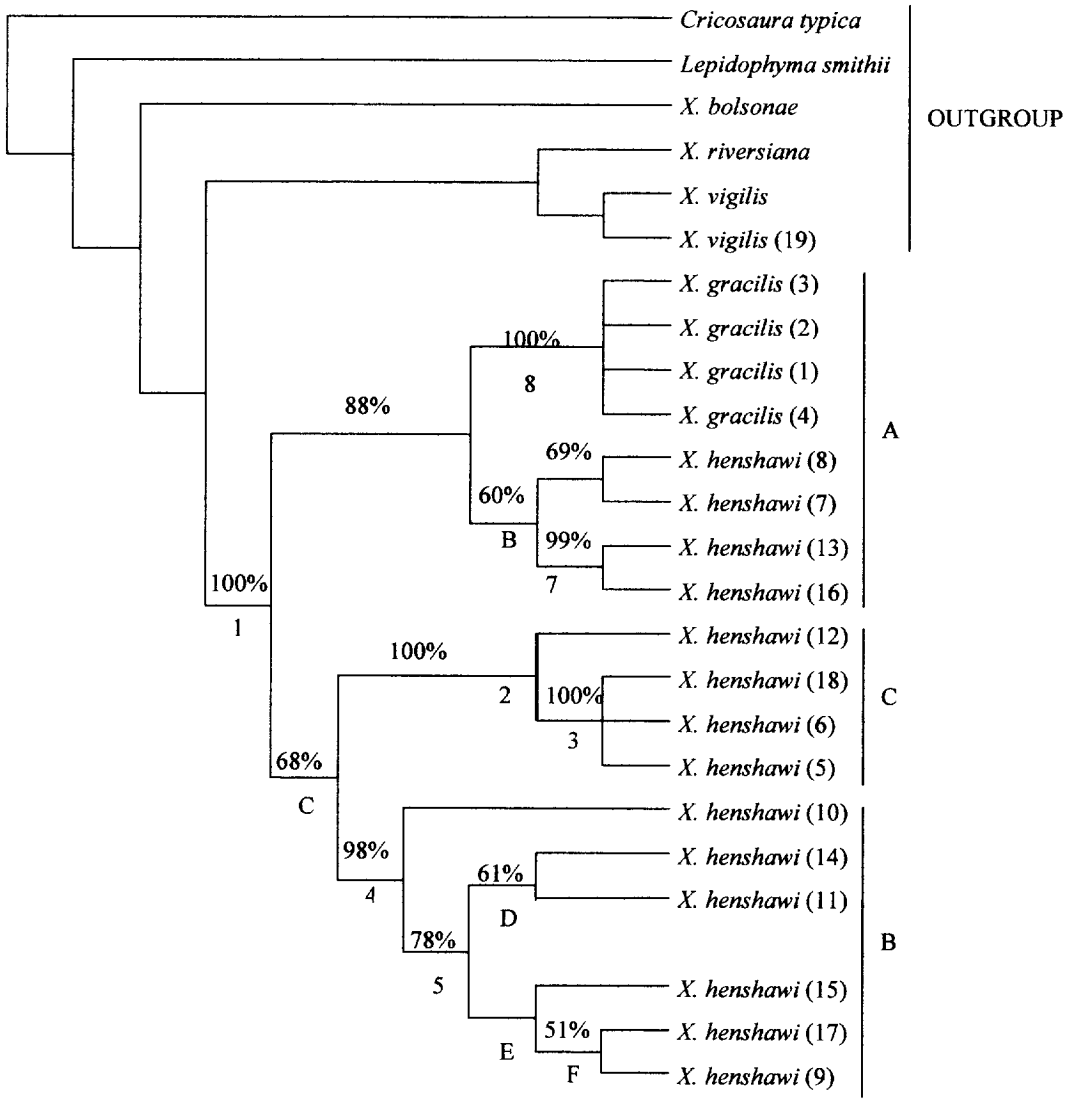

FIG. 3.—Strict consensus tree from weighted (Ti/Tv = 4.0) maximum parsimony analysis. C.I. = 0.72, R.I. = 0.78.

the same designations (i.e., 1–9) as seen in Fig. 2, and additional resolution is designated by A–F (Fig. 3).

Haploclade A is well supported by 13 nucleotide changes and an 88% bootstrap value (Fig. 3). The individuals from Ranchita and Culp Valley are supported by eight nucleotide changes and a 99% bootstrap value (node 7). A sister group relationship between Lake Perris and Chihuahua Valley is formed (node A), based on nine nucleotide changes (bootstrap value of 69%). Both of the two trees generated in the weighted analysis, as well as the strict consensus tree (Fig. 3), place *X. h. gracilis* as the sister group to the group (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 60%.

Haploclade C is identical to the results from the unweighted analysis and is supported by a 100% bootstrap value and 14

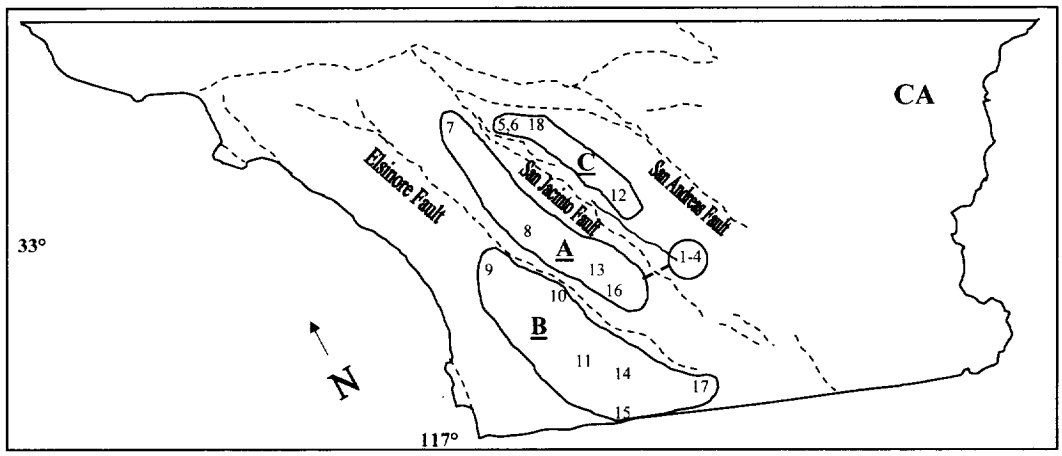

FIG. 4.—Geographic locations of the haploclades (A–C, in bold and underlined) indicated in the weighted strict consensus tree (Fig. 3). The San Jacinto and Elsinore Faults separate these haploclades. Sample numbers are those stated in Table 1.

nucleotide changes. Within this haploclade, the three Banning samples form a sister group relationship (node 3) supported by a 100% bootstrap value and 12 nucleotide changes.

Haploclade B is congruent with the results from the unweighted analysis, but the weighted strict consensus tree contains added resolution with bootstrap values of <70%. This haploclade as a whole is supported by a 98% bootstrap value and 10 nucleotide changes. The Santa Ysabel individual is the most basal 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.

DISCUSSION

Taxonomy

The gene tree relationships from the unweighted and weighted analyses indicate that *Xantusia henshawi gracilis* represents an exclusive lineage (sensu Frost and Kluge, 1994; Graybeal, 1995) that evolved from within *X. h. henshawi*. *Xantusia henshawi henshawi* thus forms a non-exclusive lineage (i.e., ferespecies), 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 (Figs. 3, 4), and that the latter was unique in having a greatly enlarged temporal scale, the absence of enlarged auriculars, lack of a vertebral furrow (in life), having a narrower head, limbs that were not as widely splayed, lack of a significant diel color phase change, a nearly complete absence of black peppering on ventral surfaces, the dorsal body pattern consisting of reduced round spots, significantly fewer scales around the upper arm and leg, a higher number of supralabials, fewer longitudinal rows of dorsal scales, a smaller interhindlimb distance/SVL ratio, a narrower body, fewer dorsal scales at midbody, and thinner limbs. Grismer and Galvan (1986) also found differences in the following life history attributes: *X. h. gracilis* inhabits sandstone, is found exclusively within a desert environment, and utilizes small burrows in addition to exfoliating sandstone. Seven out of 26 presumptive gene loci (Bezy and Sites, 1987) and the intensity of expression of two subunits of the lactate dehydrogenase isozyme (Sites et al., 1986) distinguish *X. h. gracilis* from *X. h. henshawi*. This study has shown that *X. h. henshawi* and *X. h. gracilis* differ by 18 cytochrome *b* nucleotide changes. The allopatry and numerous differences between *X. h. gracilis* and *X. h. henshawi* indicate they are on separate phylogenetic trajectories and should be considered dif-

ferent species under the evolutionary species concept (sensu Frost and Hillis, 1990). The exclusive nature of this taxon adds additional support for this hypothesis. Based on the results of this study, in conjunction with lines of evidence from previous studies, *X. gracilis* is recognized here as a species.

The results of this study seem to indicate that *Xantusia henshawi* is a paraphyletic species. Upon closer inspection of the data, this is not the case because the results of this study represent a tokogeny and not a species tree (Frost and Kluge, 1994). Issues of monophyly and paraphyly refer to clades and species are not clades (Frost and Kluge, 1994). Support for the elevation of *X. gracilis* is available from several previously studied lines of evidence, and is independent of the cytochrome *b* data set generated in this study. The gene tree relationships of this analysis simply confirm from which section of *X. henshawi* that *X. gracilis* is derived. The data generated here indicate that *X. gracilis* forms an exclusive rather than non-exclusive lineage. In addition, previous allozyme studies (Bezy and Sites, 1987; Sites et al., 1986) showed fixed allelic differences between *X. henshawi* and *X. gracilis* to indicate that there is no gene flow between these species. Some might argue that *X. henshawi* is comprised of three different species corresponding to the three haploclades shown in this study (Figs. 2, 3), but at the present time there are no data (i.e. ecological, morphological, or nuclear/allozyme) that indicate that these distinct haploclades are on separate evolutionary trajectories.

Phylogeography and Historical Biogeography

Two major fault zones geographically separate the three haploclades of *Xantusia henshawi*: the San Jacinto Fault in the north and the Elsinore Fault in the south (Fig. 4). Results of this study suggest that these faults may have fragmented the range of *X. henshawi* by bisecting it with fault valleys, canyons, and arroyos. It is likely that such valleys, canyon floors, and arroyos generally consisting of sedimentary material, alluvium, and cobble (Jennings, 1958) are apparently disturbed too frequently by erosional forces to generate stable exfoliating boulder outcrops and their respective microhabitats, such that *X. henshawi* is not found in association with these areas. The low vagility of these lizards (Fellers and Drost, 1991; Fellers et al., 1998; Lee, 1975) is attributed to their highly specialized crevice dwelling habits and their preference for stable microhabitats, and the dynamic nature of rock formations along fault zones and associated features is likely to prohibit colonization by *X. henshawi*. Genetic substructuring within the three haploclades of *X. henshawi* (Figs. 2, 3) is the result of habitat discontinuities and the isolation of boulder and mountain features by areas devoid of suitable boulder habitat.

Haploclade B in both the weighted and unweighted 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 relationships within this group. This haploclade roughly approximates the distributional center of range for *Xantusia henshawi*, and suitable habitat corridors exist throughout the geographic area composing this haploclade. The presence of continuous suitable habitat with no major geographic barriers is believed to have facilitated dispersal and gene flow within this haploclade, which spans nearly twice the geographic area as the other haploclades and is not bisected by any major fault zones. Haploclade C (Figs. 2, 3) 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 a variety of habitat types over the 48 km, such as redshank chaparral, oak woodland, and sub-alpine forest.

Xantusia gracilis shares a most recent

common ancestor with individuals of *X. henshawi* from Culp Valley, Ranchita, Lake Perris, and Chihuahua Valley (Haploclade A; Figs. 2, 3). 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* that inhabit portions of the Santa Rosa Mountains do not share a close affinity with *X. gracilis* and are in accord with its noted absence from the southern slopes of the Santa Rosa Mountains (Grismer and Galvan, 1986). In both the unweighted and weighted analyses, *X. gracilis* is most closely related to individuals from 30 (Fig. 1: no. 16) to 135 (Fig. 1: no. 7) km away. In the unweighted analysis, 30 of the 50 most parsimonious trees support the sister relationship between *X. gracilis* and samples from Ranchita (Fig. 1: no. 13) and Culp Valley (Fig. 1: no. 16). 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 (Fig. 1: no. 16), the geographically most proximate locality to the Truckhaven Rocks.

Circumscribed distributions of haploclades A, B, and C are indicated in Fig. 4. *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). 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, personal communication, 1999), in the Miocene.

The molecular evidence of this study indicates that the evolution of *Xantusia henshawi* and *X. gracilis* has been associated with the formation of the three distinct erosional surfaces bounded by the respective fault zones of this region (Norris and Webb, 1990). 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/yr, and lands to the south of the Elsinore Fault are moving to the northwest at a rate of 5 mm/yr (Harold Magistrale, personal communication, 1999).

Members of haploclade A (Figs. 2, 3) in the parsimony analyses are loosely confined to the Perris Plain. *Xantusia henshawi* in this haploclade is confined to the Perris Plain, while *X. gracilis* are confined to the Truckhaven Rocks. This relationship indicates that the Truckhaven Rocks may have once been part of the Perris Plain but are now disjunct as a result of tectonic movements. 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). Haploclade B is confined to the erosional surface area of inland San Diego County. Haploclade C is confined to areas northwest of the Perris Plain, in the Santa Rosa and San Jacinto Mountains. There is still some question as to whether any of the above mentioned erosional surfaces predate the Pleistocene in their present form (Norris and Webb, 1990).

The formation of the Truckhaven Rocks is likely to have played a role in the evo-

lution of *Xantusia gracilis*. This species is 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 km^2 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 m in depth and 1 m wide. This location is situated 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). 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 (Paul Remeika, personal communication, 1999). 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 (Paul Remeika, personal communication, 1999). The results of this study support this model because 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).

It is believed that post-Pleistocene desertification and associated climatic changes have eliminated xantusiids from suitable substrates between the Truckhaven Rocks and Vallecito Mountains. 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.

Recommendations and Conclusions

Genetic studies such as this provide data that are useful in formulating effective conservation and management plans (Dimmick et al., 1999; Moritz, 1994; Soltis and Gitzendanner, 1999). Differential management and conservation of populations that contain unique haplotypes are recommended in light of rapid urban development throughout 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. The case for conservation of *Xantusia gracilis* is more pressing, because 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 increasing monitoring of this species. The extreme geographic isolation of *X. gracilis* puts this species at risk of extinction due to catastrophic or stochastic events, introduced or subsidized predators, habitat loss, and/or the potential for illegal collecting pressure in this region. It is not currently known what impacts urban pressure, habitat loss, or exotic species may be having on either *X. henshawi* or *X. gracilis*.

Xantusia henshawi in southern California is a non-exclusive species comprised of three well-supported haploclades. *Xantusia gracilis* forms an exclusive lineage and is elevated to full species based on differences in morphology, allozymes, life history, and allopatry with respect to *X. henshawi*. Results from this study indicate high levels of intraspecific sequence divergence (raw mtDNA sequence variation within *X. henshawi* of 15%) relative to other species (Frost et al., 1998; Hollingsworth, 1999). The high levels of mtDNA sequence divergence may be associated with the ecological limitations and evolutionary age of *Xantusia henshawi* and *X. gracilis*. Members of the family Xantusiidae are generally characterized by delayed reproductive maturity, low reproductive potential, limited dispersal capability (low vagility), long life span, exhibit low mortality, and lack differential mortality

(Lee, 1975). The specialized nature and life history of these lizards, in conjunction with dynamic geologic processes, the relatively old age of this group, and its disjunct distribution are likely to have contributed significantly to the high levels of sequence divergence indicated in this study. Results of this study are also useful in developing a better understanding of the biogeographic factors that have helped to shape species diversity in a region considered to be a global "biodiversity hotspot" (Myers et al., 2000; Wilson, 1992). While no samples from México were included in this study, there are numerous additional fault zones and habitat discontinuities throughout the southern half of the range of *Xantusia henshawi* in Baja California, México, and additional haplotypes/haploclades separated by fault zones and associated features are predicted from this region. As a result of this study, predictions can also be made that other specialized and/or low vagility vertebrates found in this region share a similar evolutionary history influenced by faults and their associated features.

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APPENDIX I

Sequence Data

A 379 base-pair sequence from the mitochondrial gene (cytochrome *b*) of four *Xantusia gracilis* (1–4), 14 *X. henshawi* (5–18), and five outgroup samples. Sample numbers are from Table 1. The symbol ":" refers to no data available, while "." refers to an identical nucleotide as that listed above.

```
                          10        20        30        40        50
                          .         .         .         .         .
X. gracilis(1)            CCCAATTCTAAAAATCATTAACAACTCATTTATTGATCTTCCAACACCAT
X. gracilis(2)            ..................................................
X. gracilis(3)            ..................................................
X. gracilis(4)            ..................................................
X. henshawi(18)           ::::::::::::::::::::::::::::::::::::::::::::::::::
X. henshawi(08)           ...C.............C.................C..............
X. henshawi(5)            ......C.....................C..C.....C..A..........
X. henshawi(14)           ...G..C........T..............C.....C..C..T.......
X. henshawi(5)            ......C.....................C..C.....C..A..........
X. henshawi(15)           ......C........T..C...........C.....C..C..T.......
X. henshawi(11)           ...G..C........T..............C.....C..C..T.......
X. henshawi(10)           ......C........T..............C.....C..C..T.......
X. henshawi(12)           ......C........T.....T.....CC.C.....C..C..........
X. vigilis (19)           ....G.C......C.T..C...........C.....C..........C.
X. henshawi(13)           ...C..C..............................C........G....
X. henshawi(17)           ..G...C........T..............C.....C..C..T..TT?..
X. henshawi(9)            ..G...C........T..............C.....C..C..T.....C.
X. henshawi(16)           ...C.................................C........G....
X. henshawi(7)            ::.C.................................C..............
X. vigilis                ::::::::::::::::::::::::::::::::::::::::::::::::::
X. bolsonae               ::::::::::::::::::::::::::::::::::::::::::::::::::
X. riversiana             ::::::::::::::::::::::::::::::::::::::::::::::::::
Lepidophyma smithii       ::::::::::::::::::::::::::::::::::::::::::::::::::
Cricosaura typica         ::::::::::::::::::::::::::::::::::::::::::::::::
```

APPENDIX I

Continued.

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                          60         70         80         90         100
                          .          .          .          .          .
X. gracilis(1)            CCAATATCTCAGCCTGGTGAAACTTTGGTTCACTATTAGGATTATGCTTA
X. gracilis(2)            ..........................................................
X. gracilis(3)            ..........................................................
X. gracilis(4)            ..........................................................
X. henshawi(18)           ::::::::::::::::::::::.C.A..C.....CC.....................
X. henshawi(8)            ................A........G..............C.................
X. henshawi(6)            ....C..........A......C.A..C.....CC.....................
X. henshawi(14)           ....C..........A........A..C..G...C.....C.....C..
X. henshawi(5)            ....C..........A......C.A..C.....CC.....................
X. henshawi(15)           ....C..........A........A..C..G..TC.....C.....C..
X. henshawi(11)           ....C..........A........A..C..G...C.....C.....C..
X. henshawi(10)           ....C.....T.....A........A..C......C.....C.....C..
X. henshawi(12)           ....C..........A......C.A..C.....CC.....................
X. vigilis(19)            T.........G.....A.....T..C..C..................C..
X. henshawi(13)           ................A.........................C.................
X. henshawi(17)           ....C..........A........A..C..G...C.....C....TC..
X. henshawi(9)            ....C..........A........A..C..G...C.....C.....C..
X. henshawi(16)           ................A.........................C.................
X. henshawi(7)            ................A...........C......C.....C.................
X. vigilis                ::::::::::::::::::::::T..C..C..........T.......C..
X. bolsonae               ::::::::::::::::::::::...C..C......C...C.C.G..TC..
X. riversiana             ::::::::::::::::::::::T.....C..TA.C......C....TC..
Lepidophyma smithii       ::::::::::::::::::::::...C..C..CA.CC.T..TA.....C..
Cricosaura typica         ::::::::::::::::::::::.....C..G..CC....TA??...C..

                          110        120        130        140        150
                          .          .          .          .          .
X. gracilis(1)            ATAATTCAAATTATTACAGGACTATTTTTAGCAATGCACTACACAGCAGA
X. gracilis(2)            ..........................................................
X. gracilis(3)            ..........................................................
X. gracilis(4)            ..........................................................
X. henshawi(18)           ..C.C.......C.............C...??..A.....T.................
X. henshawi(8)            ............C.C...........C.....................................
X. henshawi(6)            ..C.C.......C.............C.......A.....T.................
X. henshawi(14)           ..C.........C.............C.......A.....................
X. henshawi(5)            ..C.C.......C.............C.......A..T..T.................
X. henshawi(15)           .CC.........C....G.........C.......A.....................
X. henshawi(11)           ..C.........C.............C.......A.....................
X. henshawi(10)           ..C.........C.............C.......A.....................
X. henshawi(12)           ..C.........C.............C.......A.....T.................
X. vigilis (19)           C.T..C..G..CT.C........T..CC.T.....A..T.....................
X. henshawi(13)           ............C.....................A.....................
X. henshawi(17)           .CC.........C.C...................A.....................
X. henshawi(9)            .CC.........C.............C.......A.....................
X. henshawi(16)           ............C.....................A.....................
X. henshawi(7)            ............C.............C.....................................
X. vigilis                C.T..C..G..CT.C........T..CC.T.....A..T.....................
X. bolsonae               C.T........C.CG.GC..G..G...C...?...A.....................
X. riversiana             C.TGC......CT..........C..CC.T..?..A.....................
Lepidophyma smithii       G.T..C......C.A.....C......C.CT....A.....T.................
Cricosaura typica         ..C.CC.....CG??..G.A.......C...??..A.....................
```

APPENDIX I

Continued.

```
                          160        170        180        190        200
                           .          .          .          .          .
X. gracilis(1)            CATCACATCAGCCTTCACATCAGTAATACACATCTGTCGAGACGTTCAAT
X. gracilis(2)            ..................................................
X. gracilis(3)            ..................................................
X. gracilis(4)            ..................................................
X. henshawi(18)           .....T.....................CT........C........C....
X. henshawi(8)            ..........................T.......................
X. henshawi(6)            .....T.....................CT........C........C....
X. henshawi(14)           ..........................C.......................
X. henshawi(5)            .....T.....................CT........C........C....
X. henshawi(15)           ..........................C.......................
X. henshawi(11)           ..........................C.......................
X. henshawi(10)           ..........................C........C.....T.......
X. henshawi(12)           .....T.....................CC.....T..C............
X. vigilis(19)            T..T..C........TT........G.C..T..T..C.....T..C....
X. henshawi(13)           ......G.....................C.......................
X. henshawi(17)           ................G..........C.......................
X. henshawi(9)            ..........................C.......................
X. henshawi(16)           ......G.....................C.......................
X. henshawi(7)            ..........................C.......................
X. vigilis                T..T..C........TT........G.C..T..T..C.....T..C....
X. bolsonae               ...T..C.....T..TT..........C.......CC..............
X. riversiana             ...T..C........TT.......TG.C..T.....C.....T..C....
Lepidophyma smithii       T...G.C..C..T...T.T...A.CGCC.....T.CC..T.....C....
Cricosaura typica         ....T.T.....T...T.......TGC.......ACC........C...C

                          210        220        230        240        250
                           .          .          .          .          .
X. gracilis(1)            ACGGCTGATTAATCCGAAATATTCATGCCAATGGGGCTTCAATCTTCTTC
X. gracilis(2)            ..................................................
X. gracilis(3)            ..................................................
X. gracilis(4)            ..................................................
X. henshawi(18)           ....T...C..........C.....C.....C..A........T..T...
X. henshawi(8)            ....T................C.........C...A..........T..T
X. henshawi(6)            ....T...C..........C.....C.....C..A........T..T...
X. henshawi(14)           ........C.............................A..C........T...
X. henshawi(5)            ....T...C..........C.....C.....C..A........T..T...
X. henshawi(15)           ........C.......................C..A..C........T...
X. henshawi(11)           ........C................C.....C..A..C........T...
X. henshawi(10)           ........C.......................C..A..C........T...
X. henshawi(12)           ........C..G.............C........A........T..T...
X. vigilis(19)            .T......C....T.....C..C..C........A..C........T..T
X. henshawi(13)           ......................C.........C..A...........T...
X. henshawi(17)           T.......C.......................C..A..C........T...
X. henshawi(9)            ........C.............................A..C........T...
X. henshawi(16)           ......................C.........C..A...........T...
X. henshawi(7)            ....T................C.........C..............T...
X. vigilis                .T......C....T.....C..C..C........A..C........T..T
X. bolsonae               ........C..........C.....C........A..C...G....T...
X. riversiana             .T..T...C..........G...C.........................
Lepidophyma smithii       ....A...C.G..T..T..C........A..C.....C...........
Cricosaura typica         ........C....T.....CC.C........C..T......C.T.....T
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APPENDIX I

Continued.

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                          260        270        280        290        300
                           .          .          .          .          .
X. gracilis(1)            ATTTGCTTATATATACACATTGCACGAGGACTATACTATGGTTCATACAT
X. gracilis(2)            ...........................................................
X. gracilis(3)            ...........................................................
X. gracilis(4)            ...........................................................
X. henshawi(18)           ..C........C.....................G..T.....................
X. henshawi(8)            ..C..T......C.....................T..........T..
X. henshawi(6)            ..C........C......T.............G..T.....................
X. henshawi(14)           ......C.............................T.....................
X. henshawi(5)            ..C........C.C.....................G..T.....................
X. henshawi(15)           ......C..........T.................T.....................
X. henshawi(11)           ......C....C.......................T.....................
X. henshawi(10)           ......C.T..C.......................T.....................
X. henshawi(12)           ..........C.....................G..........G.....
X. vigilis(19)            ..C...C.G..CT.....G..........C..G.....C..G..C.....
X. henshawi(13)           .....................................C.........
X. henshawi(17)           ......C....C.......................T.....................
X. henshawi(9)            ......C.............................T.....................
X. henshawi(16)           .....................................C.........
X. henshawi(7)            ....................................T.....................
X. vigilis                ..C...C.G..CT.....G..........C..G.....C..C..C.....
X. bolsonae               ..C........CC....T..C........GT.G.....C..C.....T..
X. riversiana             ...........C.......................T..C..C.........
Lepidophyma smithii       ..C.CAA.C..CC.T.....C..C..G.....C....TC..C..C...C.
Cricosaura typica         ........G..CC.......C........C..G.....C..A..G..T..

                          310        320        330        340        350
                           .          .          .          .          .
X. gracilis(1)            ATACACTATCACATGAAACATTGGTGTAATTCTACTGTTCCTGGTTATAG
X. gracilis(2)            ...........................................................
X. gracilis(3)            ...........................................................
X. gracilis(4)            ...........................................................
X. henshawi(18)           G.......................A...........AC....A......A
X. henshawi(8)            ....CG.TCAT..CATG.::::::::::::::::::::::::::::
X. henshawi(6)            G.......................A...........AC....A......A
X. henshawi(14)           .....TC..T..............AC...C.....TC.T..A.......
X. henshawi(5)            G.......................A...........AC....A......A
X. henshawi(15)           ......C..T..............AC...C.....TC....A.......
X. henshawi(11)           .....TC..T..............AC...C.....TC....A.......
X. henshawi(10)           ......C..T..............AC...C.....TC....A.......
X. henshawi(12)           .........T.........................AC....A......A
X. vigilis(19)            G.....A.............C..C........C..A..TT.A..C....
X. henshawi(13)           .....T.....................................................
X. henshawi(17)           ......C..T..............AC...C.....TC....A.......
X. henshawi(9)            .....TC..T..............AC...C.....TC.T..A.......
X. henshawi(16)           .....T.....................................................
X. henshawi(7)            ....G.....................G.C.....................
X. vigilis                G.....A.............C...........C..A..TT.A..C....
X. bolsonae               ...T..C...........T...........C.....A.....A..C....
X. riversiana             G....TA.........................C..A..T..A..C....
Lepidophyma smithii       .....AA.............C......C..C......C..T.AACC....
Cricosaura typica         ..TT.AAGAA..............G...G.AT....AC.A..TC.C..G.
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APPENDIX I

Continued.

	360 370 380
X. gracilis(1)	CAACAGCCTTCGTAGGTTATGTCCTACCA
X. gracilis(2)	.....
X. gracilis(3)	.....
X. gracilis(4)	.....
X. henshawi(18)	.....T..G.....AT.....
X. henshawi(8)	:::::::::::::::::::::::::::
X. henshawi(6)	.....T..G.....AT.....
X. henshawi(14)	.....A.....T.....
X. henshawi(5)	.....T..G.....AT.....
X. henshawi(15)	.....A.....
X. henshawi(11)	.....A.....T.....
X. henshawi(10)	.....A.....
X. henshawi(12)	.....T..G.....A.....
X. vigilis(19)	.....T..G.....C.....
X. henshawi(13)	.....C..C.....
X. henshawi(17)	.....A.....
X. henshawi(9)	.....A.....G....
X. henshawi(16)	.....C..C.C.....
X. henshawi(7)	.....A.....
X. vigilis	.....T..G.....A.....
X. bolsonae	.....C...T.....
X. riversiana	.....T.....CA.....
Lepidophyma smithii	.....T.....AC.A.A.....G
Cricosaura typica	.C.....T..T..T.....AA...G...