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MITOCHONDRIAL PHYLOGEOGRAPHY OF THE ENDANGERED BLUNT-NOSED LEOPARD LIZARD, *GAMBELIA SILA*

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ABSTRACT—To assess the genetic diversity and phylogeography of the blunt-nosed leopard lizard (*Gambelia sila*), we sequenced 1,285 base pairs (bp) of the mitochondrial cytochrome oxidase-*b* (cyt-*b,* 682 bp) and cytochrome oxidase III (CO3, 603 bp) genes from 33 individuals representing eight natural populations in central California. Phylogenetic analysis indicated that 17 observed haplotypes are partitioned into two major clades, which correspond geographically to where the lizards were collected. We also conducted a focused analysis of individuals collected from the canyons leading into the Cuyama Valley in Ventura and Santa Barbara counties, a geographic area with lizards possibly representing a remnant hybrid (with *G. wislizenii*) population. All lizards from the Cuyama Valley and adjacent canyons exhibited the mitochondrial haplotype of *G. sila* and were embedded within one clade. Our morphological analysis placed some leopard lizards collected from Cuyama Valley with true *G. sila*, whereas some individuals aggregated with *G. wislizenii*. This finding suggests that the quantitative morphological characteristics often used to distinguish between the two species are fairly labile and may be influenced by prevailing environmental conditions.

RESUMEN—Para evaluar la diversidad genética y la filogeografía de la lagartija leopardo de nariz chata (*Gambelia sila*), se secuenciaron 1,285 pares de bases de los genes de oxidase citocromo mitocondrial *b* (cyt *b,* 682 bp) y de oxidase III citocromo (CO3, 603 bp) de 33 individuos, representantes de ocho poblaciones naturales en California central. El análisis filogenético indicó que los 17 haplotipos encontrados se dividen en dos clades principales, que corresponden geográficamente a los sitios donde se recogieron las lagartijas. También hicimos un análisis enfocado en individuos recogidos de los cañones que van hacia el valle de Cuyama en los condados de Ventura y Santa Bárbara, un área geográfica con lagartijas que posiblemente representan una población híbrida remanente (con *G. wislizenii*). Todas las lagartijas del valle de Cuyama y cañones adyacentes exhibieron el haplotipo mitocondrial de *G. sila* y se incluyeron en un solo clade. Nuestro análisis morfológico colocó algunas lagartijas leopardo recogidas del valle Cuyama con verdaderos *G. sila*, mientras que otros individuos se agregaron a *G.wislizenii*. Este hallazgo sugiere que las características morfológicas cuantitativas a menudo empleadas para distinguir entre las dos especies son bastante lábiles y pueden ser influenciadas por condiciones ambientales actuales.

The blunt-nosed leopard lizard (*Gambelia sila*) has been federally listed as endangered since 1967 and listed as endangered in California since 1971 because ca. 85% of its original habitat has been lost to agriculture, oil development, and urbanization (Germano and Williams, 1992; Jennings, 1995; United States Fish and Wildlife Service, in litt.). The range of *G. sila* was once spread throughout the western and southern portions of the San Joaquin Valley and its surrounding foothills, portions of the Cuyama Valley, and the Carrizo Plain in California (Montanucci, 1965; McGuire, 1996). Understanding the genetic variation within and among isolated populations of species is important to their conservation, especially so for *G. sila*, which exists now in a highly fragmented range. Identifying unique haplotypes can lead to protecting significant sites for conservation and can be useful for enhancing movements of populations between them (United States Fish and Wildlife Service, in litt.). Small isolated populations of *G. sila* on fragmented land caused by the destruction of habitat in the San Joaquin Valley likely has resulted in decreased gene flow among adjacent populations and may result in increased inbreeding within each of the fragmented populations. Empirical studies on low genetic variation support the hypothesis that inbreeding lowers individual and mean population fitness and increases the possibility of inbreeding depression and the risk for extinction of populations (Charlesworth and Charlesworth, 1987; Lynch, 1991; Newman and Pilson, 1997).

The use of molecular phylogenies for conservation can be important when developing effective strategies for conservation and management (Mace, 2004). Molecular

characters provide a key source of information facilitating the identification of geographic lineages, reproductively isolated populations, or both (Moritz, 1995). No phylogenetic study of *G. sila* has been conducted. Besides the possibility of a phylogeographic pattern across the range of *G. sila*, at the extreme southwestern end of its range, there is a purported hybrid zone between *G. sila* and *G. wislizenii* (Sanders, 1950; Montanucci, 1970). Studies by Montanucci (1970, 1978) described the electrophoretic and morphological characters of leopard lizards in this purported hybrid zone, and he mapped an area that he suggested contained individuals of a remnant hybrid population. A comparison of mitochondrial DNA of known *G. wislizenii* and known *G. sila* to the DNA of lizards inhabiting the Cuyama River area should help elucidate the genetic identity of leopard lizards currently inhabiting the purported hybrid zone of Montanucci (1970, 1978).

To understand the phylogenetic relationships of populations of *G. sila* across its range, we sequenced portions of two mitochondrial genes from *G. sila* from various localities throughout their range. Besides this range-wide analysis and as a first attempt at elucidating the genetic identity of lizards inhabiting the Cuyama Valley, we compared the DNA sequences of lizards of known *G. wislizenii* and known *G. sila* to the DNA of lizards inhabiting the Cuyama River. Specifically, we wanted to determine if both *G. wislizenii* and *G. sila* mitochondrial haplotypes were represented in the Cuyama River area and if there is any correspondence between the morphological designations of sampled lizards and their mitochondrial haplotype.

Materials and Methods—In 2008–2009, we caught 28 *Gambelia sila* at seven sites across the range of the species (Fig. 1a). We obtained genetic data for two lizards for site 2 from GenBank. We caught lizards at site 8 in 2001–2002 and 2008–2009. We removed the right fifth (innermost) toe of lizards collected as the source of genetic material. Similarly, we caught nine *G. wislizenii* (three were used for genetic analysis and served as outgroup taxa) from three sites in the western Mojave Desert of Kern County, California. We analyzed 30 concatenated mitochondrial gene sequences from 30 *G. sila* and three *G. wislizenii* (Table 1, Fig. 1a). We extracted DNA from toe-clippings using the DNAeasy tissue kit (QIAGEN, Valencia, California) according to instructions from the manufacturer. We used primer sequences from Pearse and Pogson (2000) to amplify and sequence a portion (ca. 682 base pairs, bp) of the mitochondrial cytochrome oxidase-*b* gene (cyt-*b*) from all *Gambelia*. We also used primer sequences from Orange et al. (1999) to amplify and sequence a portion (ca. 603 bp) of the mitochondrial cytochrome oxidase III (CO3) gene from all *Gambelia*. This primer set, however, inconsistently amplified extracts of *G. sila*, so an internal primer set was designed from a single sequence of *G. sila* (Table 2) and used to amplify and sequence the cytochrome oxidase III (CO3) gene from all remaining samples of *G. sila*.

We conducted polymerase-chain-reaction (PCR) amplifications in 20-µl volume and annealing temperatures ranging between 48 and 53°C for the cyt-*b* gene. We also conducted PCR amplifications in 20-µl volume and annealing temperatures ranging between 48 and 50°C for the CO3 gene. Successfully amplified PCR products were purified by using shrimp phosphatase or exonuclease (ExoSAPit). We submitted purified PCR products to the DNA Sequencing Core Facility, University of Florida (Gainesville) for sequencing of the forward and reverse strands on an ABI 377 DNA sequencer. Sequence-electropherograms were read, edited, and aligned using Geneious v5.0 (A. J. Drummond et al., http://www.geneious.com/). Alignment of the DNA sequences was straight-forward and did not necessitate the insertion of any gaps.

We calculated summary statistics for the DNA-sequence data using MEGA 5.0 (Tamura et al., 2011). We estimated phylogenetic relationships with maximum parsimony and maximum likelihood analyses using MEGA 5.0 (Tamura et al., 2011). Bayesian analysis was performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) as implemented in Geneious v5.0.

We conducted maximum parsimony analysis using the tree-bisection-reconnection algorithm and 100 random addition-sequence replicates. The phylogeny based on the maximum likelihood method was inferred using the HKY + G model (Hasegawa et al., 1985). This model was selected using the Best-Fit Substitution Model option in MEGA 5.0 and exhibited a log likelihood of −2,319.5007. The gamma distribution was used to model differences in evolutionary rates among sites. We estimated support for specific nodes on the phylogenetic trees using bootstrapping (Felsenstein, 1985; 500 replicates).

For morphometric analysis, we recorded six morphometric measurements from nine *G. sila* and nine *G. wislizenii* collected from areas in their range and from 31 *Gambelia* collected from the purported hybrid zone in the Cuyama Valley (Fig. 1b). For each captured individual, we measured snout–eye length (tip of the snout to the anterior of the eye), snout–ear length (tip of snout to the anterior of the ear), head width (widest part of the head), total length, and snout–vent length (tip of snout to posterior of vent) to the nearest 0.1 mm, and mass to the nearest 0.5 g. We used MANCOVA with snout–vent length as the covariate (Minitab Inc., 2007) to determine if there were significant differences in morphometric variables among *G. sila*, *G. wislizenii*, and leopard lizards from the hybrid area. Significance of MANCOVA was determined using the greatest characteristic root (Harris, 1985). If the overall MANCOVA was significant, lower-order discriminant functions were similarly tested.

Results—We analyzed 1,285 aligned bases of DNA sequence from portions of the mitochondrial cyt-*b* (682 bp) and CO3 (603 bp) genes from 33 individuals. The average base frequencies across genes were 28.0, 27.3, 29.8, and 14.9% for A, C, G, and T, respectively. Of the 1,285 characters, 78 (6%) were variable, and 60 (4.7%) were parsimony-informative. We found 17 haplotypes that could be distinguished based on diagnostic nucleotide sites (Table 3).

Maximum parsimony analysis recovered 23 equally parsimonious trees (length = 85, consistency index = 0.89, retention index = 0.96). An inferred-consensus tree resulted from 1,425 most parsimonious trees (Fig. 2).

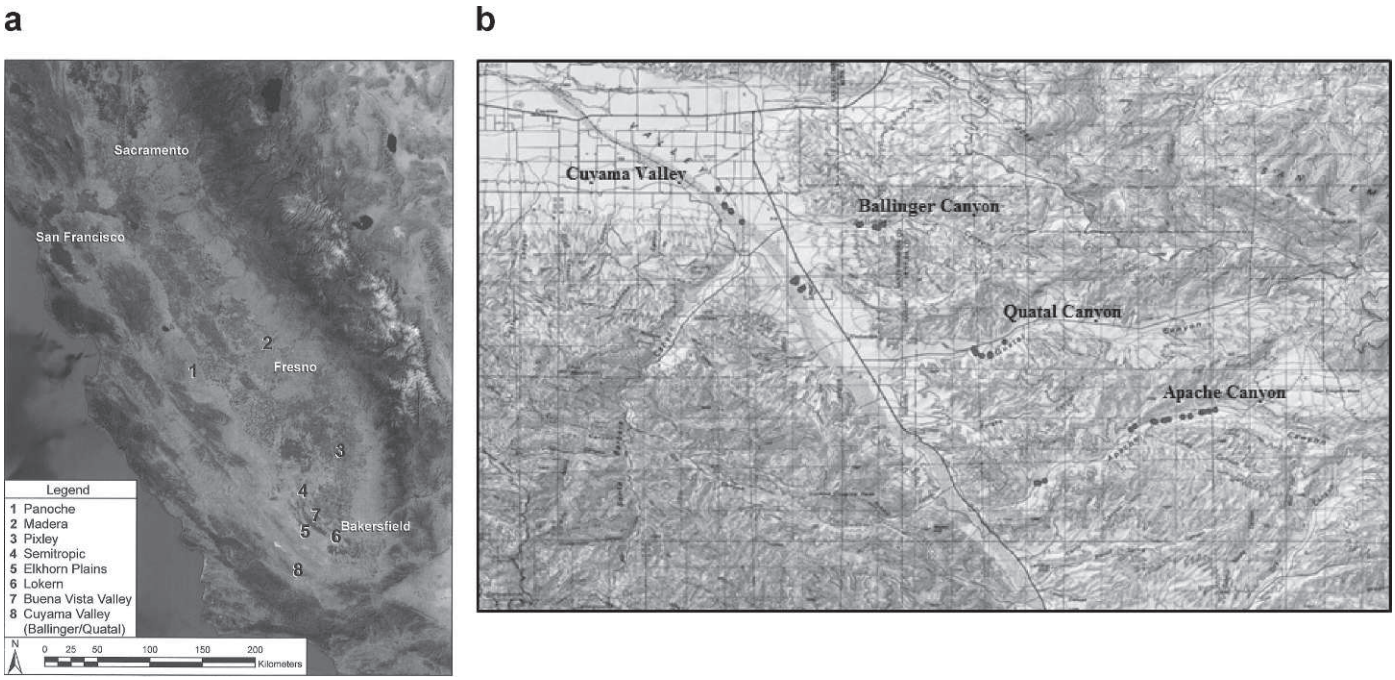

FIG. 1—a) Locations of populations of leopard lizards (*Gambelia*) sampled in the San Joaquin Valley (site 1 = Panoche, 3 = Pixley, 4 = Semitropic, 5 = Elkhorn Plain, 6 = Buena Vista, 7 = Lokern) and Cuyama Valley (site 8), California. Genetic data for site 2 (Madera grasslands) were from GenBank. b) Specific locations of *Gambelia* captured 2000–2001 and 2008–2009 in the Cuyama Valley and adjoining canyons in Santa Barbara, San Luis Obispo, and Ventura counties, California.

Branches corresponding to partitions reproduced in <50% of the trees are collapsed. The inferred tree using maximum parsimony recovered all individuals of *G. sila* as monophyletic. Within *G. sila*, two major clades were recovered (groups 1 and 2). Group 1 is comprised of individuals collected from northern populations, whereas group 2 is comprised of individuals collected from southern populations, including the southern (i.e., Apache and Ballinger) canyons (Fig. 2). Within group 1, the two most northern populations, Panoche and Madera, were recovered as a monophyletic lineage that was joined as a sister group to a grouping Semitropic and Pixley. Within group 2, there exists a grouping comprised of all noncanyon individuals (Fig. 3). The major differences between competing parsimony trees concerned the placement of individuals within group 2. The topologies of the trees resulting from the maximum likelihood analysis (Fig. 3) are largely congruent with the parsimony tree.

All lizards collected from the hybrid zone exhibited the mitochondrial signature of true blunt-nosed leopard lizards and together with other southern lizards formed a well-supported lineage that was distinct from the northern lizards (group 1; Figs. 2 and 3). We used MEGA versus 5.0 to analyze divergence of sequences within and among groups. Divergence of sequences within groups, estimated by averaging over all pairs of sequences within each group, was 0.014 for group 1 and 0.003 for group 2. The divergences of sequences between groups were 0.028 for outgroup versus group 1, 0.029 for outgroup versus group 2, and 0.019 for group 1 versus group 2. As expected, there was higher mean divergence of sequences among groups than within groups. The low mean divergence of sequences among samples within group 2 (0.003) suggests that there is minimal difference between all southern populations, including lizards collected from the hybrid zone.

For morphometric analysis, the overall MANCOVA was significant ($F_{[2,45]} = 42.73$, $P < 0.001$), as was the second discriminant function ($F_{[2,45]} = 6.74$, $P < 0.001$). The first two discriminant functions separated *G. sila* from *G. wislizenii* and accounted for all the variance. Leopard lizards from the hybrid zone occupied morphometric space within and between the two known species (Fig. 4).

DISCUSSION—There are two important findings from our analysis of the sequences of mitochondrial DNA from *G. sila*. First is that there is structure along the range of the species with two distinct groupings of haplotypes ordered from north to south. Second, we found that, despite apparent morphological distinctiveness of lizards in the hybrid zone, all individuals were genetically *G. sila*. Both of these findings are of conservational concern for the recovery of the species and the protection of unique populations.

The phylogenetic analysis provides support for the partitioning of 18 haplotypes of *G. sila* into two distinct clades that are partitioned geographically as northern and southern groups of haplotypes. The northern group (group 1) is composed of *G. sila* from Panoche and

TABLE 1—Haplotypes, locations (in California), and GenBank accession numbers of samples of *Gambelia sila* and *G. wislizenii* used for genetic assessment of *G. sila* from central California. Because samples of *G. wislizenii* were used only as an outgroup, haplotypes were not analyzed for this species.

Species	Haplotype	Locality	GenBank accession no.
G. wislizenii	-	Mojave (Long nosed 1)	KM058714
	-	Mojave (Long nosed2)	KM058715
	-	Mojave (Long nosed 3)	KM058716
G. sila	A	Ballinger Canyon 3	KM058717
	B	Ballinger Canyon 1	KM058718
	C	Buena Vista 1	KM058719
	D	Buena Vista 2	KM058720
	E	Buena Vista 3	KM058721
	F	Buena Vista 4	KM058722
	G	Elkhorn Plains	KM058723
	H	Lokern 1	KM058724
	I	Lokern 2	KM058725
	J	Lokern 3	KM058726
	K	Lokern 4	KM058727
	L	Panoche 1	KM058728
	M	Pixley 1	KM058742
	N	Pixley 2	KM058743
	O	Semitropic 1	KM058730
	P	Semitropic 2	KM058744
	Q	Buena Vista 2a	KM058731
	L	Panoche 2	KM058729
	B	Apache Canyon 10	KM058732
	B	Apache Canyon 11	KM058733
	B	Apache Canyon 13	KM058734
	B	Apache Canyon 14	KM058735
	A	Apache Canyon 4	KM058736
	B	Apache Canyon 5	KM058737
	A	Apache Canyon 6	KM058738
	A	Apache Canyon 8	KM058739
	B	Apache Canyon 9	KM058740
	F	Buena Vista 1a	KM058741

Madera plus Pixley and Semitropic. Group 1 is differentiated from group 2 by almost 2%. These two groups differ from *G. wislizenii,* its sister species, by ca. 3%. These results suggest that group 1 may be reproductively isolated from group 2. Two clades (east and west) also were detected for the wide-ranging *G. wislizenii,* which differed by 6.1% (Orange et al., 1999). Within the western clade, which includes populations from the Great Basin and Mojave deserts and the Colorado Plateau, are two geographically distinct clades that differed by 5.4%. The low level of divergence between *G. sila* and *G. wislizenii* indicates a fairly recent divergence of these species. Parham and Papenfuss (2008) determined the genetic relatedness of populations of the California legless lizard (*Anniella pulchra*) across their range in California and Baja California. They found genetic divergence as high as 14.3% in the most distant populations. Even between geographically close populations in Bakersfield in the southern San Joaquin Valley, they found high levels of divergence (5.7%). The much lower levels of genetic divergence that we found across the whole range of *G. sila* is likely the result, at least in part, of the much higher vagility of leopard lizards compared to the slow-moving fossorial legless lizard.

The formation of distinct mitochondrial lineages throughout the range of *G. sila* may be explained by a past geographic barrier not apparent now. Samples from Panoche, Madera, and Pixley are north of former Tulare Lake and Los Gatos Creek. In the past 100,000 years before present, Tulare Lake has fluctuated greatly in size due to glacial melt from the Sierra Nevada and more recently (last 2,500 years before present) because of wetter conditions after the Hypsothermal (Atwater, 1986). At some periods, Los Gatos Creek, which flows across the west side of the San Joaquin Valley, had substantial flows and created part of the fan dam that increased the size of Tulare Lake (Atwater, 1986). Leopard lizards that eventually evolved into *G. sila* may have entered the San Joaquin Valley sometime during the Pleistocene; Brattstrom (1953) recovered fragmented portions of two maxillae that he assigned to *G. wislizenii* from the McKittrick tar pits in the southwestern edge of the San Joaquin Valley. However, the fairly unspecific dating of the Pleistocene places the occurrence of leopard lizards in the southern San Joaquin Valley as early as 2.6 million years ago to as late as 11,700 years before present. A much more recent date seems unlikely if the material can correctly be assigned to *G. wislizenii,* although the evidence presented is equivocal in our view and also could be from *G. sila.* Leopard lizards may have entered the valley and spread to the north during periods of low lake levels. Subsequent high lake-levels may have separated northern from southern populations.

Although there is no direct evidence of a barrier

TABLE 2—Oligonucleotide primers (with GenBank accession numbers for sequences) used for genetic analysis of *Gambelia sila* from central California.

Gene and primer code	Sequence (5′-3′)	Reference	GenBank accession no.
CO3-F(L8618)	CATGATAACACATAATGACCC	Orange et al. (1999)	AF095613–AF095616 AF095619–AF095621
CO3-R(H9323)	ACTACGTCTACGAAATGTCAG	Orange et al. (1999)	
CO3-F	CCTTCTAATGACCTCCG		AF095613–AF095616 AF095619–AF095621
CO3-R	AAATGTCAGTATCATGCGG		

TABLE 3—List of diagnostic nucleotide sites that distinguish the 18 haplotypes (A–R) of mitochondrial cytochrome oxidase-*b* of *Gambelia sila* from central California.

Haplotype	Diagnostic nucleotide sites
A	CTTCTCTTTCCACTACTAAGCCTCGCAGATTTTAACCTGCGCTGTCTTC
B	CTTCTCTTTCCACTACTAAGCCTCGCAGGTTTTAACCTGCGCTGTCTTC
C	CTTCTCTTTCCACTACTAAGCCTCGTAGATTTTAACCTGCGCTGTCTTC
D	CTTCTCTTTCCACTACTAAGCCTCGTAGATTTTAACCTGCGCTGTCTCT
E	CTTCTCTTTCCACTACTAAGCCTCGTAGATTTTAACCTGCGCTGTATTT
F	CTTCTCTTTCCACTACTAAGCCTCGTAGATTTTAACCTGCGCTGTCTTT
G	CTTCTCTTTCCACTACTAAGCCTCGTAGATTTTAACCTGTGCTGTCTTT
H	CTTCTCTTTCCACTACTAAGCCCCGTAGATTTTAACCTGCGCTGTCTTC
I	CTTCTCTTTCCACTACTAAGCCTCGTAGATTTTGACCTGCGCTGTCTTT
J	CTTGTCTTTCCACTACTAAGCCTCGTAGATTTTAACCTGCGCTGTCTTT
K	CTTCTCTTTCCACTACTAAGCCTTGTAGATTTTAACCTGCGCTGTCTTT
L	CCTCCCTTTCCATTATTGAATCTCGCAAATCTTGATCTACACTGTCCTC
M	CTTCCCTTTCCATTATTGAATCTCGCAAATCTTGATCTACACTGTCCTC
N	CTCCCTTCTTCACTACTGAGCCTCGCAGATTTTGACCTGCGCTGCCTTC
O	CTCCCTTCTTCACTACTGAGCTTCGCAGATTTTGACCTGCGCTGCCTTC
P	TTCCCTTCTTCACTACTGAGCCTCGCAGATTTTGACCTGCACTGCCCTC
Q	TTCCCTCCTTCACTACTGAGCCTCGCAGATTTTGACCTGCACTGCCCTC
R	CTTCTCTTTCTACTACTAAGCCTCGCAGATTTTAACCTGCGCTGTCTTC

between leopard lizards in the north and the more southerly groups below Tulare Lake-Los Gatos Creek in the past, it is possible. Until damming in the Sierras significantly reduced inflow to Tulare Lake, the lake overflowed its basin, even in the past century (Atwater, 1986). Extensive marshes surrounding the lake would have broadened the extent of the lake as a barrier to leopard lizards. However, there is occupied habitat on the west side of the valley that currently is higher than the barrier of Tulare Lake. A full barrier to movement of lizards to the north would only be accomplished if Los Gatos Creek also carried much more substantial flows of water than has occurred in the recent past. Otherwise, leopard lizards could have bypassed even an enlarged Tulare Lake. Lizards from the Semitropic area, which group genetically with other northern individuals, occur south of former Lake Tulare. If Lake Tulare indeed served as a barrier to gene flow between these lizards and those further to the south in the San Joaquin Valley, then the existence of the Semitropic population may best be explained as a post-barrier migrant population that originated from a population in the north; however, this hypothesis is tentative and remains subject to further verification.

Our analysis also showed that all the lizards from the Cuyama Valley exhibited the DNA signature of *G. sila* and formed part of group 2. Montanucci (1970) described and designated hybrid leopard lizards in the Cuyama Valley based mainly on intermediate morphological traits and electrophoretic characters. Montanucci (1970) found these morphological hybrids in Ballinger, Quatal, and Burges canyons and in the Cuyama River next to these canyons. The hybrid zone extended to much of the area along Highway 33 in Santa Barbara County. He also found what he called *G. wislizenii* in Apache Canyon, in Dry Canyon, and throughout Lockwood Valley to east of the hybrid area. He determined that *G. sila* were north of Ballinger Canyon. Montanucci (1970) also hypothesized that interbreeding had been occurring between *G. sila* and a remnant hybrid population inhabiting the area. Because hybrid leopard lizards may not be protected under the federal Endangered Species Act, or by the designation of Fully Protected Species by the State of California, infrastructure projects that occur in the hybrid zone and along Highway 33 potentially would not have to take these purported hybrids into account when studying environmental effects. Although mitochondrial DNA is not sufficient for uncovering ancient (or other) hybridization, it can provide a real-time designation of species of lizards currently inhabiting the Cuyama Valley, and our analysis of mitochondrial DNA indicate that the lizards inhabiting the area of the Cuyama River are *G. sila*.

We found individuals during our surveys that morphologically appeared to be hybrids as well as leopard lizards that we would classify as *G. sila* or *G. wislizenii* based on morphology. Yet, the molecular genetic techniques we employed did not support these morphological designations. Indeed, all lizards from the purported hybrid zone exhibited the mitochondrial DNA signature of *G. sila*. If *G. wislizenii* were indeed inhabiting the canyons and Cuyama Valley, we should have detected mitochondrial DNA of true *G. wislizenii* in our analysis. Because we failed to recover a single lizard from the canyons that exhibited the mitochondrial DNA signature of known *G. wislizenii* (even from those lizards that morphologically congregated with *G. wislizenii*), we can conclude that a stable hybrid zone is currently not being maintained. Therefore, we think that true *G. wislizenii* and true *G. sila* are allopatric

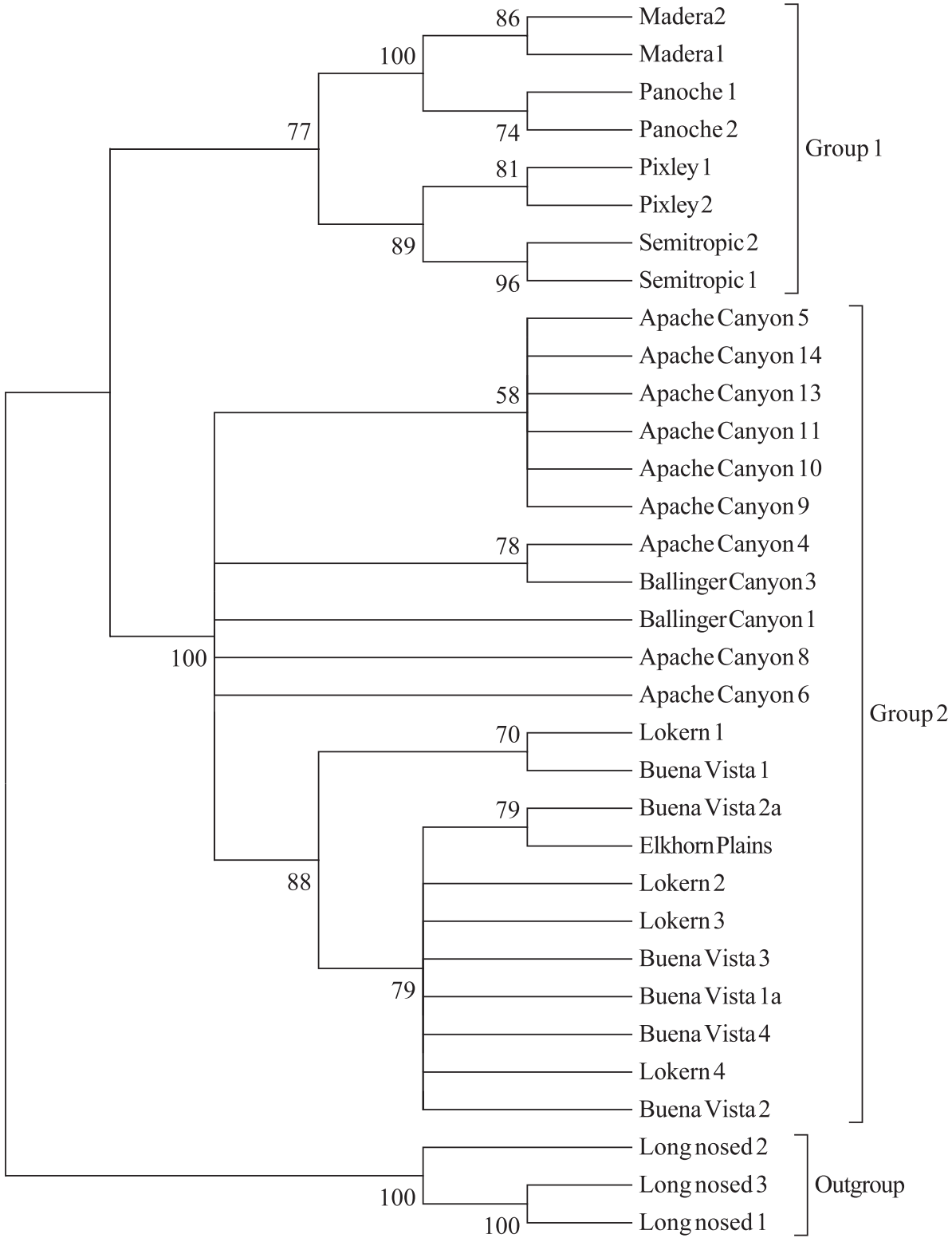

FIG. 2—Strict consensus of 23 equally parsimonious trees based on unweighted parsimony analysis of a 1,285-base pair portion of the mitochondrial cytochrome oxidase-*b* and cytochrome oxidase III genes for 30 *Gambelia sila* (groups 1 and 2) and 3 *G. wislizenii* (= long nosed) from central California. Tree length = 85; consistency index = 0.89; retention index = 0.96. Numbers at branches are bootstrap values (percentage; only bootstrap values >50% are shown). See Fig. 1 for explanation of names of samples.

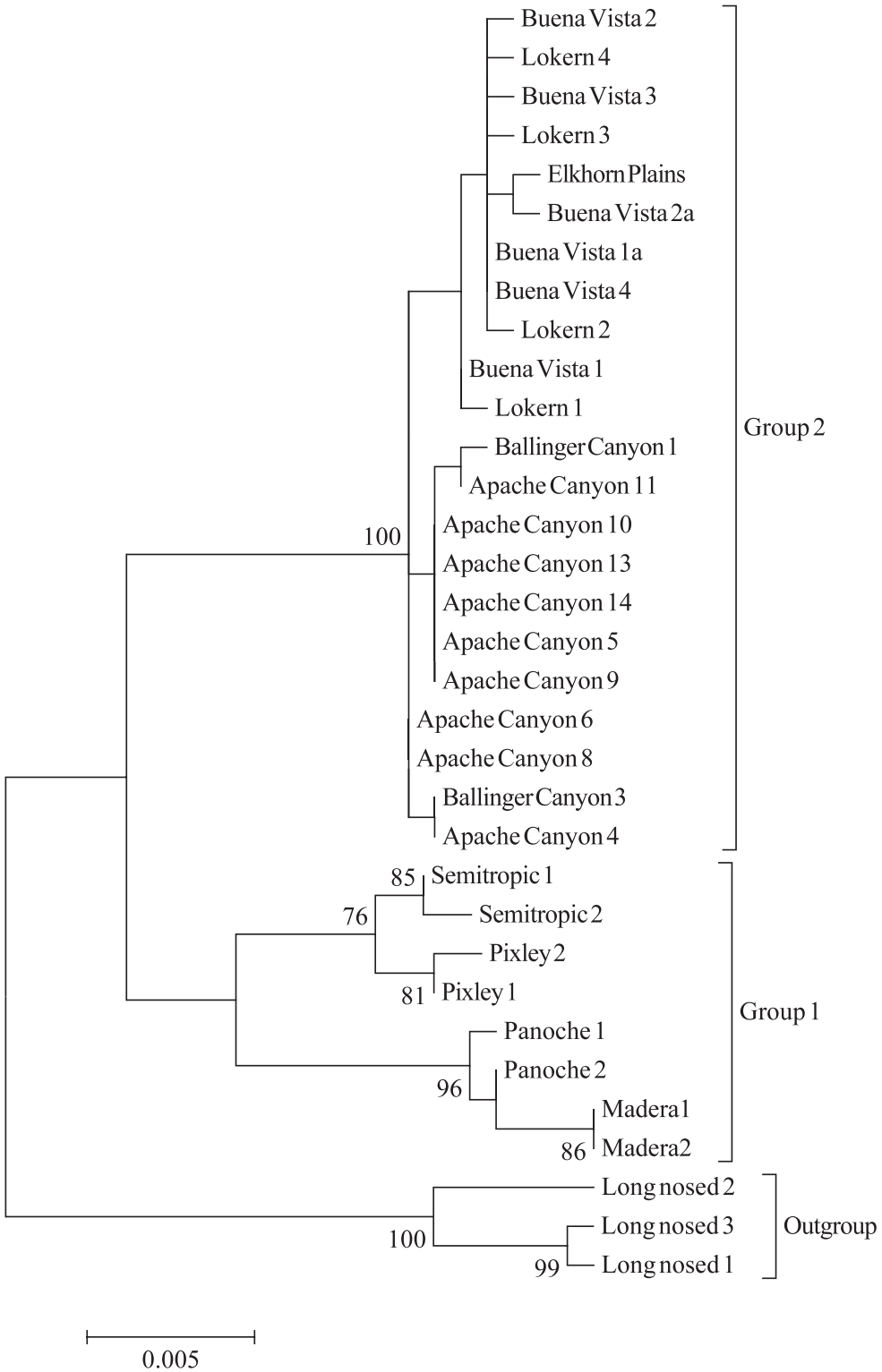

FIG. 3—A phylogenetic tree with the highest log likelihood (−2,319.5007) inferred using the maximum likelihood method based on the Hasegawa-Kishino-Yano model + Gamma (G) distribution to model differences in evolutionary rates among 30 *Gambelia sila* (groups 1 and 2) and 3 *G. wislizenii* (= long nosed) from central California. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. See Fig. 1 for explanation of names of samples.

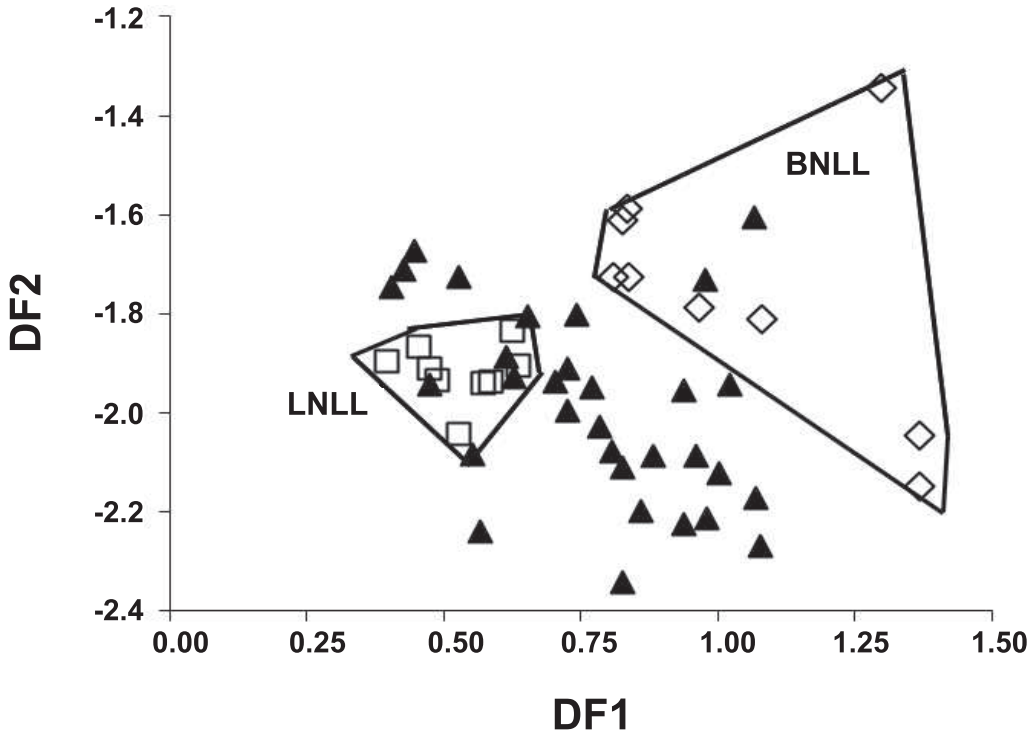

Fig. 4—Morphometric space of three groups of *Gambelia* based on the first two discriminant functions (DF) from multivariate analysis of covariance of size-adjusted morphometric measurements. LNLL = long-nosed leopard lizard (*G. wislizenii*; $n = 9$; open squares); BNLL = blunt-nosed leopard lizard (*G. sila*; $n = 9$; open diamonds). Cuyama Valley leopard lizards (solid triangles) are from the purported hybrid zone ($n = 31$). Discriminant functions 1 and 2 account for 81.9 and 18.1% of the variance, respectively,.

and are not actively producing hybrids in the canyons and Cuyama Valley. Furthermore, our results indicate that all leopard lizards in the hybrid zone should be classified as *G. sila* and not as hybrids. Thus, our findings suggest that the significant difference in morphometric characteristics of the three populations of *G. sila* disagree with the phylogenetic analysis. The morphological similarity of some *G. sila* that inhabit the canyons and Cuyama Valley to *G. wislizenii* is likely due to yet undetermined environmental factors.

One significant result of this analysis is that, although the leopard lizards from Cuyama Valley grouped with blunt-nosed and long-nosed leopard lizards, all leopard lizards from Cuyama Valley exhibited the mitochondrial DNA signature of *G.sila* and were part of the unique southern clade. Lastly, the genetic differentiation of haplotypes between the group 1 and group 2 is notable and significant with respect to future conservational efforts. The two groups of haplotypes are as different genetically from each other as either is to true *G. wislizenii*. Future efforts of conservation should refrain from translocating lizards across the geographic boundaries of the respective haplotype-groups until further study using microsatellites is completed. To do so could result in outbreeding depression, loss of unique alleles, or both. Additional populations of *G. sila* should be sampled to refine the phylogenetic structure of this species.

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