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PHYLOGENETIC RELATIONSHIPS OF THE *LAMPROPELTIS MEXICANA* COMPLEX (SERPENTES: COLUBRIDAE) AS INFERRED FROM MITOCHONDRIAL DNA SEQUENCES

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ABSTRACT

Mitochondrial DNA sequences were used to determine the phylogenetic relationships of the *Lampropeltis mexicana* complex to other taxa of the *Lampropeltis* genus. Several major nodes were consistently recovered, though the relationships of these nodes to each other was not fully resolved. Results revealed two polyphyletic clades with taxa from the *mexicana* and *triangulum* groups in both clades, referred to as a "northern" and a "southern" clade. There was very little sequence divergence among taxa of the northern clade. The concept of reticulate evolution and the genealogical species concept were used to interpret the historical biogeography of these two groups. During the eastward radiation of these two groups from a common ancestor on the Mexican Plateau, a geographic bottleneck in the Sierra Madre Oriental may have induced gene flow between the two groups and subsequent lineage sorting may have occurred. Additionally, contact between these two groups in southern refugia during Pleistocene glaciations may have again resulted in gene flow. This possibility, combined with the strong morphological and ecological differences between the *mexicana* and *triangulum* groups, suggests the gene trees derived from the mtDNA analyses in this study may not be congruent with the species tree that represents the true evolutionary history of these two groups.

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I would also like to thank my parents for allowing me to study and keep reptiles and amphibians throughout my childhood. Finally, I would like to thank my wife for her support and patience while I was out in the field and in the lab in SWT.

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INTRODUCTION

The use of molecular techniques, specifically mitochondrial DNA (mtDNA), to estimate phylogenetic relationships has dramatically increased in popularity in the past few decades. Consequently, debates over molecules versus morphology have ensued (Patterson et al., 1993). Some have argued that morphological characters are weak or misleading (Kluge, 1983; Frelin and Vuilleumier, 1979; Sibley and Ahlquist, 1987; Lamboy, 1994) or that morphological data have less utility in systematic studies than do molecular data (Hedges and Maxson, 1996; Givnish and Sytsma, 1997). Though homology and homoplasy (convergence, parallelism, and reversals of character states) can be problematic when using morphology (Hedges and Maxson, 1996), this can be true for molecular data sets as well (Weins and Hillis, citation). Baker et al. (1998) found that morphology is generally less homoplasious than molecular data. In truth, both approaches have distinct advantages and disadvantages (Moritz and Hillis, 1996).

Often, morphological and molecular data are in agreement. On some occasions, though, the relationships resulting from molecular approaches differ from those proposed by previous studies based on morphology (e.g., Sites et al., 1996; Zamudio and Greene, 1997; Zamudio et al., 1997; Rodríguez-Robles et al., 1999; Pook et al., 2000; Rodríguez-Robles and Jésus-Escobar, 2000; Alfaro and Arnold, 2001; Puerto et al., 2001; Douglas et al., in press). Baker et al. (1998) found that of 25 studies they examined, 12 showed significant incongruence between the morphological and the molecular data partitions. Determining the source of conflict is imperative in order to fully understand

phylogenetic relationships. Potential errors in molecular inferences exist, such as lineage sorting and introgressive hybridization (Moore, 1995). Gene trees resolved from molecular analyses may also not be representative of species trees (Pamilo and Nei, 1988; Moore, 1995).

The phylogenetic relationships of the polytypic species allied to *Lampropeltis mexicana* Garman, 1883 to other taxa in the genus *Lampropeltis* are unresolved. Distributed in disjunct populations from extreme southeast New México and western Texas in the United States south through the Sierra Madre Oriental and Mexican Plateau to the Cordillera Volcánica of México (Gartska, 1982; Painter et al., 1992), this species complex has received little attention since Garstka (1982). Various phylogenies and taxonomic arrangements have been proposed. Gelbach and Baker (1962) stated that the *mexicana* group was composed of five subspecies of *L. mexicana* (*L. m. mexicana*, *L. m. alterna*, *L. m. blairi*, *L. m. thayeri*, and *L. m. greeri*) and *L. leonis*. Gartska (1982) considered the *mexicana* group be composed of a monotypic *L. mexicana*, *L. alterna*, and *L. ruthveni*. Smith (1942, 1944) proposed a phylogeny for Mexican *Lampropeltis* that placed the *mexicana* group (*mexicana*, *leonis*, *thayeri*, and *alterna*) as being derived from a member of the *triangulum* group. Webb (1962) instead suggested that three distinct lineages have evolved from *mexicana*: *greeri*, *blairi*, and *alterna*; *leonis* and the *calligaster-getulus* group; and *thayeri* and the *triangulum* group. Garstka (1982) later hypothesized that the *mexicana* group was derived from *triangulum* based on shared derived characters. Under all proposed phylogenies, however, both the *mexicana* and *triangulum* groups are considered

monophyletic, and the divergence of these two groups into independent monophyletic lineages has not been disputed.

The currently accepted monophyly of the *mexicana* and *triangulum* groups is well supported by morphological characteristics and general ecology. Taxa of the *mexicana* group have a widened temporal region rendering the head very distinct from the neck (Gelbach and Baker, 1962; Garstka, 1982). The head is "very slightly distinct to distinct" in the *triangulum* group (Williams, 1988). A clinal variation in ventral scales is present in both groups (Garstka, 1982). There is a general increase in the number of ventrals in the *triangulum* group from north to south (Williams, 1988), while the reverse is true for the *mexicana* group, as ventral counts are highest in the north and lowest in the south (Garstka, 1982). In areas of sympatry, these counts rarely overlap (Garstka, 1982). The color and patterning of the head and body between these two groups differ as well (Smith, 1942; Garstka, 1982), and only in the *mexicana* group are alternating markings between the bands evident (Garstka, 1982). Collectively, these morphological characters adequately diagnose these two groups, though within-group relationships remain problematic.

Ecologically, the *mexicana* and *triangulum* groups also differ. Members of the *mexicana* group have a rather limited distribution confined to mountainous regions of central México and the southwestern United States The *triangulum* group, however, is wide spread, ranging from southern Canada to South America, and occupies incredibly diverse habitats (Williams, 1988). Members of the *triangulum* group are also found sympatrically throughout most of the range

of the *mexicana* group (Garstka, 1982), but no hybrids have been reported, suggesting reproductive isolation. In captivity, taxa from the *mexicana* group are reported to breed freely with each other but not with any of the *triangulum* group (Garstka, 1982), furthering this hypothesis.

The divergence of the *mexicana* and *triangulum* groups is perhaps best illustrated in the forms of these groups found in western Texas and adjacent northern México. *Lampropeltis alterna* is found sympatrically with *L. t. celaenops* and *L. t. annulata* throughout this area. Morphologically, *L. alterna* have 211-230 ventral scales, 56-67 subcaudal scales, 9-32 body rings, variable head color (primarily grey), and a significantly triangular-shaped head (Garstka, 1982). In contrast, *L. t. celaenops* have 170-194 ventrals, 40-53 subcaudals, 17-25 body rings, and a primarily black head that is slightly distinct from the neck (Williams, 1988). Additionally, *L. t. annulata* have 181-207 ventrals, 39-56 subcaudals, 14-20 body rings, and a black head that is slightly distinct from the neck (Williams, 1988).

There is a general agreement that the *triangulum* and *mexicana* groups (or their common ancestor) originated in central or southern México (Tanner, 1953; Williams, 1988). Tanner (1953) proposed a dispersal route for these two groups, suggesting a radiation of the *triangulum* group from the Mexican Plateau along an east-west axis to the coastal areas and then north and south. He further hypothesized that the *mexicana* group radiated eastward from the *pyromelana* group.

The purpose of the present study was multifaceted. First, mtDNA was utilized to assess the phylogenetic relationships of the *L. mexicana* complex to other members of the *Lampropeltis* genus. The genealogical species concept was then used to explain the phylogeography of the two main clades resolved containing taxa from both the *mexicana* and *triangulum* groups. Shortcomings of molecular approaches are discussed, and the conflict between previous phylogenetic relationships of the *mexicana* and *triangulum* groups based on morphology and the mtDNA topologies derived in this study are analyzed.

MATERIALS AND METHODS

Taxon Sampling and Laboratory Methods

Samples of the genus *Lampropeltis*, with emphasis on those representing the *mexicana* complex, were obtained from institutional and private collections. Care was taken to restrict all samples to vouchered specimens of precise locality (Fig. 1). Additional sequence data from several species representing ingroup and outgroup taxa were retrieved from GenBank and used in the analyses (Table 1). Two taxa, *Pituophis catenifer* and *Elaphe gutatta*, were used as outgroups based on previously hypothesized close relationships with the genus *Lampropeltis* (Keogh, 1996; Rodríguez-Robles and Jesús-Escobar, 1999). All unique samples utilized in this study are listed in Table 2.

Total genomic DNA was extracted from shed skin, ventral scale clippings, or tissue samples using standard SDS-proteinase K digestion and a modified protocol of the Puregene® DNA Isolation Kit (Gentra Systems, Minneapolis, MN). This modified protocol can be found in the Appendix. An 868 bp region of the mitochondrial DNA was amplified in this study, encompassing a 697 bp section of the NADH-dehydrogenase subunit 4 (ND4) gene and a 169 bp section of the adjacent $tRNA^{His}$, $tRNA^{Ser}$, and $tRNA^{Leu}$ genes. Primers used for amplification can be found in Table 3. The template DNA was amplified in 100 µl reactions using 0.06 M Tris, 0.015 M $(NH_4)_2SO_4$, 0.0015 M $MgCl_2$, 0.78 M dimethyl sulfoxide, 0.025 mM each dNTP, 1 mM each primer, and 2.5 U *Taq* polymerase in a Geneamp® PCR System 9700 thermal cycler. Amplification conditions consisted of 35 cycles of denaturing at 95°C for 30 s, primer annealing at 50°C

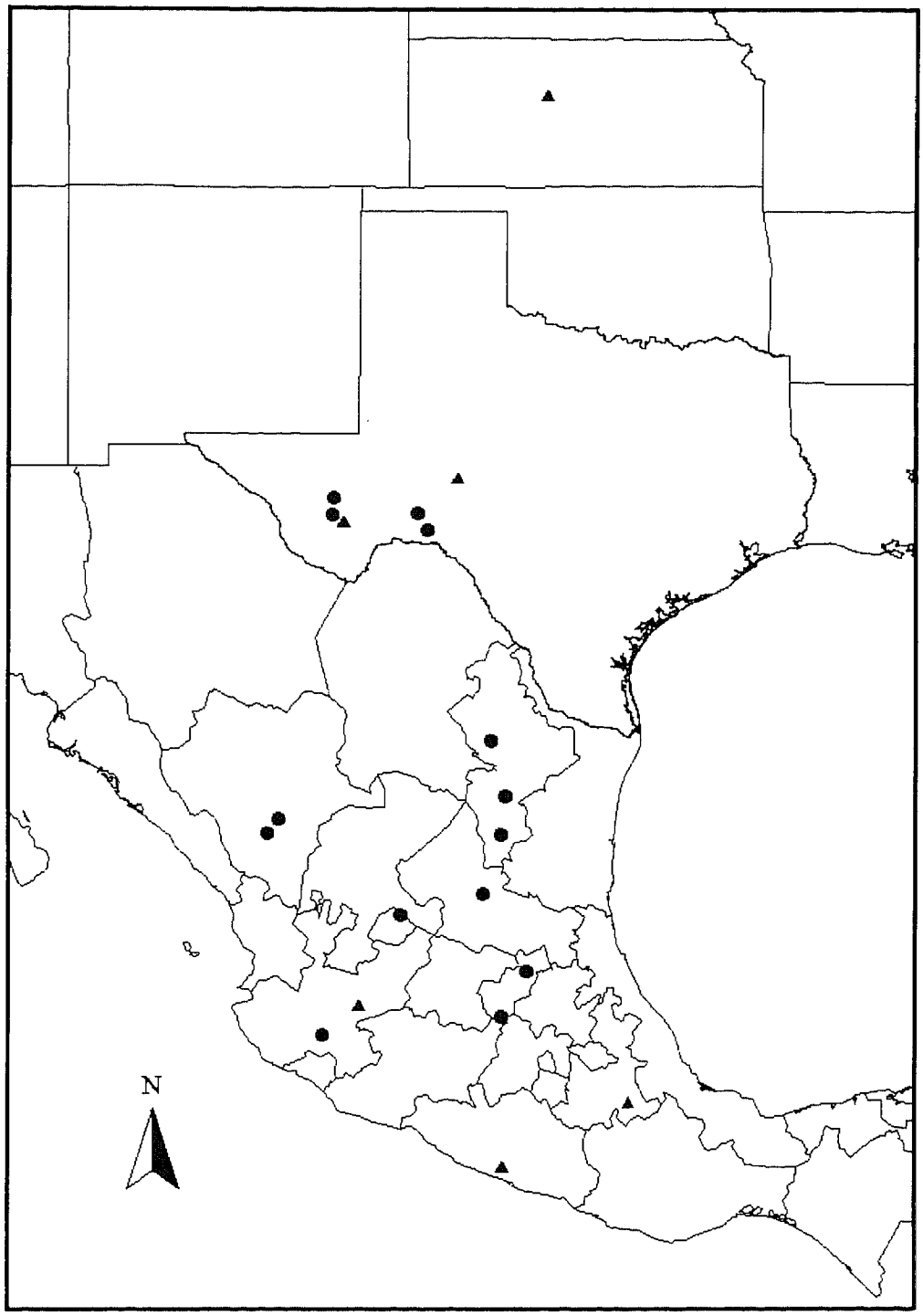

Figure 1. Map of the United States and México depicting the localities of individual specimens of the *Lampropeltis mexicana* (circles) and *L. triangulum* (triangles) groups used in this study.

Table 1. Previously published GenBank sequences used in this study. With the exception of *Lampropeltis zonata*, subspecific status of the ingroup taxa was inferred from the locality data.

Taxon	Genbank Accession No.	Locality	Source
Pituophis catenifer	AF138763	US: California, San Diego Co.	Rodríguez-Robles and Jesús-Escobar, 1999
Elaphe guttata	AF138756	US: Georgia, Fort Benning	Rodríguez-Robles and Jesús-Escobar, 1999
Stilosoma extenuatum	AF138776	US: Florida, Hillsborough Co.	Rodríguez-Robles and Jesús-Escobar, 1999
Lampropeltis getula californiae	AF138759	US: California, San Benito Co.	Rodríguez-Robles and Jesús-Escobar, 1999
L. pyromelana pyromelana	AF138761	US: Arizona, Cochise Co.	Rodríguez-Robles and Jesús-Escobar, 1999
L. zonata multicincta 8	AF136195	US: California, Mariposa Co.	Rodríguez-Robles et al., 1999
L. z. multicinta 9	AF136196	US: California, Plumas Co,	Rodríguez-Robles et al., 1999
L. z. multifasciata 14	AF136200	US: California, Monterey Co.	Rodríguez-Robles et al., 1999
L. z. parvirubra 21	AF136207	US: California, Riverside Co.	Rodríguez-Robles et al., 1999
L. z. pulchra 24	AF136210	US: California, San Diego Co.	Rodríguez-Robles et al., 1999

Table 2. Unique specimens of the genus *Lampropeltis* included in this study. The specimen sample number, voucher number, and locality for each specimen is listed. Museum and private collection abbreviations are as follows: SRSU, Sul Ross State University; UAA, Universidad Autónoma de Aguascalientes; UANL, Universidad Autónoma de Nuevo León; FWZ, Forth Worth Zoo; GRQ, George Raymond Queen; GTS, Gerard T. Salmon; SD, Stan Draper; SH, Stephen Hammack; SRB, Scott Ballard; TG, Timothy Gebhard.

Taxon	Sample No.	Voucher No.	Locality
L. getula splendida	50LM	SRSU R-6543	US: Texas, Crane Co., in Crane
L. calligaster calligaster	52LM	SRSU R-6565	US: Missouri, Jefferson Co.
L. pyromelana knoblochi	46LM	GRG 00701	México: Chihuahua, near Mojarachic
L. sp.	07LM	UANL 5684	México: Sinaloa, Hwy 40 near El Palmito
L. triangulum celaenops	18LM	SRSU R-6519	US: Texas, Jeff Davis Co., Musquiz Canyon
L. t. celaenops	47LM	SRSU R-6540	US: Texas, Crockett Co., near Barnhart
L. t. gentilis	53LM	SRSU R-6564	US: Kansas, Russell Co.
L. t. arcifera	55LM	SD LtLCF3	México: Jalisco, north shore Laguna de Chapala
L. t. campbelli	56LM	FWZ 817102	México: Puebla, Zapotitlán Basin
L. t. conanti	57LM	SRB-078	México: Guerrero, Laguna de Coyuca, NW Acapulco
L. alterna	12LM	UANL 5018	México: Nuevo León, Cerro de la Silla
L. alterna	15LM	SRSU R-6516	US: Texas, Jeff Davis Co., Hwy 17

Table 2. continued.

Taxon	Sample No.	Voucher No.	Locality
L. alterna	16LM	SRSU 6517	US: Texas, Jeff Davis Co., Musquiz Canyon
L. alterna	17LM	SRSU 6518	US: Texas, Val Verde Co., west of Langtry
L. alterna	44LM	SRSU 6521	US: Texas, Crockett Co., Howard Draw
L. mexicana thayeri	08LM	UANL 5773	México: Nuevo León, near Iturbide
L. m. thayeri	45LM	TG LMT2321	México: Nuevo León, north of Dr. Arroyo
L. m. mexicana	09LM	UANL 5603	México: San Luis Potosí, Valle de los Fantasmas
L. m. mexicana	51LM	UAA uncat.	México: Aguascalientes, near Asientos
L. m. greeri	43LM	UANL 5940	México: Durango, Hwy 40 west of Cd. Durango
L. m. greeri	41LM	GRG 00711	México: Durango, Rancho Santa Barbara
L. ruthveni	42LM	*	México: Jalisco, near Tapalpa
L. ruthveni	48LM	GTS R-12	México: Querétaro, near Amealco
L. ruthveni	58LM	SH89lts01	México: Querétaro, near Jalpan

* shed skin found in the wild

Table 3. Primers used for DNA amplification and sequencing. The position of each primer is given relative to the published sequence of *Dinodon semicarinatus* mitochondrial DNA (Kumazawa et al., 1998).

Primer	Position	Primer Sequence	Reference
ND4	11671-11702	5'-CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC-3'	Arévalo et al., 1994
Leu	12569-12594	5'-CAT TAC TTT TAC TTG GAT TTG CAC CA-3'	Arévalo et al., 1994
CornF3	12256-12278	5'-CTA CAY ATA TTY CTA TCA ACA CA-3'	Current study
CornR2	12101-12123	5'-GTT AAT TAG TAR TCA YCA GGT TG-3'	Current study
LND4#2	11863-11888	5'-CTA CAA CAA ACA GAC CTA AAA TCC CT-3'	Current study

for 60 s, and an extension at 72°C for 60 s, followed by a final extension at 72°C for 5 min. PCR products were verified in agarose minigels and then prepared for sequencing using QIAquick PCR Purification Kit (QIAGEN, Valencia, CA) and Concert™ Rapid PCR Purification System (Life Technologies, Carlsbad, CA). The cleaned products were electrophoresed alongside pGEM-3Zf(+) sequencing standard (Applied Biosystems, Norwalk, CT) in an agarose minigel to estimate final template concentration. The sequencing reactions were performed with the original primers using BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Norwalk, CT). Cycling parameters were 25 cycles of 96°C for 30 s, 50°C for 60 s, and 60°C for 4 min. The completed sequencing reactions were cleaned of excess dyes by Sephadex G-50 in CENTRI-SEP Columns (Princeton Separations, Inc., Adelphia, NJ). The reactions were electrophoresed and analyzed on an ABI Prism 377 DNA Sequencer (Applied Biosystems, Norwalk, CT).

Phylogenetic Analyses

The sequences generated were entered into PAUP* 4.0b8a (Swofford, 1999). Sequences from the light and heavy strands were aligned by eye to each other and to published GenBank sequences of related taxa, and edited for correct peak calls by the ABI software. Structural confirmation in the alignments of each of the tRNA genes was checked closely.

The aligned sequences were analyzed using three methods: maximum parsimony (MP; Swofford et al., 1996), neighbor-joining (NJ; Saitou and Nei, 1987), and maximum likelihood (ML; Felsenstein, 1981). Each method attempts

to infer the phylogeny of the taxa in the data set by searching for trees with the best score (MP, ML) or by defining a specific sequence of steps resulting in a tree (NJ). Assumptions about the data set are made by each method and as such resultant topologies may differ. Additionally, depending on the amount of taxa being analyzed, three different search algorithms can be employed which may or may not discover the exact optimal trees. For data sets of less than 20 taxa, exhaustive (11 or fewer taxa) and branch-and-bound searching methods can be used to generate all optimal trees. Larger data sets (typically 20 or more taxa) require a heuristic approach to be used. This algorithm seeks to recover optimal trees by approximate methods, sacrificing the guarantee of finding the global optimum in favor of reduced computational time. The number of taxa utilized in this study (34) necessitated the use of heuristic searches in MP and ML analyses.

MP analyses were conducted using heuristic searches with starting trees obtained via stepwise addition with 2,500 random addition sequences, accelerated character transformation (ACCTRAN), and tree-bisection-reconnection (TBR) branch swapping. All characters were treated as unordered. To judge the levels of saturation, uncorrected *p* distances (pairwise sequence divergence) were plotted against total uncorrected *p* distances for transitions (Ti) and transversions (Tv) at each codon position in the coding region of ND4. MP analyses were then performed using unweighted and weighted schemes to assess the effects of saturation.

ML analyses were performed using heuristic searches. Data were input into Modeltest 3.06 (Posada and Crandall, 1998) to determine the best model of sequence evolution. Starting trees were obtained from the most-parsimonious tree derived in MP analyses. The initial model parameters estimated by Modeltest were input into PAUP* and successive interations run. When a tree of higher likelihood was found, the parameters were reoptimized and the search run again. This was repeated until the same tree was found in successive interations to ensure that the result was a global and not a local optimum (Swofford et al., 1996). Stability of the internal branches in ML analyses were determined by quartet puzzling (QP) analyses (Strimmer and von Haeseler, 1996) of 2,500 steps.

Corrections for nucleotide sequence data suggested by Kimura (1980) were used for distance measures in NJ analyses. Gaps were considered as missing data in MP and NJ analyses. For MP and NJ methods, nonparametric bootstrap (BP) analyses (Felsenstein, 1985) of 2,500 psuedoreplicates and 10 random addition sequence replicates were performed to examine the relative support of each relationship in the resultant topologies. Values of 70% or greater are believed to represent a strongly supported clade (Hillis and Bull, 1993). Tree length, consistency index, retention index, and the number of transitions and transversions were obtained from MacClade (Maddison and Maddison, 1992) using the most parsimonious tree topologies.

RESULTS

Sequence Variation

The nucleotide sequences for ND4 and the adjacent tRNAs span a total of 868 bp. Of these, 217 were phylogenetically informative with a transition:transversion ratio of 8:1. Scatter plots of uncorrected sequence divergences (not shown) revealed slight sequence saturation at third position transitions. Therefore, third position transition changes were downweighted by a factor of five for MP analyses. Uncorrected sequence divergences are presented in Table 4, and ranged between 0 and 11.0% in the ingroup taxa, between 10.4% and 13.6% between *Lampropeltis* and *Pituophis catenifer*, and between 11.2% and 13.9% between *Lampropeltis* and *Elaphe guttata*.

Phylogenetic Relationships

The unweighted MP analyses utilizing *Pituophis catenifer* and *Elaphe guttata* as outgroups resulted in 10 trees each 705 steps in length with a consistency index (CI) of 0.4752 and a retention index (RI) of 0.7049 (Fig. 2). The weighted MP analyses resulted in four trees (tree length = 707, CI = 0.4738, RI = 0.7033) (Fig. 3). The results of the ML analyses (Fig. 4) yielded a final log-likelihood score of - ln L = 4439.87199 obtained by inputting into PAUP* the model HKY+I+G suggested by Modeltest. The ML phylogram presented in Fig. 5 maintains branch lengths proportional to the number of changes. The distance matrices constructed using Kimura 2-parameter corrections (Table 4) were

Table 4. Kimura two-parameter distance (above the diagonal) and uncorrected *p* distance (under the diagonal) matrices derived from the 868-bp sequence data set of the mitochondrial ND4 and tRNA genes.

	L.m.t. (8LM)	*L.m.t.* (45LM)	*L.a.* (12LM)	*L.a.* (17LM)	*L.a.* (44LM)	*L.t.c.* (18LM)	*L.t.c.* (47LM)	*L.t.g.* (53LM)
L. m. thayeri (8LM)	-	0.002	0.011	0.011	0.011	0.015	0.015	0.015
L. m. thayeri (45LM)	0.002	-	0.013	0.013	0.013	0.018	0.018	0.018
L. alterna (12LM)	0.010	0.013	-	0.014	0.014	0.016	0.016	0.019
L. alterna (17LM)	0.010	0.013	0.014	-	0	0.005	0.005	0.014
L. alterna (44LM)	0.010	0.013	0.014	0	-	0.005	0.005	0.014
L. t. celaenops (18LM)	0.015	0.017	0.016	0.005	0.005	-	0	0.019
L. t. celaenops (47LM)	0.015	0.017	0.016	0.005	0.005	0	-	0.019
L. t. gentilis (53LM)	0.015	0.017	0.019	0.014	0.014	0.019	0.019	-
L. alterna (15LM)	0.021	0.023	0.024	0.015	0.015	0.017	0.017	0.019
L. alterna (16LM)	0.021	0.023	0.024	0.015	0.015	0.017	0.017	0.016
L. g. californiae	0.067	0.069	0.067	0.066	0.066	0.068	0.068	0.067
L. g. splendida (50LM)	0.062	0.065	0.061	0.059	0.059	0.061	0.061	0.061
Stilosoma extenuatum	0.067	0.069	0.066	0.066	0.066	0.068	0.068	0.068
L. m. mexicana (51LM)	0.074	0.074	0.070	0.072	0.072	0.077	0.077	0.078
L. m. mexicana (9LM)	0.074	0.074	0.069	0.072	0.072	0.076	0.076	0.078
L. m. greeri (41LM)	0.087	0.089	0.088	0.088	0.088	0.090	0.090	0.090
L. m. greeri (43LM)	0.087	0.089	0.088	0.088	0.088	0.090	0.090	0.090
L. ruthveni (42LM)	0.088	0.090	0.089	0.087	0.087	0.089	0.089	0.092
L. ruthveni (48LM)	0.086	0.088	0.087	0.085	0.085	0.087	0.087	0.089
L. ruthveni (58LM)	0.086	0.088	0.087	0.085	0.085	0.087	0.087	0.089
L. t. arcifera (55LM)	0.083	0.086	0.083	0.082	0.082	0.085	0.085	0.087
L. t. campbelli (56LM)	0.088	0.090	0.086	0.084	0.084	0.089	0.089	0.087
L. t. conanti (57LM)	0.090	0.093	0.088	0.089	0.089	0.094	0.094	0.098
L. c. calligaster (52LM)	0.092	0.094	0.089	0.089	0.089	0.089	0.089	0.095
L. z. multicincta 8	0.099	0.102	0.099	0.094	0.094	0.097	0.097	0.101
L. z. multicincta 9	0.102	0.102	0.102	0.097	0.097	0.099	0.099	0.103
L. z. multifasciata 14	0.101	0.101	0.101	0.096	0.096	0.099	0.099	0.101
L. z. parvirubra 21	0.098	0.098	0.098	0.091	0.091	0.093	0.093	0.096
L. z. pulchra 24	0.097	0.097	0.097	0.089	0.089	0.092	0.092	0.096
L. sp. (7LM)	0.086	0.088	0.081	0.080	0.080	0.082	0.082	0.085
L. p. pyromelana	0.086	0.088	0.081	0.081	0.081	0.081	0.081	0.082
L. p. knoblochi (46LM)	0.082	0.085	0.078	0.078	0.078	0.078	0.078	0.082
Pituophis catenifer	0.111	0.113	0.104	0.108	0.108	0.108	0.108	0.110
Elaphe guttata	0.118	0.120	0.114	0.112	0.112	0.114	0.114	0.116

Table 4. Continued.

	L.a. (15LM)	*L.a.* (16LM)	*L.g.c.*	*L.g.s.* (50LM)	*S.e.*	*L.m.m.* (51LM)	*L.m.m.* (9LM)	*L.m.g.* (41LM)
L. m. thayeri (8LM)	0.021	0.021	0.071	0.066	0.072	0.079	0.079	0.094
L. m. thayeri (45LM)	0.024	0.024	0.074	0.069	0.074	0.079	0.079	0.097
L. alterna (12LM)	0.025	0.025	0.071	0.065	0.070	0.074	0.074	0.095
L. alterna (17LM)	0.015	0.015	0.070	0.062	0.070	0.077	0.076	0.095
L. alterna (44LM)	0.015	0.015	0.070	0.062	0.070	0.077	0.076	0.095
L. t. celaenops (18LM)	0.018	0.018	0.073	0.065	0.073	0.082	0.082	0.098
L. t. celaenops (47LM)	0.018	0.018	0.073	0.065	0.073	0.082	0.082	0.098
L. t. gentilis (53LM)	0.019	0.016	0.071	0.065	0.073	0.083	0.083	0.098
L. alterna (15LM)	-	0.002	0.073	0.062	0.073	0.087	0.087	0.092
L. alterna (16LM)	0.002	-	0.070	0.063	0.076	0.090	0.090	0.092
L. g. californiae	0.068	0.066	-	0.048	0.076	0.091	0.094	0.105
L. g. splendida (50LM)	0.059	0.060	0.046	-	0.072	0.098	0.100	0.105
Stilosoma extenuatum	0.068	0.071	0.071	0.067	-	0.102	0.102	0.114
L. m. mexicana (51LM)	0.081	0.084	0.085	0.090	0.094	-	0.005	0.068
L. m. mexicana (9LM)	0.081	0.083	0.087	0.093	0.094	0.005	-	0.071
L. m. greeri (41LM)	0.086	0.086	0.096	0.096	0.103	0.064	0.067	-
L. m. greeri (43LM)	0.086	0.086	0.096	0.096	0.103	0.064	0.067	0
L. ruthveni (42LM)	0.091	0.091	0.096	0.101	0.104	0.059	0.060	0.038
L. ruthveni (48LM)	0.087	0.087	0.094	0.098	0.102	0.056	0.057	0.037
L. ruthveni (58LM)	0.087	0.087	0.094	0.098	0.102	0.056	0.057	0.037
L. t. arcifera (55LM)	0.083	0.086	0.103	0.100	0.109	0.056	0.060	0.064
L. t. campbelli (56LM)	0.086	0.088	0.091	0.100	0.107	0.058	0.058	0.064
L. t. conanti (57LM)	0.096	0.098	0.099	0.102	0.110	0.070	0.070	0.070
L. c. calligaster (52LM)	0.094	0.094	0.090	0.094	0.099	0.099	0.101	0.105
L. z. multicincta 8	0.093	0.093	0.106	0.102	0.104	0.088	0.091	0.104
L. z. multicincta 9	0.095	0.095	0.110	0.108	0.107	0.088	0.091	0.103
L. z. multifasciata 14	0.095	0.095	0.101	0.104	0.104	0.077	0.082	0.092
L. z. parvirubra 21	0.089	0.089	0.103	0.103	0.103	0.082	0.082	0.088
L. z. pulchra 24	0.088	0.088	0.107	0.102	0.102	0.083	0.083	0.089
L. sp. (7LM)	0.084	0.084	0.093	0.096	0.094	0.073	0.075	0.089
L. p. pyromelana	0.083	0.083	0.096	0.094	0.099	0.084	0.083	0.095
L. p. knoblochi (46LM)	0.082	0.082	0.093	0.094	0.093	0.084	0.083	0.096
Pituophis catenifer	0.108	0.110	0.120	0.119	0.122	0.116	0.116	0.136
Elaphe guttata	0.114	0.116	0.119	0.122	0.123	0.120	0.117	0.138

Table 4. Continued.

	L.m.g. (43LM)	*L.r.* (42LM)	*L.r.* (48LM)	*L.r.* (58LM)	*L.t.a.* (55LM)	*L.t.c.* (56LM)	*L.t.c.* (57LM)	*L.c.c.* (52LM)
L. m. thayeri (8LM)	0.094	0.095	0.092	0.092	0.090	0.095	0.098	0.099
L. m. thayeri (45LM)	0.097	0.098	0.095	0.095	0.093	0.098	0.101	0.102
L. alterna (12LM)	0.095	0.097	0.094	0.094	0.090	0.093	0.095	0.096
L. alterna (17LM)	0.095	0.094	0.091	0.091	0.089	0.091	0.096	0.096
L. alterna (44LM)	0.095	0.094	0.091	0.091	0.089	0.091	0.096	0.096
L. t. celaenops (18LM)	0.098	0.097	0.094	0.094	0.091	0.097	0.102	0.096
L. t. celaenops (47LM)	0.098	0.097	0.094	0.094	0.091	0.097	0.102	0.096
L. t. gentilis (53LM)	0.098	0.099	0.097	0.097	0.094	0.094	0.107	0.103
L. alterna (15LM)	0.092	0.099	0.094	0.094	0.090	0.093	0.104	0.102
L. alterna (16LM)	0.092	0.099	0.094	0.094	0.093	0.095	0.107	0.102
L. g. californiae	0.105	0.105	0.102	0.102	0.113	0.099	0.109	0.098
L. g. splendida (50LM)	0.105	0.111	0.108	0.108	0.109	0.109	0.111	0.102
Stilosoma extenuatum	0.114	0.115	0.112	0.112	0.121	0.118	0.122	0.108
L. m. mexicana (51LM)	0.068	0.062	0.058	0.058	0.059	0.061	0.074	0.107
L. m. mexicana (9LM)	0.071	0.064	0.060	0.060	0.064	0.061	0.074	0.110
L. m. greeri (41LM)	0	0.040	0.038	0.038	0.068	0.068	0.074	0.116
L. m. greeri (43LM)	-	0.040	0.038	0.038	0.068	0.068	0.074	0.116
L. ruthveni (42LM)	0.038	-	0.008	0.008	0.061	0.069	0.082	0.122
L. ruthveni (48LM)	0.037	0.008	-	0	0.060	0.065	0.077	0.116
L. ruthveni (58LM)	0.037	0.008	0	-	0.060	0.065	0.077	0.116
L. t. arcifera (55LM)	0.064	0.058	0.057	0.057	-	0.040	0.073	0.117
L. t. campbelli (56LM)	0.064	0.065	0.061	0.061	0.038	-	0.073	0.113
L. t. conanti (57LM)	0.070	0.076	0.072	0.072	0.068	0.068	-	0.121
L. c. calligaster (52LM)	0.105	0.110	0.105	0.105	0.107	0.103	0.110	-
L. z. multicincta 8	0.104	0.100	0.097	0.097	0.098	0.102	0.107	0.103
L. z. multicincta 9	0.103	0.100	0.097	0.097	0.096	0.097	0.110	0.109
L. z. multifasciata 14	0.092	0.094	0.091	0.091	0.087	0.094	0.097	0.102
L. z. parvirubra 21	0.088	0.092	0.090	0.090	0.088	0.084	0.098	0.103
L. z. pulchra 24	0.089	0.091	0.088	0.088	0.089	0.086	0.101	0.097
L. sp. (7LM)	0.089	0.086	0.082	0.082	0.083	0.083	0.092	0.096
L. p. pyromelana	0.095	0.089	0.087	0.087	0.089	0.089	0.093	0.094
L. p. knoblochi (46LM)	0.096	0.090	0.089	0.089	0.086	0.090	0.095	0.100
Pituophis catenifer	0.136	0.130	0.123	0.123	0.132	0.124	0.129	0.125
Elaphe guttata	0.138	0.139	0.139	0.139	0.129	0.128	0.124	0.129

Table 4. Continued.

	L.z.m. 8	*L.z.m.* 9	*L.z.m.* 14	*L.z.p.* 21	*L.z.p.* 24	*L.*sp. (7LM)	*L.p.p.*	*L.p.k.* (46LM)
L. m. thayeri (8LM)	0.109	0.112	0.111	0.107	0.106	0.093	0.092	0.089
L. m. thayeri (45LM)	0.112	0.112	0.111	0.107	0.106	0.095	0.095	0.091
L. alterna (12LM)	0.109	0.112	0.111	0.107	0.106	0.087	0.087	0.083
L. alterna (17LM)	0.103	0.106	0.105	0.098	0.097	0.086	0.087	0.083
L. alterna (44LM)	0.103	0.106	0.105	0.098	0.097	0.086	0.087	0.083
L. t. celaenops (18LM)	0.106	0.109	0.108	0.101	0.100	0.089	0.087	0.083
L. t. celaenops (47LM)	0.106	0.109	0.108	0.101	0.100	0.089	0.087	0.083
L. t. gentilis (53LM)	0.111	0.114	0.111	0.105	0.104	0.091	0.089	0.089
L. alterna (15LM)	0.101	0.104	0.103	0.096	0.095	0.091	0.090	0.089
L. alterna (16LM)	0.101	0.104	0.103	0.096	0.095	0.091	0.090	0.089
L. g. californiae	0.117	0.122	0.111	0.114	0.118	0.101	0.105	0.101
L. g. splendida (50LM)	0.112	0.120	0.114	0.114	0.112	0.105	0.102	0.103
Stilosoma extenuatum	0.114	0.119	0.115	0.114	0.112	0.103	0.108	0.102
L. m. mexicana (51LM)	0.095	0.095	0.082	0.088	0.089	0.078	0.090	0.090
L. m. mexicana (9LM)	0.098	0.098	0.088	0.088	0.089	0.080	0.090	0.090
L. m. greeri (41LM)	0.114	0.114	0.100	0.095	0.097	0.097	0.104	0.106
L. m. greeri (43LM)	0.114	0.114	0.100	0.095	0.097	0.097	0.104	0.106
L. ruthveni (42LM)	0.110	0.110	0.102	0.100	0.099	0.093	0.097	0.099
L. ruthveni (48LM)	0.106	0.106	0.099	0.097	0.095	0.089	0.094	0.097
L. ruthveni (58LM)	0.106	0.106	0.099	0.097	0.095	0.089	0.094	0.097
L. t. arcifera (55LM)	0.108	0.105	0.094	0.095	0.097	0.090	0.097	0.093
L. t. campbelli (56LM)	0.113	0.107	0.102	0.091	0.092	0.090	0.097	0.099
L. t. conanti (57LM)	0.118	0.122	0.106	0.107	0.110	0.100	0.101	0.104
L. c. calligaster (52LM)	0.113	0.121	0.112	0.113	0.105	0.105	0.102	0.109
L. z. multicincta 8	-	0.012	0.047	0.052	0.050	0.094	0.084	0.095
L. z. multicincta 9	0.011	-	0.044	0.048	0.046	0.090	0.082	0.090
L. z. multifasciata 14	0.045	0.042	-	0.033	0.033	0.084	0.078	0.083
L. z. parvirubra 21	0.050	0.046	0.032	-	0.010	0.085	0.084	0.085
L. z. pulchra 24	0.048	0.045	0.032	0.010	-	0.081	0.085	0.087
L. sp. (7LM)	0.087	0.083	0.078	0.079	0.076	-	0.052	0.056
L. p. pyromelana	0.078	0.077	0.073	0.078	0.079	0.050	-	0.038
L. p. knoblochi (46LM)	0.087	0.083	0.077	0.080	0.081	0.053	0.037	-
Pituophis catenifer	0.124	0.120	0.117	0.120	0.116	0.111	0.112	0.117
Elaphe guttata	0.130	0.125	0.134	0.124	0.127	0.115	0.116	0.116

Table 4. Continued.

	P.c.	*E.g.*
L. m. thayeri (8LM)	0.123	0.131
L. m. thayeri (45LM)	0.125	0.134
L. alterna (12LM)	0.114	0.125
L. alterna (17LM)	0.118	0.124
L. alterna (44LM)	0.118	0.124
L. t. celaenops (18LM)	0.118	0.125
L. t. celaenops (47LM)	0.118	0.125
L. t. gentilis (53LM)	0.122	0.128
L. alterna (15LM)	0.118	0.125
L. alterna (16LM)	0.121	0.128
L. g. californiae	0.134	0.132
L. g. splendida (50LM)	0.133	0.135
Stilosoma extenuatum	0.136	0.137
L. m. mexicana (51LM)	0.128	0.132
L. m. mexicana (9LM)	0.128	0.129
L. m. greeri (41LM)	0.154	0.156
L. m. greeri (43LM)	0.154	0.156
L. ruthveni (42LM)	0.146	0.158
L. ruthveni (48LM)	0.137	0.158
L. ruthveni (58LM)	0.137	0.158
L. t. arcifera (55LM)	0.149	0.144
L. t. campbelli (56LM)	0.139	0.143
L. t. conanti (57LM)	0.144	0.138
L. c. calligaster (52LM)	0.141	0.144
L. z. multicincta 8	0.139	0.147
L. z. multicincta 9	0.134	0.140
L. z. multifasciata 14	0.130	0.151
L. z. parvirubra 21	0.134	0.138
L. z. pulchra 24	0.129	0.142
L. sp. (7LM)	0.123	0.127
L. p. pyromelana	0.124	0.128
L. p. knoblochi (46LM)	0.130	0.128
Pituophis catenifer	-	0.120
Elaphe guttata	0.109	-

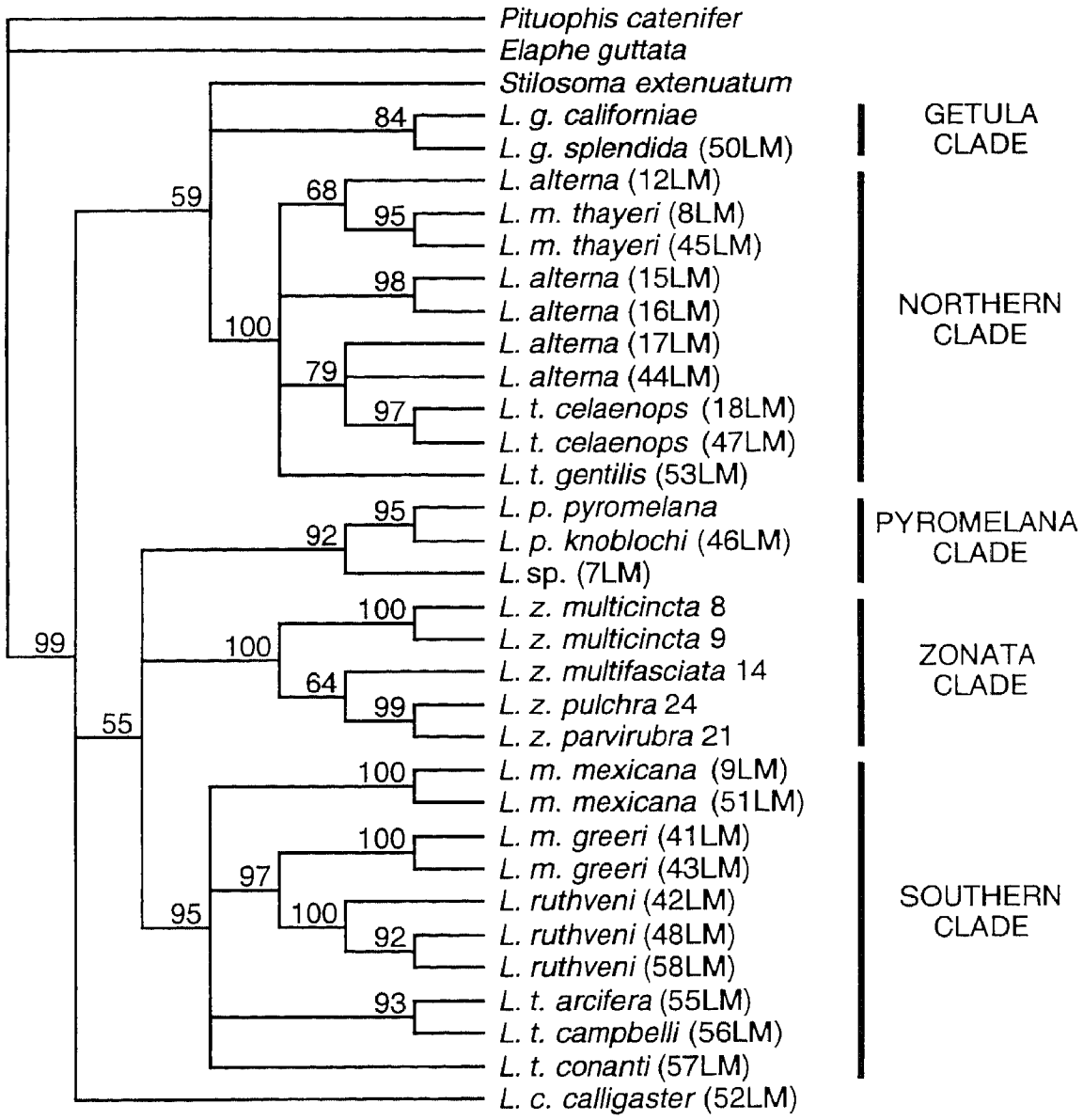

Figure 2. Unweighted maximum parsimony tree based on the sequences of part of the mitochondrial ND4 gene and the adjacent $tRNA^{His}$, $tRNA^{Ser}$, and partial $tRNA^{Leu}$. Numbers at nodes represent percentage of bootstrap replicates supporting that node, with nodes retained by less than 50% not shown.

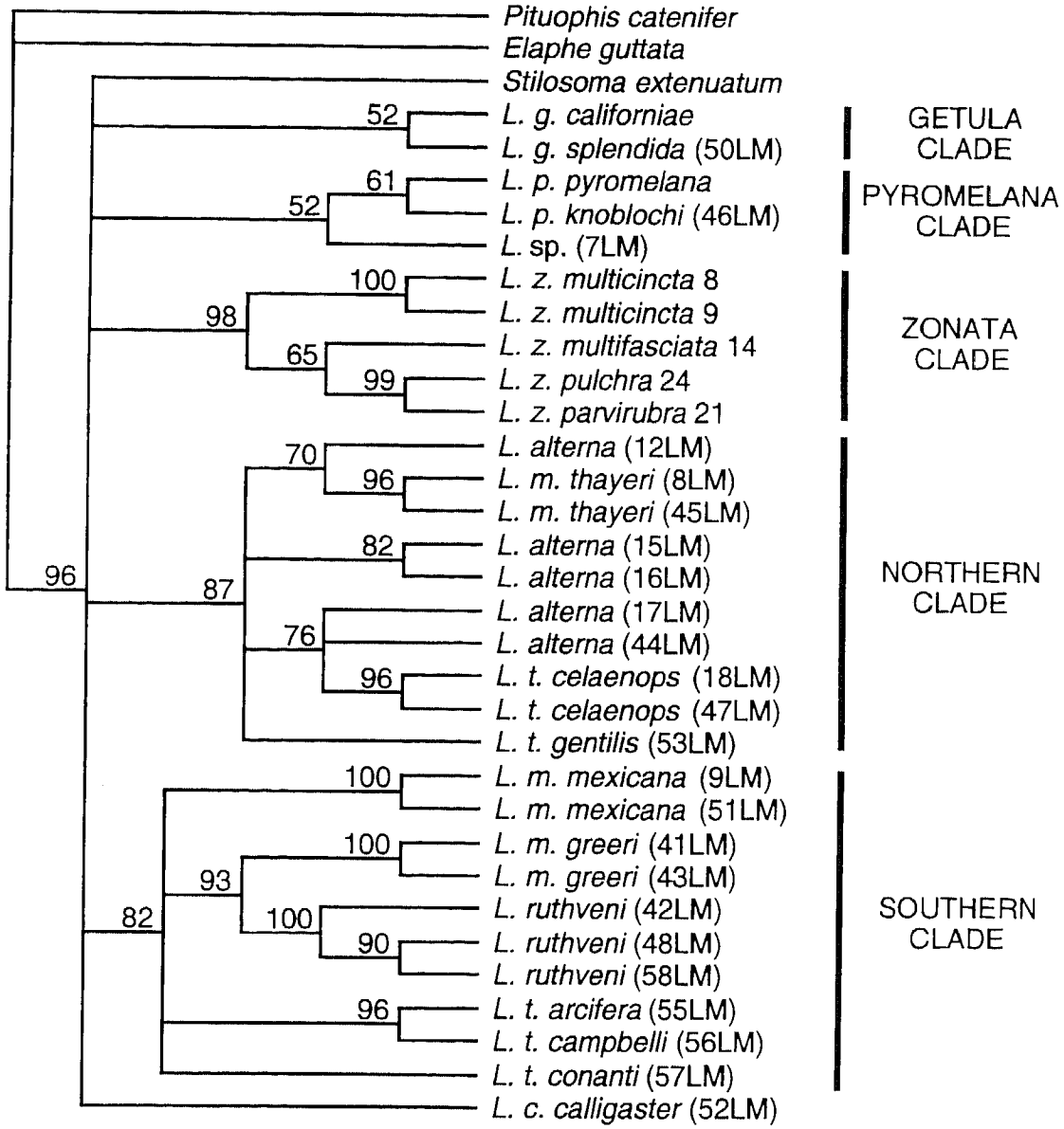

Figure 3. Weighted maximum parsimony tree based on the sequences of part of the mitochondrial ND4 gene and the adjacent $tRNA^{His}$, $tRNA^{Ser}$, and partial $tRNA^{Leu}$. Third codon transition changes downweighted by a factor of five. Numbers at nodes represent percentage of bootstrap replicates supporting that node, with nodes retained by less than 50% not shown.

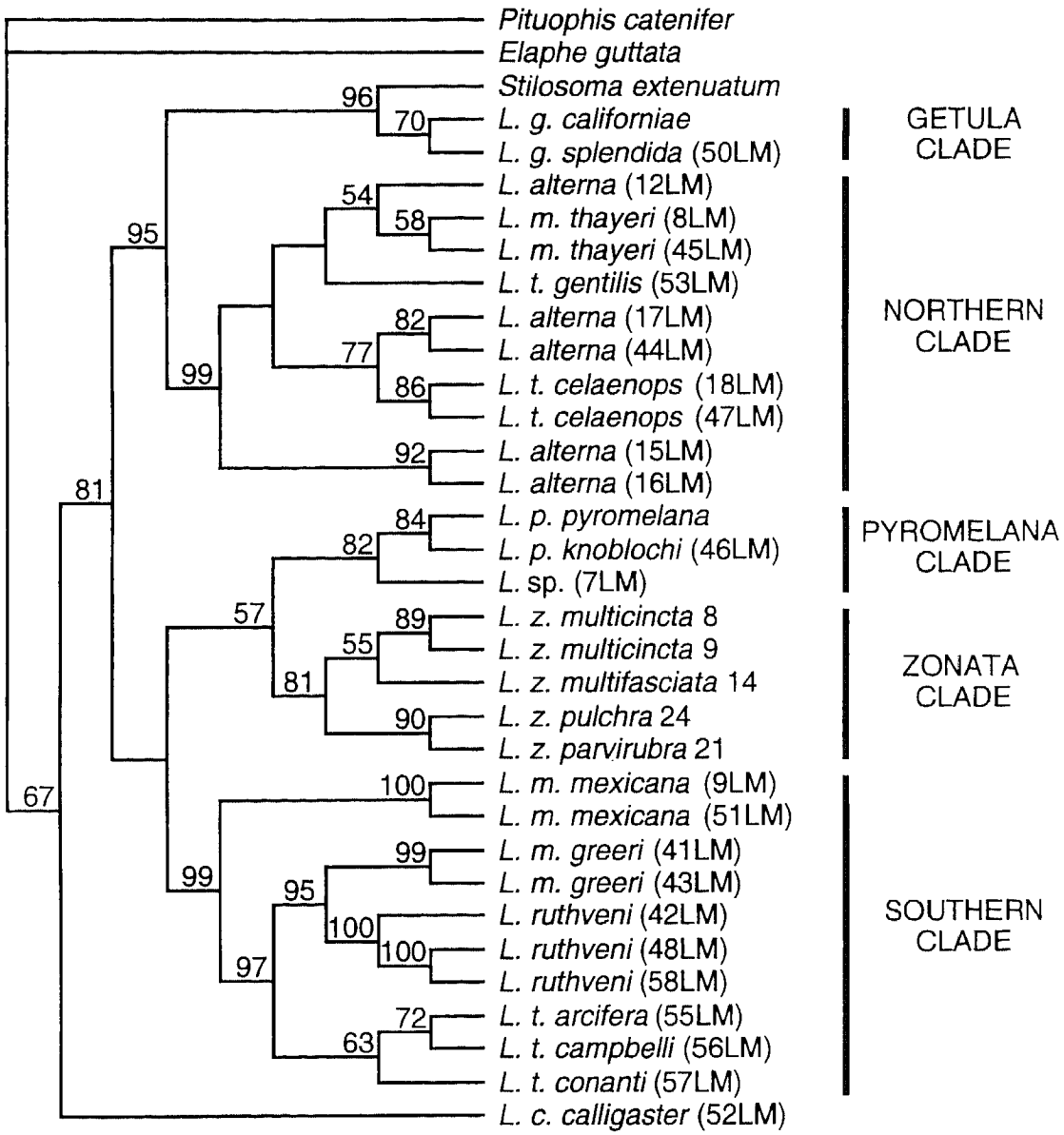

Figure 4. Maximum likelihood quartet puzzling tree based on the sequences of part of the mitochondrial ND4 gene and the adjacent $tRNA^{His}$, $tRNA^{Ser}$, and partial $tRNA^{Leu}$. Numbers at nodes represent as a percentage how often the corresponding cluster was found among the 2,500 intermediate trees. Nodes retained by less than 50% not shown.

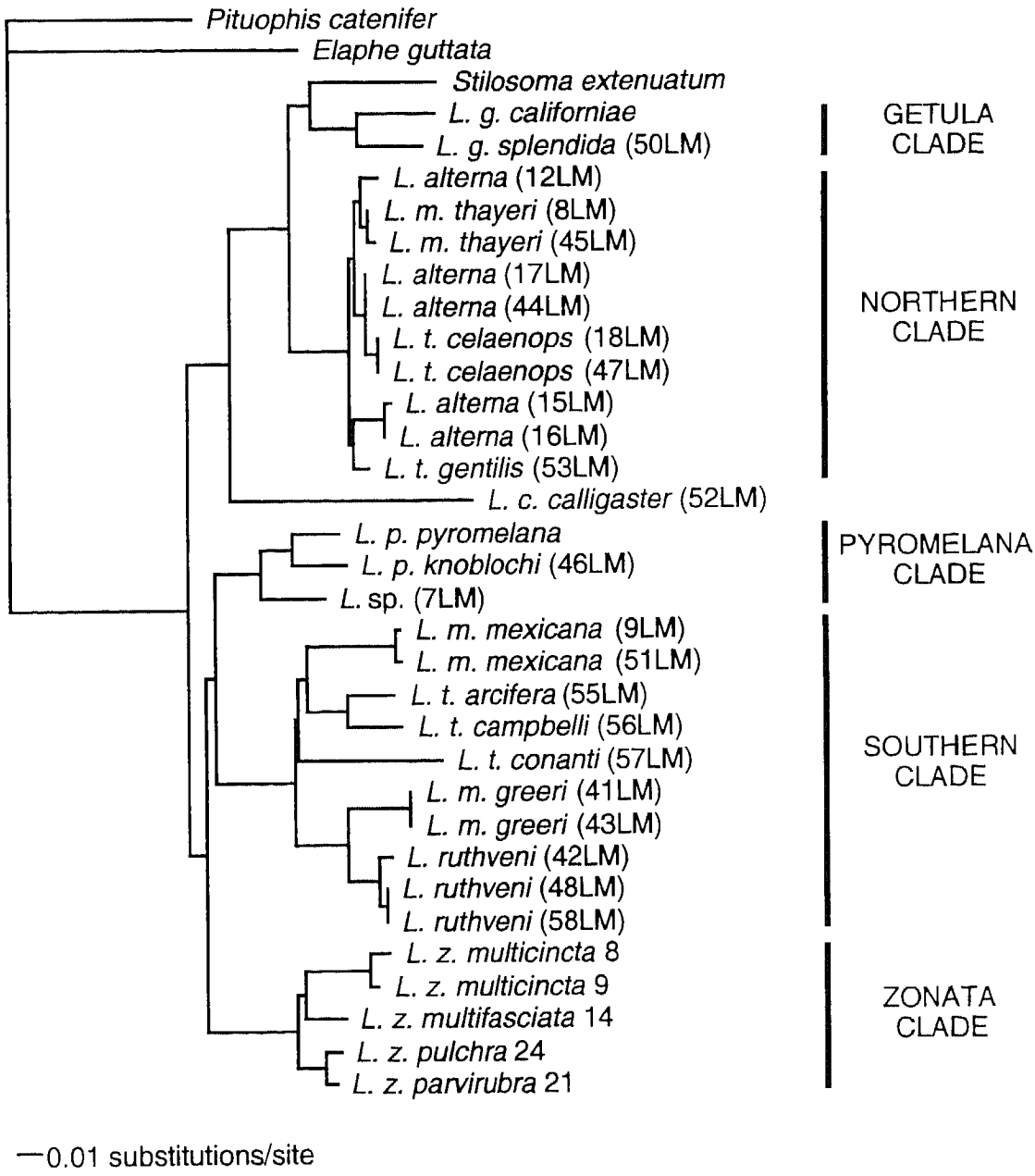

Figure 5. Maximum likelihood phylogram based on the sequences of part of the mitochondrial ND4 gene and the adjacent $tRNA^{His}$, $tRNA^{Ser}$, and partial $tRNA^{Leu}$. Branch lengths drawn in proportion to the amount of changes.

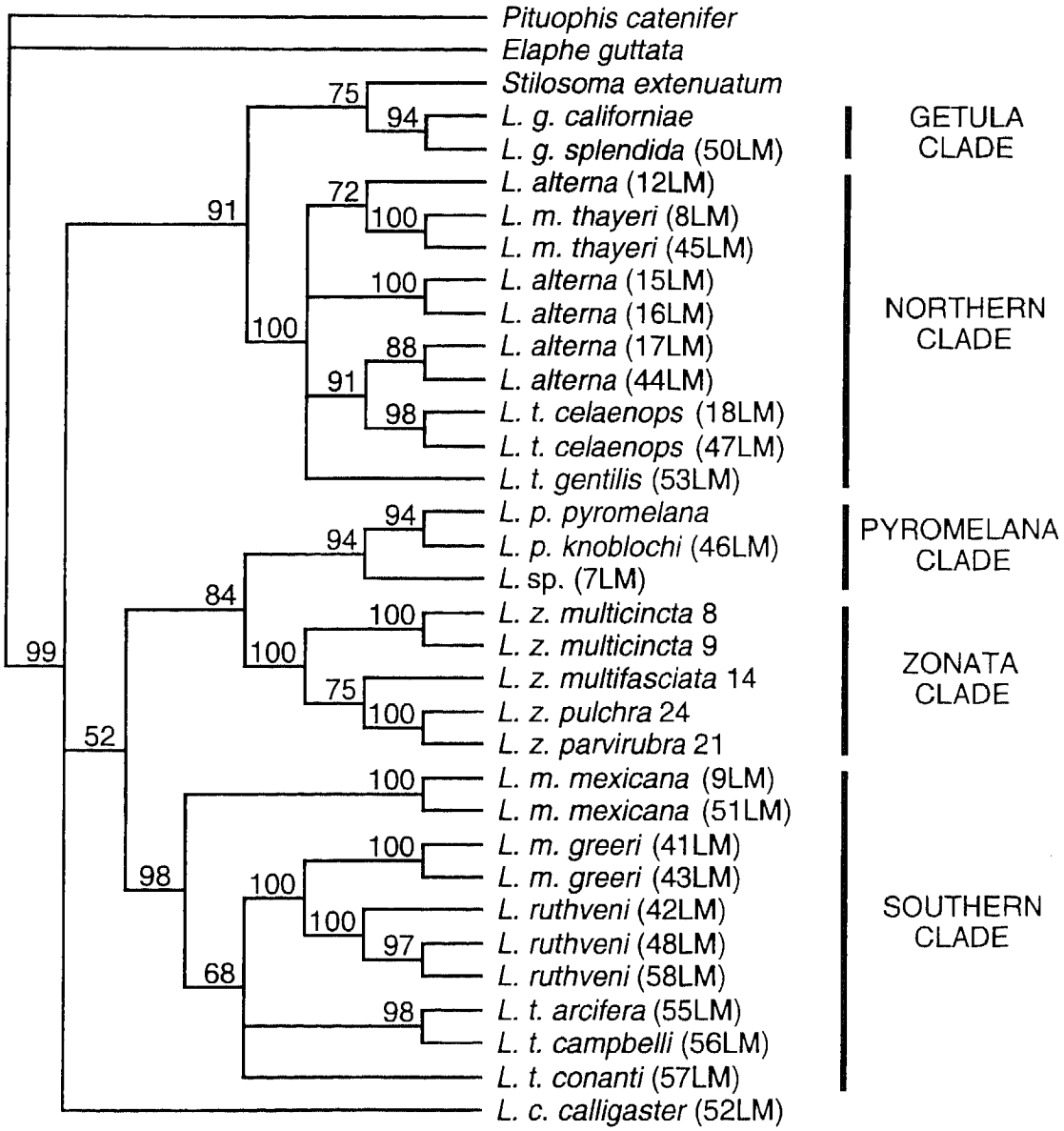

Figure 6. Neighbor-joining tree using Kimura 2-parameter distance correction based on the sequences of part of the mitochondrial ND4 gene and the adjacent $tRNA^{His}$, $tRNA^{Ser}$, and partial $tRNA^{Leu}$. Numbers at nodes represent percentage of bootstrap replicates supporting that node, with nodes retained by less than 50% not shown.

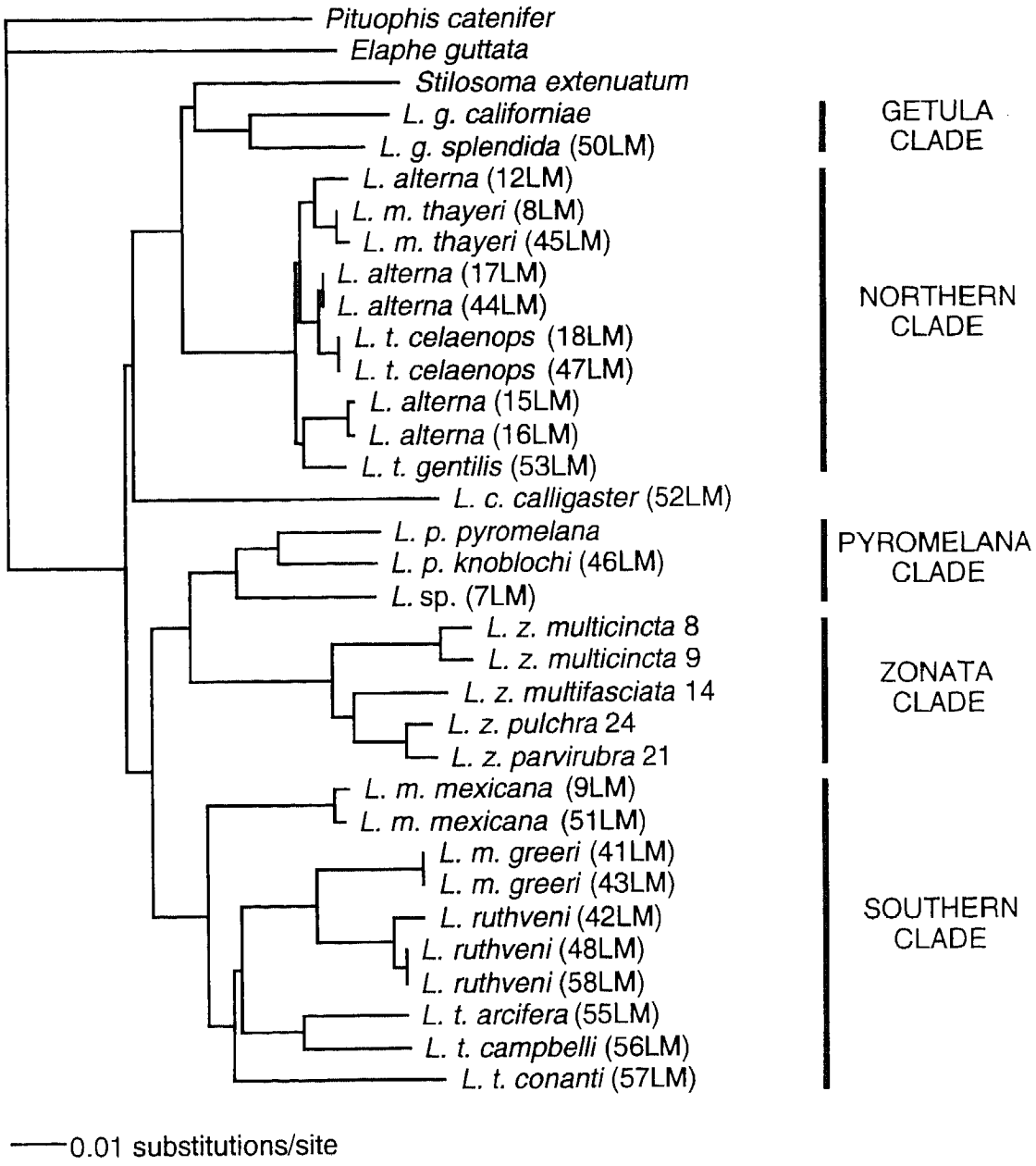

Figure 7. Neighbor-joining phylogram using Kimura 2-parameter distance correction based on the sequences of part of the mitochondrial ND4 gene and the adjacent $tRNA^{His}$, $tRNA^{Ser}$, and partial $tRNA^{Leu}$. Branch lengths drawn in proportion to the amount of changes.

analyzed by NJ and presented in Fig. 6. The NJ phylogram with proportional branch lengths is shown in Fig. 7.

All methods (MP unweighted and weighted, NJ, and ML) recovered the same major nodes and were well supported by bootstrap (MP and NJ) and quartet puzzling (ML) values. The *mexicana* and *triangulum* groups were polyphyletic, with taxa from each group falling out into a "northern" and "southern" clade. Five other nodes were consistently recovered: *Lampropeltis getula* ("*getula*" clade), *L. pyromelana* and *L.* sp. ("*pyromelana*" clade), *L. zonata* ("*zonata*" clade), *L. c. calligaster*, and *Stilosoma extenuatum*. The relationships of these seven nodes to each other, however, remain largely unresolved. ML and NJ analyses strongly support (>90%) the position of the getula clade and *Stilosoma extenuatum* as the sister group to the northern clade. Relationships among the remaining nodes are more problematic. ML and NJ analyses place the *zonata* and *pyromelana* clades as sister groups (ML, 57%; NJ, 84%), and weakly suggest they are the sister group to the southern clade (ML, <50%; NJ, 52%). MP analyses align these three groups into an unresolved trichotomy (55%). However, the ML phylogram positions the southern clade as the sister group to the *pyromelana* clade, though the connecting branch length is short. The position of *L. c. calligaster* remains unresolved. While supported MP, ML, and NJ trees place this taxon separate from all other *Lampropeltis* groups, both ML and NJ phylograms place this taxon as the sister group to the getula, *Stilosoma extenuatum*, and northern clades. The branch leading to *L. c. calligaster* is relatively long suggesting numerous substitutions, so it is possible

that long branch attraction (LBA; Felsenstein, 1978; Huelsenbeck, 1997) is responsible for its topological variation.

DISCUSSION

Polyphyly in L. mexicana *and* L. triangulum

The topologies derived from the mtDNA sequences analyzed in this study suggest that both the *mexicana* and *triangulum* groups are polyphyletic, in obvious conflict with previously hypothesized phylogenies (Smith 1942, 1944; Webb, 1962; Garstka, 1982). Though not all currently recognized subspecies of the *triangulum* group were sampled in the current study, it is clear that this group is composed of at least two divergent mtDNA lineages, as evidenced by the northern and southern clades resolved in all analyses. These two clades contain taxa from both the *mexicana* and *triangulum* groups; *L. alterna*, *L. m. thayeri*, *L. t. celaenops*, and *L. t. gentilis* in the northern clade, and *L. m. greeri*, *L. ruthveni*, *L. t. arcifera*, *L. t. campbelli*, and *L. t. conanti* in the southern clade. Additionally, it appears that the *getula* clade and *Stilosoma extenuatum* are the sister groups to the northern clade. Although weakly supported, the *pyromelana* and *zonata* clades seem to form the sister groups to the southern clade. These relationships further demonstrate the divergence of the *mexicana* and *triangulum* groups into genetically distinct polyphyletic lineages.

Rates of Divergence and the Fossil Record

Between the northern and southern clades resolved by the mtDNA data set presented in this study, the percent sequence divergence found by utilizing the formula proposed by Avise et al. (1992) to correct for within-lineage variation ($p_{corr}=p_{xy} - 0.5(p_x+p_y)$ where p_{xy} is the mean pairwise genetic distance between

individuals in populations x and y, and p_x and p_y are nucleotide diversities within regions or populations) is 5.2%. If a molecular clock is imposed on these values with the mtDNA clock rate estimated by Zamudio and Greene (1997) for small to medium-sized ectotherms (0.47 to 1.32% per million years), the northern and southern clades diverged approximately 11.0-3.9 million years ago (MYA). This places the time of divergence in the late Miocene to middle Pliocene. Rodríguez-Robles et al. (1999) estimated a similar time of divergence between *L. zonata* and *L. pyromelana* using the same molecular clock (middle to late Miocene). The mean sequence divergence among taxa within the northern clade is 1.41%, suggesting a recent expansion dating back 3.0-1.0 MYA (late Pliocene to early Pleistocene), in agreement with the fossil record for *L. triangulum*. Mean sequence divergence within the southern clade is 5.27% (11.2-3.9 MYA), suggesting a much earlier divergence in the late Miocene to middle Pliocene.

Several Pliocene records of *L. triangulum* exist from Kansas and Oklahoma (Brattstrom, 1967), though most records are from the Pleistocene (Guilday, 1962; Auffenberg, 1963; Holman, 1963; Holman, 1964; Brattsrom, 1965; Holman 1966; Brattstrom, 1967; Holman, 1967; Holman, 1969; Meylan, 1982). A single record of *L. triangulum* from the late Miocene of Nebraska (Parmley and Holman, 1995) may be referable to *L. similis*, an extinct species of *Lampropeltis* with a similar vertebral morphology to *L. triangulum* (Parmley, 1994) (see below). Van Devender (pers. comm., cited in Miller, 1979) recorded fossil records for *L altema* in western Texas from the late Pleistocene.

Species Concepts and Biogeographical Implications

Since the advent of the Linnaean classification system, the formal definition of a species has proven to be problematic and the source of a long-standing debate. Any set of statements about relationships must be logically consistent with the recovered history of evolution. The long entrenched Biological Species Concept (BSC) has been challenged in the past few decades by the proponents of the Evolutionary Species Concept (ESC), the Phylogenetic Species Concept (PSC), and other species concepts. Since the BSC emphasizes reproductive compatibility rather than evolutionary descent, it is argued that as such it is essentially flawed and obstructs the recovery of the history of evolution (Hennig, 1966; Rosen, 1978; Wiley, 1978; Cracraft, 1983; Donoghue, 1985; Frost and Hillis, 1990). The ESC, as proposed by Simpson (1961) and later modified by Wiley (1978), defines a species as a lineage from a common ancestral descent separate from other such lineages with its own evolutionary future. This view is problematic since predictions must be made as to how lineages will evolve in the future; thus, though in part based on the evolutionary past, the ESC is dependent on the future (Cracraft, 1983; Donoghue, 1985; Frost and Hillis, 1990). Cracraft (1983) defined the PSC as "the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent." Frost and Hillis (1990) criticized this view, arguing that the criteria used to detect the smallest detectable lineages (one or more synapomorphies) may instead recover ephemeral units or local demes.

All species concepts, as stated by Frost and Hillis (1990), suffer from "operational difficulties." Comparable to different phylogenetic methodologies used to obtain optimal trees, assumptions must be made about the data set. The BSC is unable to resolve relationships within a genealogical relationship since it is based on the assumption of reproductive compatibility. The ESC makes the assumption that a population is on an independent phylogenetic trajectory. The genealogical species concept as proposed by Baeum and Shaw (1995) is similar to the PSC and draws on the coalescence theory. Futuyma (1998:452) provided the following summation of this species concept: "At first, gene copies in each population will often be genealogically more closely related to some gene copies in the other population than to some gene copies in their own population. Due to genetic drift and natural selection, however, all copies will eventually be more closely related to each other within their own population to those in the other population—that is, each population will acquire a monophyletic gene tree." Drawing on the biogeographic evolution of the *mexicana* and *triangulum* groups as proposed by Tanner (1953) and this study (see below), this genealogical approach can be used to hypothesize the phylogeny of the northern and southern clades and help explain the polyphyly in these two groups.

The *mexicana* and *triangulum* groups diverged from a common ancestor in central México in the late Miocene to early Pliocene. This period was marked by increased volcanic activity and mountain formation along the Mexican Plateau and the formation of the Sierra Madre Oriental (Morafka, 1977). Both groups radiated out to the east, west, and south. To the north, the xeric habitat of the

Mojavia Desert (Axelrod, 1958; Morafka, 1977) may have acted as a barrier to dispersal, thus effectively funneling the *mexicana* and *triangulum* groups that had dispersed to the east up through the newly formed Sierra Madre Oriental and adjacent mesic foothills and northward. Three alternative hypotheses can explain the eastward radiations of these two groups to the Sierra Madre Oriental. If the origin of dispersal was south or southeast of Durango, as hypothesized by Tanner (1953), then an eastern radiation of the *mexicana* and *triangulum* groups may have been through the Trans-Plateau Corridor and Anticlinorium (Anticline) of Arteaga (Martin, 1958; Morafka, 1977) to the Sierra Madre Oriental. The Anticline of Arteaga is a narrow belt of mountains that subdivides the Mapimian subprovidence of the modern Chihuahuan Desert in the north from the Saladan portion to the south (Morafka, 1977). Alternatively, the *mexicana* group may have passed through this corridor, and the *triangulum* group may have radiated out to the east along the southern edges of the desert and then north upon reaching the Sierra Madre Oriental (as proposed by Tanner, 1952), coming back in contact with the *mexicana* group. Finally, both the *mexicana* and *triangulum* groups may have dispersed to the east along the southern periphery of the Mojavia Desert and then up the Sierra Madre Oriental together. Regardless of the route, both eastward radiations were in contact with each other along the narrow belt of the Sierra Madre Oriental and surrounding foothills. The newly formed modern Chihuahuan Desert (late Pliocene; Morafka, 1977) prevented dispersal to the west and continued to funnel both groups northward.

Consistent with the genealogical approach summarized by Futuyma (1998:452), as these two groups became isolated, "the reticulating pattern of ancestral population gave way to a divergent population of ancestry and decent." Though still closely related genetically to ancestors of the *mexicana* and *triangulum* groups, gene flow between the two groups in this geographic bottleneck increased their relatedness to each other. Smith (1942), Gelbach and Baker (1982),and Gartska (1988) all remarked that hemipenal characteristics were very similar in the *mexicana* and *triangulum* groups. Once entering the United States, the *triangulum* group radiated further out to the north and east, probably in the Pliocene as indicated by the earliest fossil records (Brattstrom, 1967). As with other North American herpetofauna (reviewed in Avise et al., 1998), Pleistocene glaciations probably affected the expansion of this group. Indeed, most of the fossil records for the *triangulum* group date back to Pleistocene, especially for the eastern United States. Glaciation events that disrupted the Gulf Circumferential Corridor between the southeastern United States and northeastern México (Auffenberg and Milstead, 1965) forced many organisms into southern glacial refugia and probably subdivided the *triangulum* group. Future molecular studies including taxa from these areas may show this genetic separation. There may have also been additional gene flow between northern *mexicana* and *triangulum* in their southern glacial refugia. The remaining two species of the *mexicana* group in the northern clade (*L. altema* and *L. m. thayeri*), or their common ancestor, remained along the northern Sierra Madre Oriental. The Chihuahuan Desert was more mesic during the Pleistocene

as indicated by packrat (*Neotoma* spp.) middens (Van Devender, 1990). *Lampropeltis alterna* may have invaded this area during this time and subsequently adapted to the more xeric conditions of the present day Chihuahuan Desert. Garstka (1982) considered *L. alterna* to be the most derived species of the *mexicana* group based on head shape and eye color, but Van Devender et al. (1992) suggested these characters may be an adaptation to subterranean crevice dwelling and nocturnal activity. Van Devender et al. (1992) also suggested possible gene flow between *L. alterna* and *L. m. thayeri* based on color pattern similarities between the groups. As mentioned previously, mean sequence divergence within the northern clade is 1.41%, suggesting a rapid recent radiation. The development of the Cerritos-Arista Filter Barrier (Morafka, 1977), a narrow xeric valley subdividing the Sierra Madre Oriental in northern San Luis Potosí, may have prevented northern *mexicana* and *triangulum* from a re-invasion south, thus isolating these groups from their southern ancestors.

As members from the *mexicana* and *triangulum* groups radiated east to the Sierra Madre Oriental in the late Miocene to early Pliocene, these groups also moved south and southeast along the Mexican Plateau. Morafka (1977:175), in reference to the Mexican Plateau, stated, "The immense size of the plateau, its continued stability, both tectonic and climactic, since Neogene [Miocene through Middle Pleistocene], and its geographical accessibility, all combine to make the region an excellent stage for speciation, radiation, and the survival of relict stocks." As such, taxa from the *mexicana* and *triangulum* groups both remained on the Mexican Plateau and continued to disperse. The Cordillera

Volcánica of México formed during the Pliocene and may have acted as a barrier to gene flow between populations that had previously radiated out to the south. The Cerritos-Arista Filter Barrier in the Sierra Madre Oriental prevented the northern expansion of these groups up through the Sierra Madre Oriental and into subsequent contact with taxa of the northern clade. Duellman (1965) suggested that the geologic changes prior to the Pleistocene were not significant in affecting animal dispersal routes in southwestern México. However, high rates of sequence divergence among taxa in the southern clade suggest pre-Pleistocene isolation.

Failures of Molecular Hypotheses: Gene Trees vs. Species Trees

Vast numbers of systematic studies in the past decade have utilized molecular markers (specifically mtDNA) to infer the evolutionary history and phylogenetic relationships of a wide array of organisms. Indeed, mtDNA has properties that make it an eminently desirable choice to investigate inter- and intraspecific relationships, especially at the lower taxonomic level. Mitochondrial DNA evolves 5-10 times faster than nuclear DNA (Brown et al., 1979) and thus has a relatively rapid rate of sequence evolution. Universal primers that amplify a wide range of gene regions within mtDNA are also readily available and results are easily interpreted (Avise et al., 1987). Additionally, mtDNA is maternally inherited and nonrecombinant. While this may reduce the number of taxa needed to assess populational variability, male-mediated gene flow is not represented. Since all 37 different genes of the animal mtDNA are then only inherited as a

single linkage group, resultant topologies (gene trees) provide only one estimate of an organism's evolutionary history (species tree).

It has been suggested that topologies derived from DNA sequences may not be congruent with actual species trees due to lineage sorting (Tajima, 1983; Takahata and Nei, 1985; Neigel and Avise, 1986; Nei, 1987; Pamilo and Nei, 1988; Avise, 1989; Avise and Ball, 1990; Wu, 1991; Hudson, 1992; Moore, 1995). Hence, a monophyletic lineage is the result of several descendent species acquiring a single lineage through genetic drift and the retention of ancestral polymorphisms (reflective of the coalescent theory). As indicated by Neigel and Avise (1986) and Pamilo and Nei (1988), this process is accelerated by smaller effective population sizes (N_e). The problem of lineage sorting is compounded when periods of internodes (periods of shared ancestry) are short. Multiple substitutions may have destroyed earlier synapomorphies, making inferences about relationships impossible (Lanyon, 1988). One alternative approach to this conflict is to include both nDNA gene trees and mtDNA gene trees when estimating phylogenetic relationships, thus increasing the probability of gene tree and species tree congruence (Pamilo and Nei, 1988; Wu, 1991). Moore (1995), however, suggested that since the effective population size of a mtDNA gene is less than that of a nDNA gene, mtDNA gene trees have a better chance of tracking the species tree. Even with the inclusion of nDNA and mtDNA gene trees, if the internodes are short, whether relatively modern or ancient, lineage sorting can obscure the relationship between gene trees and species trees (Moore, 1995).

Conclusions

The data derived from the mtDNA sequences in the present study suggest that the *mexicana* and *triangulum* groups are polyphyletic, with taxa sequenced from both groups comprising a northern and southern clade. Under the genealogical species concept, the northern clade would be considered a species as it consists of a monophyletic lineage with little sequence divergence. However, examination of the probable biogeography of the northern clade suggests a reticulate evolution during which lineage sorting may have occurred due to a geographic bottleneck. The general morphology and ecology of *mexicana* and *triangulum* indicate these two groups are independent monophyletic lineages. The two sympatric taxa *L. altema* and *L. t. celaenops*, which are significantly distinct morphologically, demonstrate little sequence divergence. Thus, the gene trees presented in the current study may not be congruent with the species tree that represents the true evolutionary history of the *mexicana* and *triangulum* groups.

Future studies might help to resolve this conflict in a number of ways. A large number of morphological characters need to be examined and cladisticly analyzed from large sample sets of both groups. In addition to mtDNA, nuclear genes capable of resolving relationships at the species level need to be employed to provide characters that are independent of the linked mtDNA sequences. However, as pointed out by Moore (1995), mtDNA is more likely to resolve discordance between gene and species trees than nDNA.

REFERENCES

ALFARO, M. E., AND S. J. ARNOLD. 2001. Molecular systematics and evolution of *Regina* and the Thamnophiine snakes. Mol. Phylogenet. Evol. 21:408-423.

ARÉVALO, E., DAVIS, S. K., AND SITES, J. W., JR. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. Syst. Biol. 43:387-418.

AUFFENBERG, W. L. 1963. The fossil snakes of Florida. Tulane Stud. Zool. 10:131-216.

AUFFENBERG, W. L., AND W. W. MILSTEAD. 1965. Reptiles in the Quaternary of North America. Pages 557-568 *in* The Quaternary of the United States (H. E. Wright, Jr. and D. G. Frey, eds.). Princeton Univ. Press, Princeton, New Jersey.

AVISE, J. C. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. Evolution 43:1192-1208.

AVISE, J. C., AND R. M. BALL. 1990. Mitochondrial DNA and avian microevolution. Acta XX Cong. Int. Orinithol. 1:514-524.

AVISE, J. C., J. ARNOLD, R. M. BALL, E. BERMINGHAM, T. LAMB, J. E. NEIGEL, C. A. REEB, AND N. C. SAUNDERS. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Ann. Rev. Ecol. Syst. 18:489-522.

AVISE, J. C., B. B. BOWEN, T. LAMB, A. B. MEYLAN, AND E. BERMINGHAM. 1992. Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic

variability and reduced microevolutionary rate in Testudines. Mol. Biol. Evol. 9:457-473.

AVISE, J. C., D. WALKER, AND G. C. JOHNS. 1998. Speciation durations and Pleistocene effects on vertebrate phylogeography. Proc. R. Soc. London B 265:1707-1712.

AXELROD, D. I. 1958. Evolution of the Madro-Tertiary geoflora. Bot. Rev. 24:433-509.

BAKER, R. H., X. YU, AND R. DESALLE. Assessing the relative contribution of molecular and morphological characters in simultaneous analysis trees. Mol. Phylogenet. Evol. 9:427-436.

BAUEM, D. A., AND K. L. SHAW. 1995. Genealogical perspectives on the species problem. Pages 289-303 *in* Experimental and molecular approaches to plant biosystematics (P.C. Hoch and A. G. Stephenson, eds.). Monographs in Systematic Botany. Missouri Botanical Garden, St. Loius, Missouri.

BRATTSTROM, B. H. 1955. Pliocene and Pleistocene amphibians and reptiles from southeastern Arizona. J. Paleontol. 29:150-154.

BRATTSTROM, B. H. 1965. Body temperatures of reptiles. Amer. Midl. Nat. 73:376-422.

BRATTSTROM, B. H. 1967. A succession of Pliocene and Pleistocene snake faunas from the high plains of the United States. Copeia 1967:188-202.

BROWN, W. M., M. GEORGE, JR., AND A. C. WILSON. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76:1967-1971.

CRACRAFT, J. 1983. Species concepts and speciation analysis. Pages 159-187 *in* Current ornithology, Vol. 1 (R. F. Johnston, ed.). Plenum Press, New York.

DONOGHUE, M. L. 1985. A critique of the biological species concept and recommendations of a phylogenetic alternative. Bryologist 88:172-181.

DOUGLAS, M. E., M. R. DOUGLAS, G. W. SCHUETT, L. W. PORRAS, AND A. T. HOLYCROSS. In press. Phylogeography of the western rattlesnake (*Crotalus virdis*) complex, with emphasis on the Colorado Plateau. *In* Biology of the vipers (G. W. Schuett, M. Höggren, M. E. Douglas, and H. W. Greene, eds.). Biological Science Press, Traverse City, Michigan.

DUELLMAN, W. E. 1965. A biogeographic account of the herpetofauna of Michoacán, México. Univ. Kansas Publs. Mus. Nat. Hist. 15:627-709.

FELSENSTEIN, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 27:401-410.

FELSENSTEIN, J. 1981. Evolutionary trees from gene frequencies and quantitative characters: finding maximum likelihood estimates. Evolution 35:1229-1242.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.

FRELIN, C., AND F. VUILLEUMIER. 1979. Biochemical methods and reasoning in systematics. Z. Zool. Syst. Evolut.-forsch. 17:1-10.

FROST, D. R., AND D. M. HILLIS. 1990. Species in concept and practice: herpetological applications. Herpetologica 46:87-104.

FUTUYMA, D. J. 1998. Evolutionary biology. 3rd edition. Sinauer, Sunderland, Massachusetts.

GARMAN, S. 1883. The reptiles and batrachians of North America, Part I, Ophidia. Mem. Mus. Comp. Zool. 8:1-185.

GARTSKA, W. R. 1982. Systematics of the *mexicana* complex species group of the colubrid genus *Lampropeltis*, with an hypothesis mimicry. Breviora 466:1-35.

GELBACH, F. R., AND J. K. BAKER. 1962. Kingsnakes allied with *Lampropeltis mexicana*: taxonomy and natural history. Copeia 1962:291-300.

GIVNISH, T. J., AND K. J. SYTSMA. 1997. Consistency, characters, and the likelihood of correct phylogenetic inference. Mol. Phylogenet. Evol. 7:320-330.

GUILDAY, J. E. 1962. The Pleistocene local fauna of the natural chimneys, Augusta County, Virginia. Ann. Carnegie Mus. 36:87-122.

HEDGES, S. B., AND L. R. MAXON. 1996. Molecules and morphology in amniote phylogeny. Mol. Phylogenet. Evol. 6:312-319.

HENNIG, W. 1966. Phylogenetic Systematics. University of Illinois Press, Urbana.

HILLIS, D. M., AND J. J. BULL. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42:182-192.

HOLMAN, J. A. 1963. Late Pleistocene amphibians and reptiles of the Clear Lake and Ben Franklin local faunas of Texas. J. Grad. Res. Center 31:152-187.

HOLMAN, J. A. 1964. Pleistocene amphibians and reptiles from Texas. Herpetologica 20:73-83.

HOLMAN, J. A. 1966. The Pleistocene herpetofauna of Miller's Cave, Texas. Texas J. Sci. 28:372-377.

HOLMAN, J. A. 1967. A Pleistocene herpetofauna from Ladd's Georgia. Bull. Georgia Acad. Sci. 25:154-166.

HOLMAN, J. A. 1969. The Pleistocene amphibians and reptiles of Texas. Publ. Mus. Michigan State Univ. 4:163-192.

HUDSON, R. R. 1992. Gene trees, species trees, and the segregation of ancestral alleles. Genetics 131:509-512.

HUELSENBECK, J. P. 1997. Is the Felsenstein Zone a fly trap? Syst. Biol. 46:69-74.

KEOGH, J. S. 1996. Evolution of the colubrid snake tribe Lampropeltini: a morphological perspective. Herpetologica 52:406-416.

KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucelotide sequences. J. Mol. Evol. 16:111-120.

KLUGE, A. G. 1983. Cladistics and the classification of the great apes. Pages 151-177 *in* New interpretations of ape and human ancestry (R. L. Ciochon and R. S. Corruccini, eds.). Plenum, New York.

KUMAZAWA, Y., H. OTA, M. NISHIDA, AND T. OZAWA. 1998. The complete nucleotide sequence of a snake (*Dinodon semicarinatus*) mitochondrial genome with two identical control regions. Genetics 150:313-329.

LAMBOY, W. F. 1994. The accuracy of the maximum parsimony method of phylogeny reconstruction with morphological characters. Syst. Bot. 19:489-505.

LANYON, S. M. 1988. The stochastic mode of molecular evolution: what consequences for systematic investigations. Auk 105:565-573.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade. Analysis of Phylogeny and Character Evolution. Version 3. Sinauer, Sunderland, Massachusetts.

MARTIN, P. S. 1958. A biogeography of reptiles and amphibians in the Gomez Farias region, Tamaulipas, Mexico. Misc. Publs. Mus. Zoo. Univ. Michigan 101:1-102.

MEYLAN, P. A. 1982. The squamate reptiles of the Inglis IA fauna (Irvingonian, Citris County, Florida). Bull. Florida State Mus. Biol. Sci. 27:1-85.

MILLER, D. J. 1979. A life history study of the gray-banded kingsnake, *Lampropeltis mexicana alterna* in Texas. Chihuahuan Desert Research Institute, Contrib. No. 87:1-48.

MOORE, W. S. 1995. Inferring phylogenies from mtDNA variation: mitochodrial-gene trees versus nuclear gene trees. Evolution 49:718-726.

MORAFKA, D. J. 1977. A biogeographical analysis of the Chihuahuan Desert through its herpetofauna. Dr. W. Junk B. V., The Hague.

MORITZ, C., AND D. M. HILLIS. 1996. Molecular systematics: context and controversies. Pages 1-13 *in* Molecular systematics, 2nd edition (D. M. Hillis, C. Mortiz, and B. K. Mable, eds.). Sinauer, Sunderland, Massachusetts.

NEI, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

NEIGEL, J. E., AND J. C. AVISE. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. Pages 515-533 *in* Evolutionary processes and theory (S. Karlin and E. Nevo, eds.). Academic Press, Orlando, Florida.

PAINTER, C. W., P. W. HYDER, AND G. SWINFORD. 1992. Three species new to the herpetofauna of New Mexico. Herpetol. Rev. 23: 62

PAMILO, P., AND M. NEI. 1988. Relationships between gene trees and species trees. Mol. Biol. Evol. 5:568-583.

PARMLEY, D. 1987. *Lampropeltis similis* from the Coffee Ranch local fauna (Hemphillian Land Mammal Age) of Texas. Texas J. Sci. 39:123-128.

PARMLEY, D. 1994. Reevaluation of the extinct kingsnake *Lampropeltis intermedius* Brattstrom (Colubridae) with comments on the ancestry of *Lampropeltis triangulum*. Herpetol. Nat. Hist. 2:83-88.

PARMLEY, D., AND J. A. HOLMAN. 1995. Hemphilian age (late Miocene) snakes from Nebraska, with comments on Arikareean through Blancan snakes of midcontinental North America. J. Vert. Paleontol. 15:79-95.

PATTERSON, C., D. M. WILLIAMS, AND C. J. HUMPHRIES. 1993. Congruence between molecular and morphological phylogenies. Annu. Rev. Ecol. Syst. 24:153-188.

POOK, C. E., W. WÜSTER, AND R. S. THORPE. 2000. Historical biogeography of the Western Rattlesnake (Serpentes: Viperidae: *Crotalus virdis*), inferred from

mitochondrial DNA sequence information. Mol. Phylogenet. Evol. 15:269-282.

POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: testing the models of DNA substitution. Bioinformatics 14:817-818.

PUERTO, G., M. G. SALOMÃO, R. D. G. THEAKSTON, R. S. THORPE, D. A. WARRELL, AND W. WÜSTER. 2001. Combining mitochondrial DNA sequences and morphological data to infer species boundaries: phylogeography of lanceheaded pitvipers in the Brazilian Atlantic forest, and the status of *Bothrops pradoi* (Squamata: Serpentes: Viperidae). J. Evol. Biol. 14:527-538.

RODRÍGUEZ-ROBLES, J. A., AND J. M. DE JESÚS-ESCOBAR. 1999. Molecular systematics of New World lampropeltinine snakes (Colubridae): implications for biogeography and evolution of food habits. Biol. J. Linnean Soc. 68:355-385.

RODRÍGUEZ-ROBLES, J. A., AND J. M. DE JESÚS-ESCOBAR. 2000. Molecular systematics of New World gopher, bull, and pinesnakes (*Pituophis*: Colubridae), a transcontinental species complex. Mol. Phylogenet. Evol. 14:35-50.

RODRÍGUEZ-ROBLES, J. A., D. F. DENARDO, AND R. E. STAUB. 1999. Phylogeography of the California mountain kingsnake, *Lampropeltis zonata* (Colubridae). Mol. Ecol. 8:1923-1934.

ROSEN, D. E. 1978. Vicariant patterns and historical explanation in biogeography. Syst. Zool. 27:159-188.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

SIBLEY, C. G., AND J. AHLQUIST. 1987. Avian phylogeny reconstructed from comparisons of the genetic material, DNA. Pages 95-121 *in* Molecules and morphology in evolution: Conflict or compromise? (C. Patterson, ed.). Cambridge University Press, Cambridge.

SIMPSON, G. G. 1961. Principles of animal taxonomy. Columbia University Press, New York.

SITES, J. W., JR., S. K. DAVIS, T. GUERRA, J. B. IVERSON, AND H. L. SNELL. 1996. Character congruence and phlylogenetic signal in molecular and morphological data sets: a case study in living iguanas (Squamata: Iguanidae). Mol. Biol. Evol. 13:1087-1105.

SMITH, H. M. 1942. Remarks on the Mexican king snakes of the *triangulum* group. Rochester Acad. Sci. 8:196-207.

SMITH, H. M. 1944. Snakes of the Hoogstraal Expeditions to northern Mexico. Zool. Ser. Field Mus. Nat. Hist. 29:135-152.

STRIMMER, K., AND A. VON HAESELER. 1996. Quartet puzzling: A quartet maximum likelihood method for reconstructing tree topologies. Mol. Biol. Evol. 13: 964-969.

SWOFFORD, D. L. 1999. PAUP*, Phylogenetic Analysis Using Parsimony (*and other methods), Version 4. Sinauer, Sunderland, Massachusetts.

SWOFFORD, D. L., G. J. OLSEN, P. J. WADDELL, AND D. M. HILLIS. 1996. Phylogenetic inference. Pages 407-514 *in* Molecular systematics, 2nd

edition (D. M. Hillis, C. Moritz, and B. K. Mable, eds.). Sinauer, Sunderland, Massachusetts.

TAJIMA, F. 1983. Evolutionary relationships of DNA sequences in finite populations. Genetics 105:437-460.

TAKAHATA, N., AND M. NEI. 1985. Gene genealogy and variance of interpopulational nucleotide differences. Genetics 110:325-344.

TANNER, W. W. 1953. A study of the taxonomy and phylogeny of *Lampropeltis pyromelana* Cope. Great Basin Naturalist 13:47-66.

VAN DEVENDER, T. R. 1990. Late Quaternary vegetation and climate of the Chihuahuan Desert, United States and Mexico. Pages 104-133 *in* Packrat middens: the last 40,000 years of biotic change (J. L. Betancourt, T. R. Van Devender, and P. S. Martin, eds). The University of Arizona Press, Tuscon.

VAN DEVENDER, T. R., C. H. LOWE, H. K. MCCRYSTAL, AND H. E. LAWLER. 1992. Viewpoint: reconsider suggested systematic arrangements for some North American amphibians and reptiles. Herpetol. Rev. 23:10-14.

WEBB, R. G. 1962. A new kingsnake from Mexico, with remarks on the *mexicana* group of the genus *Lampropeltis*. Copeia 1961:326-333.

WILEY, E. O. 1978. The evolutionary species concept reconsidered. Syst. Zool. 27:17-26.

WILLIAMS, K. L. 1988. Systematics and natural history of the American milk snake, *Lampropeltis triangulum*. 2nd edition. Milwaukee Pub. Mus., Milwaukee.

WU, C. -I. 1991. Inferences of species phylogeny in relation to segregation of ancient polymorphisms. Genetics 127:429-435.

ZAMUDIO, K. R., AND H. W. GREENE. 1997. Phylogeography of the bushmaster (*Lachesis muta*: Viperidae): implications for neotropical biogeography, systematics, and conservation. Biol. J. Linnean Soc. 62:421-442.

ZAMUDIO, K. R., K. B. JONES, AND R. H. WARD. 1997. Molecular systematics of short-horned lizards: biogeography and taxonomy of a widespread species complex. Syst. Biol. 46:284-305.

Modified Protocol for DNA Isolation*

1. Place tissue sample in a 1.5 ml microfuge tube containing 500 µl of 1X STE.
2. Add 25 µl of a 20 mg/ml stock solution of proteinase K in STE to the microfuge tube.
3. Add 25 µl of 20% SDS and vortex thoroughly.
4. Incubate the mixture at 55°C overnight. Mix occasionally during the incubation to keep the tissue suspended.
5. Following the incubation, cool the sample to room temperature.
6. Add 150 µl of Protein Precipitation Solution (Gentra Systems, Inc.) to the microcentrifuge tube and vortex at high speed for 20 s.
7. Incubate the sample on ice for 5 min.
8. Vortex again at high speed for 20 s.
9. Centrifuge the sample at 13,000-16,000 x g for 3 min. A tight protein pellet should be visible.
10. Pour the supernatant containing the DNA (leaving behind the protein pellet) into a clean 1.5 ml microfuge tube containing 500 µl of ice-cold 100% isopropanol. Throw the old tube away.
11. Invert the sample gently approximately 50 times to mix the solution. At this point precipitated DNA typically will be visible, although in some samples where the yield is low, no precipitate will be visible.
12. Centrifuge the sample at 13,000-16,000 x g for 5 min.
13. Pour the supernatant off and drain any excess isopropanol on to clean, absorbent paper.
14. Add 300 µl of 70% ethanol to the tube and invert several times to wash the pellet.
15. Centrifuge the sample for 1 min at 13,000-16,000 x g, and then carefully pour the ethanol off. Caution should be taken when pouring off the ethanol since the pellet may have become dislodged from the microfuge tube during the washing step.
16. Drain any excess ethanol on to clean, absorbent paper and allow the DNA pellet to air dry for 15 min to overnight.
17. Hydrate the DNA in 50 µl of TE buffer overnight. Periodically tap the tube to aid in dispersing the DNA.
18. Store DNA at 4°C for short term storage, or at -20°C or -80°C for long term storage.

* Modified from the Puregene® DNA Isolation Kit (Gentra Systems, Minneapolis, MN)

VITAE

Education:

M. S., Biology, May 2002
Sul Ross State University, Alpine, Texas

B. S., Wildlife Management, May 1998
Sul Ross State University, Alpine, Texas

Professional Experience:

September 2000 - *present* — Assistant Curator/Research Assistant
Sul Ross State University, Alpine, Texas

- Responsible for the accessioning of mammals, birds, and herpetofauna into the SRSU vertebrate collection.
- Maintain the preserved herpetological collection.
- Initiated the development of the herpetological tissue collection.

June 2000 - *present* — Researcher
Sul Ross State University, Alpine, Texas

- Chief scientist on an international joint program for the captive study and natural history of montane Mexican rattlesnakes (genus *Crotalus*) with Universidad Autónoma de Nuevo León in Monterrey, Nuevo León.
- Assisting scientists at Universidad Autónoma de Aguascalientes in Aguascalientes, Aguascalientes, and Bosque Tropical, A. C., Guadalajara, Jalisco on various herpetological projects.
- Conducted field research in Mexican states of Chihuahua, Durango, Coahuila, Nuevo León, Aguascalientes, and México.
- Issued the following Mexican scientific research permits: SEMARNAP D00.02.-2546, D00.02.-6390 (2000-2001); SEMARNAT NÚM/SGPA/DGVS/3394, 4267, 5431(2001-2002)

Spring 2001 — Teaching Assistant
Sul Ross State University, Alpine, Texas

- Taught Herpetology Lab to 21 undergraduate and 3 graduate students.
- Subject matter included taxonomy and identification of reptiles and amphibians of the United States, with emphasis on those occurring in the Chihuahuan Desert.

Publications:

Bryson, R. W., and A. T. Holycross. 2001. *Crotalus willardi amabilis*. Diet. Herpetological Review 32: 262.

Bryson, R. W., and D. Lazcano. 2001. *Thamnophis errans*. Diet. Herpetological Review 32: 265.

Bryson, R. W., and J. M. Mueller. 2001. Geographic distribution. *Crotalus lepidus lepidus*. Herpetological Review 32: 122.

Bryson, R. W., G. T. Salmon, and D. Lazcano. 2001. Geographic distribution. *Lampropeltis mexicana*. Herpetological Review 32: 123.

Quintero-Díaz, G., G. G. Adame, J. Vázquez-Díaz, J. S. Rodríguez, R. W. Bryson, and G. T. Salmon. 2001. Geographic distribution. *Lampropeltis mexicana*. Herpetological Review 32: 278.

Salmon, G. T., R. W. Bryson, and D. Lazcano. 2001. Geographic distribution. *Lampropeltis mexicana*. Herpetological Review 32: 123.

Bryson, R. W., and D. Lazcano. In press. Reproduction and mating behavior in the Del Nido ridgenose rattlesnake, *Crotalus willardi amabilis*. Southwestern Naturalist.

Bryson, R. W., J. Banda, and D. Lazcano. In press. *Crotalus lepidus maculosus*. Diet. Herpetological Review.

Bryson, R. W., J. Banda, and D. Lazcano. In press. *Crotalus pricei pricei*. Habitat selection. Herpetological Review.

Banda-Leal, J., R. W. Bryson, and D. Lazcano. In press. A new record of *Elgaria parva* (Lacertilla: Anguidae) from Nuevo León, México. Southwestern Naturalist.