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A phylogeny of the *Lampropeltis mexicana* complex (Serpentes: Colubridae) based on mitochondrial DNA sequences suggests evidence for species-level polyphyly within *Lampropeltis*

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Abstract

The systematic relationships of snakes in the *Lampropeltis mexicana* complex (*L. mexicana*, *L. alterna*, and *L. ruthveni*) are poorly known despite several taxonomic studies over the last 80 years. Mitochondrial DNA sequences were used to infer the phylogeny of the *L. mexicana* complex. At least one representative sample from the nine currently recognized species of *Lampropeltis* was sequenced. Our results suggest that a deep basal split resulted in the divergence of two groups of *Lampropeltis*, with one group occupying the upland areas of western United States and most of western and central Mexico, and the other northeastern Mexico and the lowland areas of the southern United States. Results also revealed that the *L. mexicana* complex and *Lampropeltis triangulum* are polyphyletic, with taxa from both groups nested together in deeply divergent northern and southern clades. These results are incongruent with previous hypotheses of phylogenetic relationships based on morphology, and suggest that morphological characters shared among the various tri-colored *Lampropeltis* (e.g., hemipenal structure and tri-colored pattern) may be difficult to interpret phylogenetically.

Keywords: *Lampropeltis*; Mitochondrial DNA; ND4; Phylogenetics; Polyphyly

1. Introduction

The widely distributed kingsnakes and milksnakes of the genus *Lampropeltis* of the snake family Colubridae are a highly variable group, whose range extends from southern Canada to northern South America. This genus is easily recognized and well-known among scientists, naturalists, and hobbyists and of great importance to ecologists, behaviorists, conservationists, and physiologists. Moreover, the taxonomy of this group has been relatively stable throughout the last 80 years (Blanchard, 1921). The majority of the taxa in this genus are relatively small (typically less than one meter) with an alternating tri-colored pattern of white, black, and red bands belonging to the species *L. triangulum*, *L. pyromelana*, *L. zonata*, *L. webbi*, and the *L. mexicana* complex (*L. mexicana*, *L. alterna*, and *L. ruthveni*). Notably, these animals represent a classic case of Batesian mimicry, where a selective advantage is conferred upon these tri-colored lampropeltinines by mimicking the venomous coralsnakes (*Micrurus*) (Brodie and Janzen, 1995; Pfennig et al., 2001). These tri-colored *Lampropeltis* share similar vertebral and hemipenal structures that are unlike those of the remaining non-tri-colored species in the genus, *L. getula* and *L. calligaster* (Gartska, 1982; Smith, 1942). Here, we examine the phylogeographic history of the poorly known

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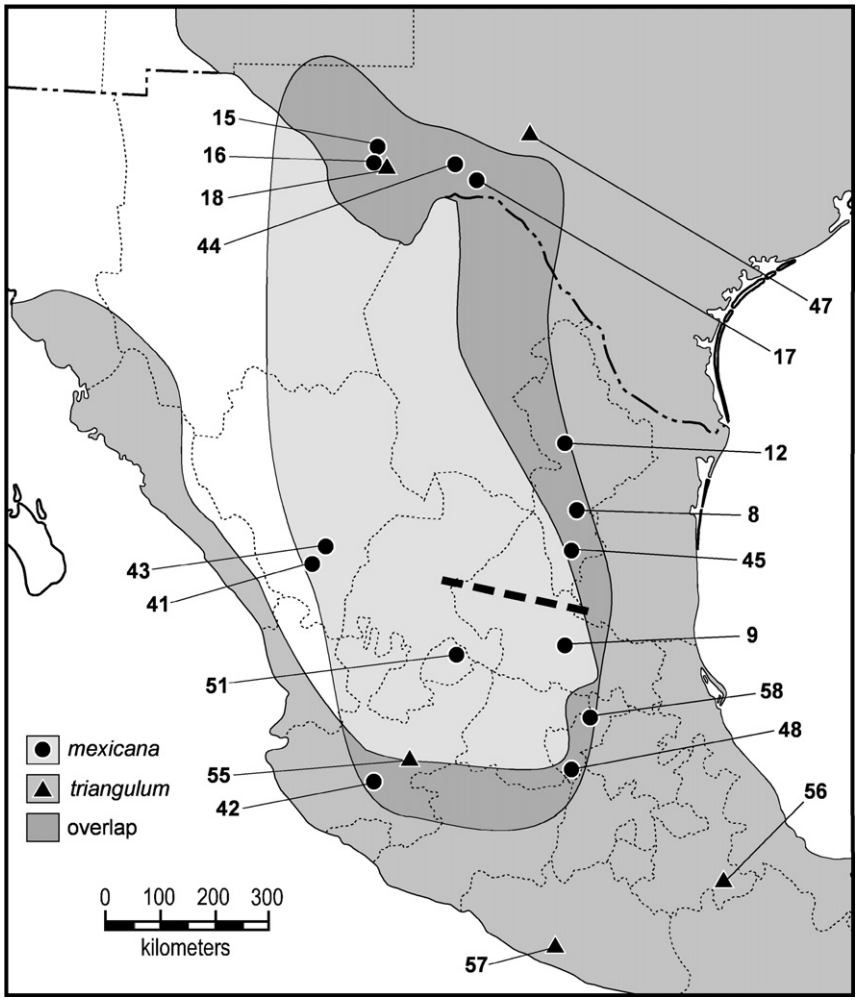

Fig. 1. Approximate distribution of the *Lampropeltis mexicana* complex (light gray) and *L. triangulum* (darker gray) in the southwestern United States and Mexico (after Gehlbach (1967) and Gartska (1982), and Williams (1988), respectively). Darkest gray area represents areas of potentially overlapping distribution. Numbers indicate the localities of specimens used in this study; circles and triangles represent taxa of the *L. mexicana* complex and *L. triangulum*, respectively. Not shown is the single sample of *L. t. gentilis* (53) from Russell County, Kansas. The dotted line indicates the combined relative positions of the Cerritos-Arista and Saladan Filter Barriers across the Sierra Madre Oriental and Mexican Plateau.

L. mexicana complex with respect to the other species within the genus *Lampropeltis*.

Species of the *L. mexicana* group are distributed across the mountainous regions of the Chihuahuan Desert and surrounding areas (Gartska, 1982). They range from extreme southeastern New Mexico and western Texas in the United States south through the Sierra Madre Oriental and Mexican Plateau to northern Michoacán (Fig. 1), and inhabit a wide range of habitats from xeric desert scrub to subhumid wooded uplands (Gartska, 1982; Painter et al., 1992). Until the 1980s, over half of the subspecies within this group were known from fewer than 10 specimens each (Gehlbach and Baker, 1962; Webb, 1961). Species composition within the *L. mexicana* group has changed several times, and the phylogenetic relationships within this group and to other *Lampropeltis* remain largely unclear. No study using DNA sequence data to assess these relationships in detail has yet to be produced.

Blanchard (1921) was the first to recognize several groups within *Lampropeltis* based on morphological similarities. Subsequently, Smith (1942, 1944), Webb (1961), Gehlbach and Baker (1962), and Gartska (1982) each attempted to assign taxa into a *L. mexicana* group based on perceived shared combinations of light-edged, red-centered blotches, mottled speckling (or alternating reduced markings), and a widened temporal region that renders the head very distinct from the neck. Recently, Hilken and Schlepper (1998) suggested that the *L. mexicana* complex should include a polytypic *L. alterna* (*L. a. alterna* and *L. a. blairi*) and *L. mexicana* (*L. m. mexicana*, *L. m. greeri*, and *L. m. thayeri*). Their decision seems to have been based solely on a review of the literature and presumably new evidence from captive specimens (a list of specimens examined was not provided). Taxonomy for the *L. mexicana* complex used in this paper follows Hilken and Schlepper (1998), although we retain *L. alterna* as a monotypic species since Hilken and Schlepper's (1998) division of this taxon into subspecies appears to be based on highly variable morphological characters obtained from snakes in the pet trade.

Few studies have utilized molecular data to assess the phylogenetic relationships within the genus *Lampropeltis*. Rodríguez-Robles and De Jesús-Escobar (1999) examined the phylogenetic relationships of the colubrid tribe Lampropeltini (which includes the New World colubrid taxa *Arizona*, *Bogertophis*, *Lampropeltis*, *Pantherophis*, *Pituophis*, *Pseudoelaphe*, *Rhinocheilus*, and *Senticolis*; Utiger et al., 2002) and included four species of *Lampropeltis* in their analysis. Additionally, Rodríguez-Robles et al. (1999) used mtDNA sequences to infer the phylogeography of *L. zonata*. Both studies utilized the same sample of *L. mexicana* in their comparisons. This sample was either inferred as being the sister taxon to *L. getula* (Rodríguez-Robles et al., 1999), or placed in an unresolved trichotomy with *L. pyromelana* and *L. zonata* or as the sister taxon to *L. pyromelana* (Rodríguez-Robles and De Jesús-Escobar, 1999). The purpose of our study is to use mtDNA sequences to (i) infer phylogeographic relationships within the *L. mexicana* complex, (ii) assess phylogenetic relationships among other species of the genus *Lampropeltis*, and (iii) explore possible biogeographic hypotheses to account for these relationships.

2. Materials and methods

2.1. Taxon sampling

Twenty-four tissue samples of the genus *Lampropeltis* were obtained from institutional and private collections (Table 1). Care was taken to restrict all samples to vouchered specimens of known locality, including those from private collections. Because the current taxonomy of *Lampropeltis* is mostly dependent on color pattern which is extremely variable, we included at least one representative sample from the nine currently recognized species of *Lampropeltis*, especially those species with a similar alternating tri-colored pattern of white, black, and red bands or triads (*L. pyromelana*, *L. triangulum*, *L. webbi*, and *L. zonata*) that resemble members of the *L. mexicana* complex. Given that taxa in the *L. mexicana* complex have been considered both derived from (Blanchard, 1921; Gartska, 1982; Smith, 1942, 1944; Tanner, 1953) and ancestral to (Webb, 1961) *L. triangulum*, six *L. triangulum* samples from Mexico and the western United States were included in our study (Fig. 1). Additional sequence data from eight ingroup and two outgroup taxa were obtained from GenBank and used in the analyses (Table 2). *Pituophis catenifer* and *Pantherophis guttatus* were used as outgroups based on previously hypothesized close relationships with *Lampropeltis* (Keogh, 1996; Rodríguez-Robles and De Jesús-Escobar, 1999). *Stilosoma extenuatum* was included with the ingroup taxa since both immunological (Dowling and Maxson, 1990) and molecular (Rodríguez-Robles and De Jesús-Escobar, 1999) data suggest that this species belongs within the genus *Lampropeltis*.

2.2. Laboratory methods

Total genomic DNA was extracted from shed skin, ventral scale clippings, or tissue samples using SDS-proteinase

Table 1
Samples of *Lampropeltis* obtained from institutional and private collections and used in this study

Taxon	Sample no.	Voucher no.	GenBank no.	Locality
Lampropeltis getula splendida	50LM	SRSU R-6543	AY739629	USA: Texas, Crane Co., in Crane
L. calligaster calligaster	52LM	SRSU R-6565	AY739644	USA: Missouri, Jefferson Co.
L. pyromelana knoblochi	46LM	GRQ 00701	AY497313	México: Chihuahua, near Mojarachic
L. webbi	07LM	UANL 5684	AY497308	México: Sinaloa, Hwy 40 near El Palmito
L. triangulum celaenops	18LM	SRSU R-6519	AY739630	USA: Texas, Jeff Davis Co., Musquiz Canyon
L. t. celaenops	47LM	SRSU R-6540	AY739631	USA: Texas, Crockett Co., near Barnhart
L. t. gentilis	53LM	SRSU R-6564	AY739632	USA: Kansas, Russell Co.
L. t. arcifera	55LM	SD LtLCF3	AY497312	México: Jalisco, north shore Laguna de Chapala
L. t. campbelli	56LM	FWZ 817102	AY739638	México: Puebla, Zapotitlán Basin
L. t. conanti	57LM	SRB-078	AY739643	México: Guerrero, Laguna de Coyuca, NW Acapulco
L. alterna	12LM	UANL 5018	AY739633	México: Nuevo León, Cerro de la Silla
L. alterna	15LM	SRSU R-6516	AY739634	USA: Texas, Jeff Davis Co., Hwy 17
L. alterna	16LM	SRSU R-6517	AY739635	USA: Texas, Jeff Davis Co., Musquiz Canyon
L. alterna	17LM	SRSU R-6518	AY739636	USA: Texas, Val Verde Co., west of Langtry
L. alterna	44LM	SRSU R-6521	AY497307	USA: Texas, Crockett Co., Howard Draw
L. mexicana thayeri	08LM	UANL 5773	AY497306	México: Nuevo León, near Iturbide
L. m. thayeri	45LM	TG LMT2321	AY739637	México: Nuevo León, north of Dr. Arroyo
L. m. mexicana	09LM	UANL 5603	AY739639	México: San Luis Potosí, Valle de los Fantasmas
L. m. mexicana	51LM	UAA uncat.	AY497309	México: Aguascalientes, near Asientos
L. m. greeri	43LM	UANL 5940	AY497310	México: Durango, Hwy 40 west of Cd. Durango
L. m. greeri	41LM	GRQ 00711	AY739640	México: Durango, Rancho Santa Barbara
L. ruthveni	42LM	[a]	AY739641	México: Jalisco, near Tapalpa
L. ruthveni	48LM	GTS R-12	AY739642	México: Querétaro, near Amealco
L. ruthveni	58LM	SH89lts01	AY497311	México: Querétaro, near Jalpan

Acronyms for vouchers 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.

[a] Shed skin found in the wild (species determined by ventral scale count).

Table 2
Previously published sequences used in this study

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

With the exception of *Lampropeltis zonata*, subspecific designation of *Lampropeltis* taxa was inferred from locality data.

K digestion and a modified protocol of the Puregene® DNA Isolation Kit (Gentra Systems, Minneapolis, MN) available from RWB. An 868 base pair (bp) region of mtDNA was amplified, 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. This was done using the primers ND4 and Leu (Arévelo et al., 1994), and CornF3, CornR2, and LND4#2 (Bryson et al., 2005). The template DNA was amplified in 100 µl reactions containing 0.06 M Tris, 0.015 M $NH_{42}SO_4$, 0.015 M $MgCl_2$, 0.78 M dimethyl sulfoxide, 0.025 mM of each dNTP, 1 mM of each primer, and 2.5 U Promega *Taq* polymerase (Madison, WI) in a Geneamp® PCR System 9700 thermal cycler (Applied Biosystems; Foster City, CA). Amplification conditions consisted of 35 cycles of denaturing at 95 °C for 30 s, primer annealing at 50 °C for 60 s, and extension at 72 °C for 60 s, followed by a final extension at 72 °C for 5 min. PCR products were visualized in ethidium bromide-stained agarose minigels and then prepared for sequencing using QIAquick PCR Purification Kit (QIAGEN, Valencia, CA) or Concert™ Rapid PCR Purification System (Life Technologies, Carlsbad, CA). The purified 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 amplification primers using BigDye v2.0 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 purified of excess dyes using Sephadex G-50 in CENTRI-SEP Columns (Princeton Separations, Inc., Adelphia, NJ). The reactions were sequenced on an ABI Prism 377 DNA Sequencer (Applied Biosystems, Norwalk, CT).

2.3. Phylogenetic analyses

Sequences were aligned manually by eye. The protein-coding ND4 sequence was conservative across all taxa in the data set with an open reading frame and no insertions/deletions (indels) being observed. The few indels in the tRNA genes were restricted to the loops of the $tRNA^{His}$ and to non-coding base positions between genes. Both samples of *L. m. thayeri* had an additional base between $tRNA^{His}$ and $tRNA^{Ser}$.

The aligned sequences were analyzed using maximum parsimony (MP) and maximum likelihood (ML) in PAUP* 4.0b10 (Swofford, 2002), and Bayesian inference (BI) in MrBayes 3.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Maximum parsimony analyses were conducted using a heuristic search with starting trees obtained via stepwise addition with 2500 random addition-sequence replicates, accelerated character transformation (ACCTRAN), and tree-bisection-reconnection (TBR) branch swapping. All characters were treated as unordered (Swofford et al., 1996). Gaps were considered as missing data. Nodal support was estimated using 2500 non-parametric bootstraps (BS) (Felsenstein, 1985) with heuristic searches involving 10 random addition sequence replicates. Tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC) were obtained from MacClade (Maddison and Maddison, 1992) using the most parsimonious tree topologies.

The most appropriate ML model was assessed using the Akaike Information Criterion (AIC) in Modeltest 3.7 (Posada and Crandall, 1998). Starting trees were obtained from the most-parsimonious tree derived in MP analyses. The initial model parameters estimated by Modeltest using AIC were input into PAUP* and a ML tree was estimated. From this tree, we estimated new model parameters and subsequently conducted another ML analysis. This procedure was repeated until the same tree was found in successive iterations. Starting trees were obtained via stepwise addition with an "as-is" addition sequence, and non-parametric bootstrapping with 100 pseudo-replicates with TBR branch swapping were performed to estimate nodal support.

To infer trees and assess tree support using models incorporating evolutionary information specific to each gene, we performed single and mixed-model analyses using BI with MrBayes 3.1. Prior to tree inference, three basic evolutionary models were evaluated for the data set using Bayes factors on the harmonic mean of the posterior probability (PP) distribution of these models. The first model accounts for differences in evolutionary rates of each of the

three codon positions in the ND4 gene and the tRNAs using the GTR + Γ + I model with estimated base pair (BP) frequencies for each codon position. For this model, we used four partitions which include the three codon positions and the tRNAs, abbreviated 4(GTR + Γ + I). A single tree was estimated for all four partitions simultaneously, but all other model parameters were unlinked among partitions. The second model simply accounted for differences between the tRNAs and the protein coding gene as a whole and was abbreviated 2(GTR + Γ + I). The third model applied the GTR + Γ + I model across all positions with no partitioning among codon positions or the tRNAs.

For each model, four independent searches were executed to insure convergence of all parameters by comparing the variance across chains within a search to the chain variance among searches using Rubin and Gelman's "r" statistic (Gelman et al., 1995). Searches were considered burned-in when the values for "r" had reached 1.000. All searches consisted of three "heated" and one "cold" Markov chain estimated for 10 million generations, with every 1000th sample being retained, and without specified priors being applied to any parameters. Parameter stationarity was assumed to have occurred when tree $-\ln L$ values for chains converge in all four replicates for each model. Trees prior to stationarity were discarded. Using Bayes factors to determine the appropriate model for the sequence data requires that the harmonic mean for the model likelihood $f(X|M_i)$ was estimated from the values in the stationarity phase of a Markov chain Monte Carlo run following the methodologies of Newton and Raftery (1994) and Nylander et al. (2004). To compare models, Bayes factors followed the form $2\log_e B_{10}$, where B_{10} is the ratio of model likelihoods. A Bayes factor value greater than 10 was considered as strong evidence favoring the more parameter rich model (Kass and Raftery, 1995). These BI trees were compared with those obtained using ML and the most credible inferences of relationship were confined to nodes where the posterior probability and non-parametric bootstraps were greater than 95%.

3. Results

3.1. Sequence variation

The nucleotide sequences for ND4 and the adjacent tRNAs span a total of 868 bp. Of these, 217 sites (25%) were phylogenetically informative. Uncorrected sequence divergences ranged between 0% and 11.0% within *Lampropeltis* (Table 3), between 10.4% and 13.6% between *Lampropeltis* and *Pituophis catenifer*, and between 11.2% and 13.9% between *Lampropeltis* and *Pantherophis guttatus*. The aligned data set and phylogenetic trees were deposited in TreeBASE.

3.2. Phylogenetic relationships

Maximum parsimony analyses with all characters equally weighted resulted in 10 equally parsimonious trees (length = 705 steps, CI = 0.48, RI = 0.70, RC = 0.33). A strict consensus of these trees produced the same basic structure as the ML and BI analyses (Figs. 2 and 3).

Maximum likelihood produced a likelihood score for the best tree of $-\ln L = 4437.22875$ using the best-fit TrN + Γ model suggested by Modeltest using AIC and BIC. Parameters for this model where rAC = 1.000, rAG = 24.55, rAT = 1.000, rCG = 1.000, rCT = 14.8461, and rGt = 1.000 with $\Gamma = 0$, and no invariable sites.

All models used for these data in the BI analyses burned-in prior to 500,000 generations as assessed using Gelman and Rubin's "r" statistic at a value of 1.00 for all parameters. The most parameter rich model, 4(GTR + Γ + I), was chosen by Bayes factors as the best model with the highest

Table 3
Uncorrected pair-wise sequence divergences within and between major groupings of *Lampropeltis* samples; averages followed by number of comparisons in parentheses above, with ranges below

Clade		Southern	Northern	*getula*	*S. extenuatum*	*pyromelana*	*zonata*	*L. calligaster*
	N	10	10	2	1	3	5	1
Southern	10	0.053 (45) 0–0.076						
Northern	10	0.085 (100) 0.069–0.098	0.014 (45) 0–0.024					
getula	2	0.096 (20) 0.085–0.103	0.064 (20) 0.059–0.069	0.046 (1) —				
S. extenuatum	1	0.103 (10) 0.094–0.110	0.068 (10) 0.066–0.071	0.069 (2) 0.067–0.071	—			
pyromelana	3	0.088 (30) 0.073–0.096	0.082 (30) 0.078–0.088	0.094 (6) 0.093–0.096	0.095 (3) 0.093–0.099	0.047 (3) 0.037–0.053		
zonata	5	0.093 (50) 0.077–0.110	0.096 (50) 0.088–0.103	0.105 (10) 0.101–0.110	0.104 (5) 0.102–0.107	0.080 (15) 0.073–0.087	0.036 (10) 0.010–0.050	
L. calligaster	1	0.105 (10) 0.099–0.110	0.091 (10) 0.089–0.095	0.092 (2) 0.090–0.094	0.099 (1) —	0.097 (3) 0.094–0.100	0.103 (5) 0.097–0.109	—

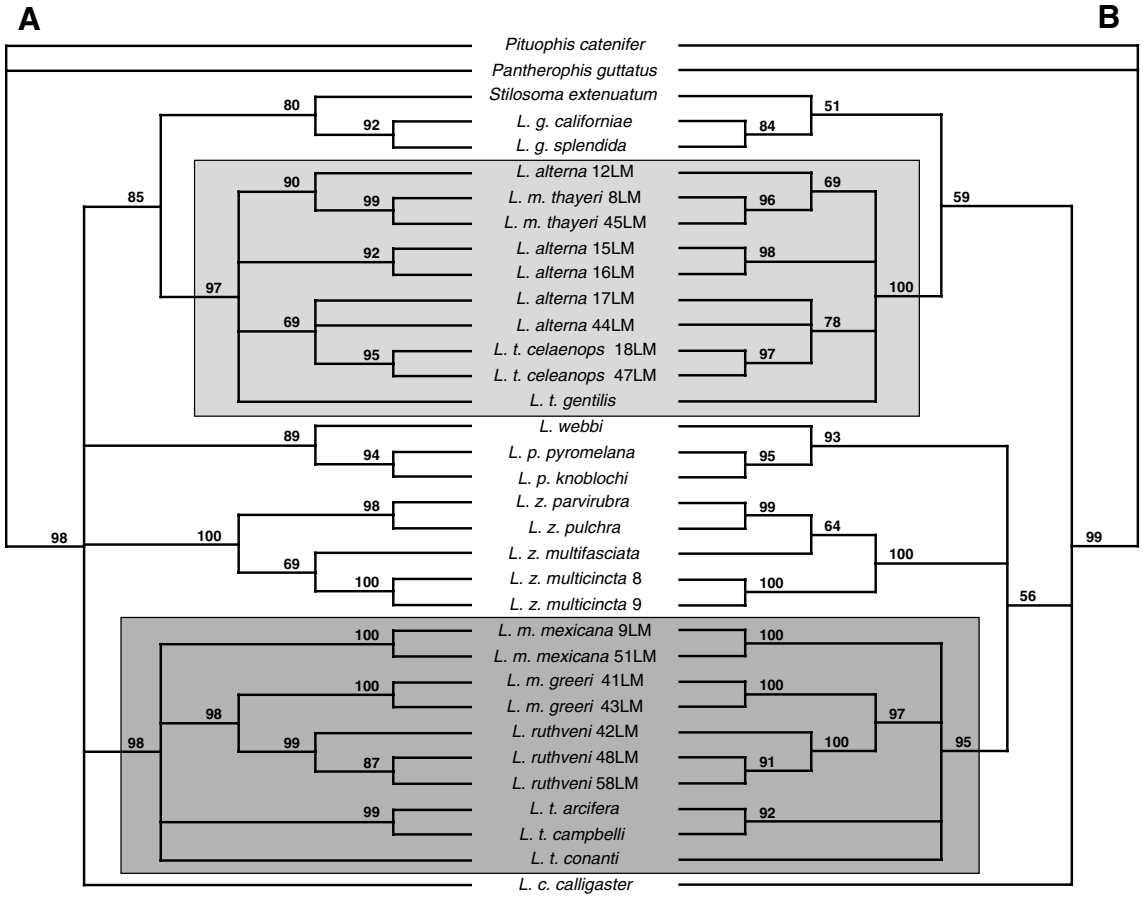

Fig. 2. Phylogenetic trees based on the sequences of part of the mitochondrial ND4 gene and the adjacent $tRNA^{His}$, $tRNA^{Ser}$, and partial $tRNA^{Leu}$. Numbers at nodes indicate percentage of bootstrap replicates supporting that node. Light gray box indicates the northern clade; dark gray box indicates the southern clade. (A) Maximum likelihood cladogram and (B) maximum parsimony cladogram with all characters weighted equally.

harmonic mean of −4316.67. This is in comparison with the 2(GTR + Γ + I) and the GTR + Γ + I model that yielded ln L values of −4506 and −4517, respectively. Given that both AIC and BIC both chose models that do not incorporate invariable sites (I), we ran the three models used for the BI analyses again without I. These three models, 4(GTR + Γ), 2(GTR + Γ), and GTR + Γ + I, produced the following three ln$L$ scores: −4366.75, −4513.26, and −4508.55. Again, Bayes factors chose the most parameter rich model, 4(GTR + Γ + I), over all models not incorporating invariable sites. These models produced similar topologies.

All phylogenetic methods consistently inferred the same major clades with similar measures of support. Given that Bayesian inference chooses a much more parameter rich model and that this model was able to partition different genes regions, a feature not yet available in ML analyses, we chose the BI tree as our preferred phylogenetic hypothesis. Samples of the *L. mexicana* group and *L. triangulum* were grouped within two major geographically defined clades. The "northern clade" is comprised of *L. alterna*, *L. m. thayeri*, and *L. triangulum* (*L. t. celaenops*, and *L. t. gentilis*) from the western US and Mexico. *Lampropeltis m. mexicana*, *L. m. greeri*, *L. ruthveni*, and *L. triangulum* from central and southern Mexico (*L. t. arcifera*, *L. t. campbelli*, and *L. t. conanti*) comprised the "southern clade". Three other clades were consistently produced in these analyses: *L. getula* ("*getula*" clade), *L. pyromelana* and *L. webbi* ("*pyromelana*" clade), and *L. zonata* ("*zonata*" clade). The relationships among these five clades, *S. extenuatum*, and *L. c. calligaster*, however, is difficult to determine using these data.

The BI tree strongly suggests a deep early division within the genus *Lampropeltis* (PP = 100%). One group includes those taxa occupying the upland areas of western United States and most of western and central Mexico (the *pyromelana, zonata*, and southern clades), and the other group includes the taxa occupying northeastern Mexico and the lowland areas of the southern United States (*L. c. calligaster*, *S. extenuatum*, the *getula* clade, and northern clade). Maximum likelihood, MP, and BI analyses consistently place the *getula* clade and *S. extenuatum* as the sister group to the northern clade, although support for this placement is variable (BS = 85%, 59%, PP = 100%, respectively).

Support for the relationships among the remaining nodes are less robust. Maximum parsimony and BI analyses weakly support a grouping between the *pyromelana*, *zonata*, and southern clades (BS = 56%, PP = 76%). This weakly supported trichotomy is not inferred using ML.

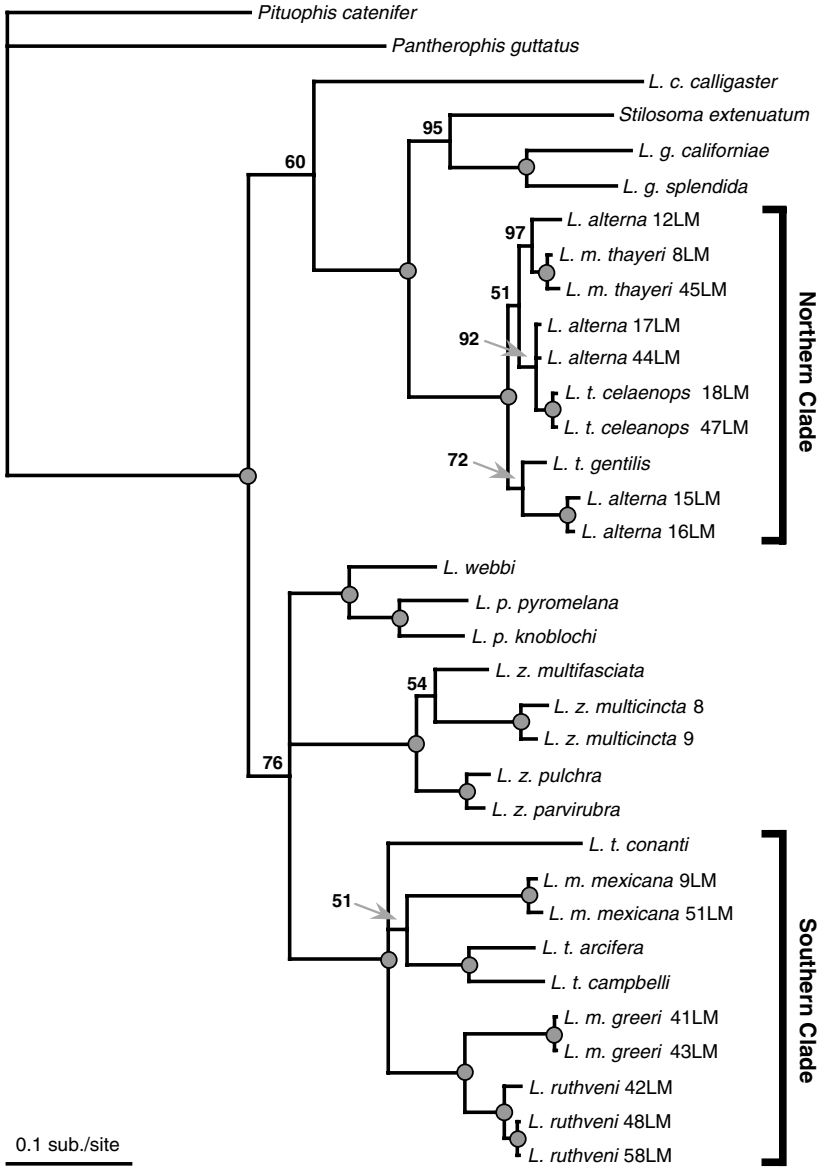

Fig. 3. Bayesian inference phylogram based on the sequences of part of the mitochondrial ND4 gene and the adjacent $tRNA^{His}$, $tRNA^{Ser}$, and partial $tRNA^{Leu}$. Numbers at nodes indicate Bayesian posterior probabilities. Nodal posterior probabilities of 100% are indicated by gray-filled circles. Branch lengths drawn in proportion to the amount of changes.

The position of *L. c. calligaster* remains uncertain. Maximum likelihood and MP analyses place this taxon in a polytomy with the *pyromelana*, *zonata*, and the southern clades, and the group comprised of *S. extenuatum*, the *getula* clade, and the northern clade. Bayesian inference weakly (PP = 60%) places this taxon as the sister group to the clade composed of *S. extenuatum*, the *getula* clade, and the northern clade.

4. Discussion

4.1. Polyphyly within the genus Lampropeltis

Our phylogenies using mtDNA sequences suggest that the current taxonomy of the *L. mexicana* complex and *L. triangulum* may be in error with respect to evolutionary history. This phylogeny is incongruent with previously hypothesized relationships based on morphology (Gartska, 1982; Smith, 1942, 1944; Webb, 1961). Typically, members of the *L. mexicana* group (*L. mexicana*, *L. alterna*, and *L. ruthveni*) have been considered distinct from other species of *Lampropeltis* because they possess a combination of light-edged, red-centered blotches, mottled speckling (or alternating reduced markings), and a widened temporal region that renders the head very distinct from the neck (Gartska, 1982; Gehlbach and Baker, 1962). In the closely related *L. triangulum*, the head is only slightly distinct from the neck (Williams, 1988). Although variable, the color and patterning of the head and body between the *L. mexicana* group and *L. triangulum* differ as well (Fig. 4) (Gartska, 1982; Gehlbach and Baker, 1962; Smith, 1944). In addition, where species from the *L. mexicana* group and *L. triangulum*

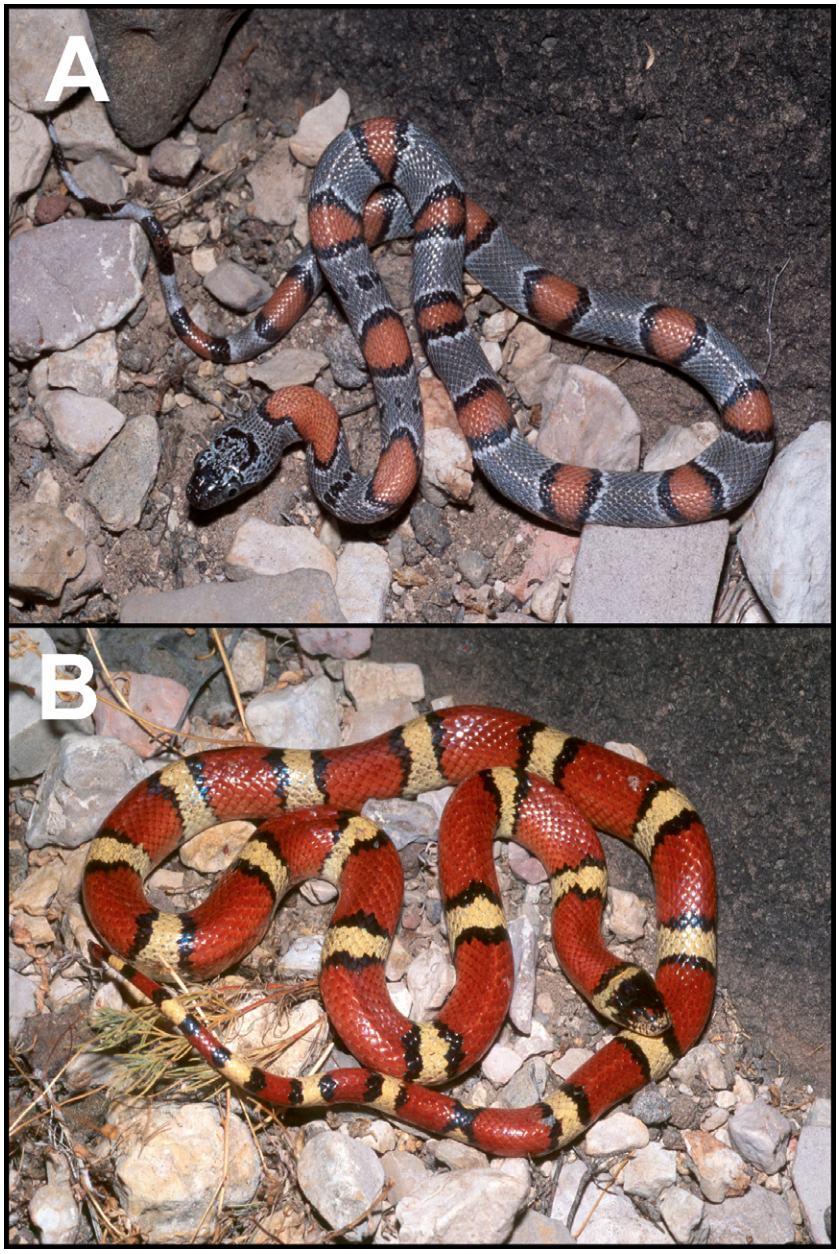

Fig. 4. (A) *Lampropeltis alterna* and (B) *Lampropeltis triangulum celaenops* from southeastern Brewster County, Texas demonstrating color and pattern differences. *Lampropeltis t. celaenops* is sympatric with *L. alterna* throughout most of western Texas.

are sympatric, their number of ventral scales are different (Gartska, 1982).

In our mtDNA phylogenies, two clades contain taxa from both the *L. mexicana* group and *L. triangulum*: (1) A geographically defined northern clade containing *L. alterna*, *L. m. thayeri*, *L. t. celaenops*, and *L. t. gentilis*, and (2) a geographically defined southern clade containing *L. m. mexicana*, *L. m. greeri*, *L. ruthveni*, *L. t. arcifera*, *L. t. campbelli*, and *L. t. conanti*. Although only five of the 25 currently recognized subspecies of *L. triangulum* (Williams, 1988) were sampled for this current study, it is clear that this species is composed of at least two divergent mtDNA lineages, each nested within both the northern and southern clades. In addition, the long branch connecting *L. t. conanti* with the clade comprised of *L. t. arcifera* and *L. t. campbelli* on the BI tree (Fig. 3) might suggest an earlier divergence or an increased relative rate of evolution. Our results suggest that the current recognition of *L. mexicana* and *L. triangulum* may be incongruent with the evolutionary history of these two groups. This is surprising given that *L. triangulum* have been treated as a distinct species in the literature for over 60 years (Smith, 1942; Williams, 1978, 1988). Conversely, the mtDNA data used here may not reflect the evolutionary history of these taxa, but might indicate recurrent patterns of gene flow among these species. Further research using nuclear markers to assess gene flow among these lineages will be necessary to determine if the currently recognized taxa do represent species and if the mtDNA data are indeed in error.

Our phylogenies also indicate that the genus *Lampropeltis* appears paraphyletic with respect to *Stilosoma*. All analyses support a sister relationship between the *getula* clade and *S. extenuatum*, consistent with previous studies (Dowling and Maxson, 1990; Rodríguez-Robles and De Jesús-Escobar, 1999). Given the morphological differences between these two species, this relationship is unexpected. *Stilosoma extenuatum* is a small (typically 35–50 cm in total length), extremely slender, fossorial snake with a unique vertebral structure restricted to the sandy pinelands of north-central Florida (Tennant and Bartlett, 2000). By contrast, the subspecies of *L. getula* are large (typically 90–120 cm in total length, with records upwards of 150 cm), robust constrictors with a wide distribution across the southern United States and northern Mexico (Tennant and Bartlett, 2000). However, both species frequently prey on snakes, and *S. extenuatum* may prey exclusively on the snakes in the genus *Tantilla* (Tennant and Bartlett, 2000). In addition, the attenuate body structure of *S. extenuatum* may be the result of adaptation to fossorial habits.

4.2. Phylogeographic relationships within the Lampropeltis mexicana complex

Our preferred phylogeny (Fig. 3) suggests that an early split resulted in the eventual diversification of two major groups of *Lampropeltis*: one group that occupies the upland areas of western United States and most of western and central Mexico, and one group that occupies northeastern Mexico and the lowland areas of the southern United States. These two clades contain taxa from both the *L. mexicana* group and *L. triangulum*, indicating that the *L. mexicana* complex and *L. triangulum*, as currently recognized, are polyphyletic. Therefore, any discussions concerning the phylogeography of *L. mexicana* must also consider members of *L. triangulum*.

All previous taxonomic studies suggesting relationships within and among the *L. mexicana* group and *L. triangulum* using morphology are in many ways contrary to our phylogenies (Figs. 2 and 3). Smith (1942) suggested that most species within *Lampropeltis* other than *L. calligaster* and *L. getula* were derived from a member of the *L. triangulum* group based on similarities in hemipenal structures. He placed *L. mexicana, L. leonis, L. thayeri* (Smith, 1944) and *L. alterna* in the *L. mexicana* subgroup, and *L. thayeri* and *L. ruthveni* in the *L. pyromelana* subgroup. Webb (1961) instead suggested that *L. mexicana* was the "most primitive" form of *Lampropeltis*, and considered the *L. mexicana* group to consist of *L. mexicana, L. thayeri, L. leonis, L. alterna, L. greeri*, and *L. blairi*. He indicated that three distinct lineages have evolved from *L. mexicana*: (1) *L. greeri*, *L. blairi*, and *L. alterna*; (2) *L. leonis* and the *L. calligaster-getula* group; and (3) *L. thayeri* and the *L. triangulum* group (which included *ruthveni*). Gartska (1982) hypothesized that the *L. mexicana* group (comprised of the monotypic species *L. mexicana*, *L. alterna*, and *L. ruthveni*) and the closely related species *L. pyromelana* and *L. zonata* are sister to *L. triangulum* based on shared derived morphological characters.

Our sequence data does not support the recognition of a monophyletic *L. mexicana* complex as previously proposed. Gartska (1982) assumed *a priori* that *L. mexicana* was a single, highly variable species. As such, he proposed that the *L. mexicana* group was comprised of three species-level taxa (*L. mexicana*, *L. alterna*, and *L. ruthveni*), and subsequently analyzed all characters within that framework. The phylogeny of the southern clade derived in our study supports Gartska's (1982) placement of *L. ruthveni* in the *L. mexicana* complex. However, our topologies would indicate that *L. mexicana* is not a single species; in fact, two individuals of *L. mexicana* (*L. m. thayeri*) in the northern clade are more closely related to *L. alterna* and *L. triangulum* then they are to other *L. mexicana*. Additionally, in the southern clade, four individuals of *L. mexicana* (two *L. m. mexicana* and two *L. m. greeri*) are most closely related to *L. triangulum* and *L. ruthveni*.

Gehlbach and Baker (1962) considered *L. mexicana* to be comprised of five subspecies: *L. m. mexicana* from the Mexican Plateau, *L. m. alterna* from the western and southern regions of the Chihuahuan Desert, *L. m. thayeri* from the northern Sierra Madre Oriental, *L. m. blairi* from the eastern region of the Chihuahuan Desert, and *L. m. greeri* from the southern Sierra Madre Occidental. In the southern clade of our study, *L. mexicana* samples 9LM and 51LM from the Mexican Plateau, and samples 41LM and 43LM from the southern Sierra Madre Occidental (Fig. 1), each form two separate clades, corresponding to Gehlbach and Baker's (1962) *L. m. mexicana* and *L. m. greeri*, respectively. Therefore, our analyses suggest that there may be a geographical component to the phylogenetic history of this group. The formation of the Cerritos-Arista and Saladan Filter Barriers (Morafka, 1977) may have significantly affected the biogeography of both the *L. mexicana* group and *L. triangulum*. These lowland barriers essentially bisect the Sierra Madre Oriental near central San Luis Potosí and correspond to the genetic break between the northern and southern clades in eastern Mexico in our analyses (Fig. 1). A geographically similar genetic break occurs within the *Sceloporus jarrovii* group (Wiens et al., 1999; Wiens and Penkrot, 2002). The inclusion of additional samples will help to infer more about the phylogeographic history of the *L. mexicana* complex. Recognition of the remaining taxa (Gelbach and Baker's *L. m. alterna*, *L. m. blairi*, and *L. m. thayeri*; and Garstka's *L. alterna*), however, remains problematic given the little sequence divergence between these taxa in our northern clade (0–2.4%; Table 3).

The pattern of species-level polyphyly inferred in our analyses among taxa of the *L. mexicana* complex and *L. triangulum* and incongruence between previous relationships between these two groups may have a variety of causes (see Funk and Omland, 2003; and Rubinoff and Holland, 2005), and future studies using a more diversified sampling should explore these patterns to prevent erroneous evolutionary

interpretations. In addition, nuclear genes capable of assessing relationships at the species level need to be employed to provide characters that are independent of the linked mtDNA sequences.

Previous relationships within the *L. mexicana* complex have been based on morphological similarities: a decrease or loss of red in between black bands, reduction of head markings, and overall dark ground color (Smith, 1942); a shared pattern of black-bordered red bands alternating with white-bordered gray bands (Webb, 1961); alternating color pattern of black, red, black, white, gray, white markings on the body; a wide head distinct from the neck; mottled gray snout; dark postocular mark; dorsal head pattern; large eye; enlarged nuchal blotch; large number of posterior dorsal scale rows; and relatively large tail (Gehlbach and Baker, 1962); and light-colored, red-centered blotches, possible alternating reduced markings, gray iris, and triangular skull (Gartska, 1982). Morphological characters shared among the various tri-colored *Lampropeltis* may actually be homoplastic and difficult to interpret phylogenetically. The use of color pattern to define species or subspecies has been shown to be problematic with respect to evolutionary history in other species of lampropeltinine snakes (Burbrink et al., 2000). The large range of overlap and frequent lack of diagnosable morphological characters within and between these groups of *Lampropeltis* may have obscured inferences of phylogenetic relationships among these species and produced erroneous taxonomic classifications for over 80 years. Finally, it should be noted that the group which tri-colored members of the genus *Lampropeltis* are mimicking, the venomous coral snakes of the genus *Micrurus*, are also wide ranging with similarly variable tri-colored patterns. In the same way, these color patterns were assumed to be diagnostic for various species of *Micrurus*, but have been found to be largely plastic (Eric N. Smith, personal communication). As a result, molecular phylogenies for the coral snakes are also inconsistent with morphologically defined species.

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