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

Robert W. Bryson Jr. a,*, Jennifer Pastorini b,c, Frank T. Burbrink d, Michael R.J. Forstner b,e

a Department of Biology, Sul Ross State University, Alpine, TX 79832, USA
b Department of Biology, Texas State University, San Marcos, TX 78666, USA
c Centre for Conservation and Research, 35 Ginasekara Gardens, Nawala Road, Rajagiriya, Sri Lanka
d Department of Biology, 6S-143, 2800 Victory Drive, College of Staten Island/CUNY, Staten Island, NY 10314, USA
e Texas Cooperative Wildlife Collection, Texas A&M University, College Station, TX 77843, USA

Received 8 May 2006; revised 27 November 2006; accepted 28 November 2006

**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.

© 2006 Elsevier Inc. All rights reserved.

**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 through-out 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 coral-snakes (*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...
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 Michoacan (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.

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.
Few studies have utilized molecular data to assess the phylogenetic relationships within the genus Lampropeltis. Rodriguez-Robles and De Jesús-Escobar (1999) examined the phylogenetic relationships of the colubrid tribe Lampropeltini (which includes the New World colubrid taxa Arizona, Bogortophis, Lampropeltis, Pantherophis, Pituophis, Pseudaelaphus, Rhinocelis, and Senticolis; Utiger et al., 2002) and included four species of Lampropeltis in their analysis. Additionally, Rodriguez-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 (Rodriguez-Robles et al., 1999), or placed in an unresolved trichotomy with L. pyromelana and L. zonata or as the sister taxon to L. pyromelana (Rodriguez-Robles and De Jesús-Escobar, 1999). The purpose of our study is to use mtDNA sequences to (i) infer phylogenetic 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 vouched specimens of known local identity, 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; Rodriguez-Robles and De Jesús-Escobar, 1999). Stilosisoma extenuatum was included with the ingroup taxa since both immunological (Dowling and Maxson, 1990) and molecular (Rodriguez-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

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Sample no.</th>
<th>Voucher no.</th>
<th>GenBank no.</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. calligaster calligaster</td>
<td>52LM</td>
<td>SRSU R-6565</td>
<td>AY739644</td>
<td>USA: Missouri, Jefferson Co.</td>
</tr>
<tr>
<td>L. pyromelana knabchi</td>
<td>46LM</td>
<td>GRQ 00701</td>
<td>AY497313</td>
<td>México: Chihuahua, near Mojarachic</td>
</tr>
<tr>
<td>L. webbi</td>
<td>07LM</td>
<td>UANL 5684</td>
<td>AY497308</td>
<td>México: Sinaloa, Hwy 40 near El Palmito</td>
</tr>
<tr>
<td>L. triangulum caleanops</td>
<td>18LM</td>
<td>SRSU R-6519</td>
<td>AY739630</td>
<td>USA: Texas, Jeff Davis Co., Musquiz Canyon</td>
</tr>
<tr>
<td>L. t. caleanops</td>
<td>47LM</td>
<td>SRSU R-6540</td>
<td>AY739631</td>
<td>USA: Texas, Crockett Co., Barnhart</td>
</tr>
<tr>
<td>L. t. gentilis</td>
<td>53LM</td>
<td>SRSU R-6564</td>
<td>AY739632</td>
<td>USA: Kansas, Russell Co.</td>
</tr>
<tr>
<td>L. t. arcifera</td>
<td>55LM</td>
<td>SD L1LCF3</td>
<td>AY497312</td>
<td>México: Jalisco, north shore Laguna de Chapala</td>
</tr>
<tr>
<td>L. t. campbelli</td>
<td>56LM</td>
<td>FWZ 817102</td>
<td>AY739638</td>
<td>México: Puebla, Zapotitlán Basin</td>
</tr>
<tr>
<td>L. t. conanti</td>
<td>57LM</td>
<td>SRB-078</td>
<td>AY739643</td>
<td>México: Guerrero, Laguna de Coyuca, NW Acapulco</td>
</tr>
<tr>
<td>L. alterna</td>
<td>12LM</td>
<td>UANL 5018</td>
<td>AY739633</td>
<td>México: Nuevo León, Cerro de la Silla</td>
</tr>
<tr>
<td>L. alterna</td>
<td>15LM</td>
<td>SRSU R-6516</td>
<td>AY739634</td>
<td>USA: Texas, Jeff Davis Co., Hwy 17</td>
</tr>
<tr>
<td>L. alterna</td>
<td>16LM</td>
<td>SRSU R-6517</td>
<td>AY739635</td>
<td>USA: Texas, Jeff Davis Co., Musquiz Canyon</td>
</tr>
<tr>
<td>L. alterna</td>
<td>17LM</td>
<td>SRSU R-6518</td>
<td>AY739636</td>
<td>USA: Texas, Val Verde Co., west of Langtry</td>
</tr>
<tr>
<td>L. alterna</td>
<td>44LM</td>
<td>SRSU R-6521</td>
<td>AY497307</td>
<td>USA: Texas, Crockett Co., Howard Draw</td>
</tr>
<tr>
<td>L. mexicana thayeri</td>
<td>08LM</td>
<td>UANL 5777</td>
<td>AY497306</td>
<td>México: Nuevo León, near Ixtaltepe</td>
</tr>
<tr>
<td>L. m. thayeri</td>
<td>45LM</td>
<td>TG LMT2321</td>
<td>AY739637</td>
<td>México: Nuevo León, north of Dr. Arroyo</td>
</tr>
<tr>
<td>L. m. mexicana</td>
<td>09LM</td>
<td>UANL 5603</td>
<td>AY739639</td>
<td>México: San Luis Potosi , Valle de los Fantasmas</td>
</tr>
<tr>
<td>L. m. mexicana</td>
<td>51LM</td>
<td>UAA uncat.</td>
<td>AY497309</td>
<td>México: Aguascalientes, near Asientos</td>
</tr>
<tr>
<td>L. m. greeri</td>
<td>43LM</td>
<td>UANL 5940</td>
<td>AY497310</td>
<td>México: Durango, Hwy 40 west of Cd. Durango</td>
</tr>
<tr>
<td>L. m. greeri</td>
<td>41LM</td>
<td>GRQ 00711</td>
<td>AY739640</td>
<td>México: Durango, Rancho Santa Barbara</td>
</tr>
<tr>
<td>L. ruthvenii</td>
<td>42LM</td>
<td></td>
<td>AY739641</td>
<td>México: Jalisco, near Tapalpa</td>
</tr>
<tr>
<td>L. ruthveni</td>
<td>48LM</td>
<td></td>
<td>AY739642</td>
<td>México: Querétaro, near Amealco</td>
</tr>
<tr>
<td>L. ruthveni</td>
<td>58LM</td>
<td></td>
<td>AY497311</td>
<td>México: Querétaro, near Jalpan</td>
</tr>
</tbody>
</table>

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.

* Shed skin found in the wild (species determined by ventral scale count).

K digestion and a modified protocol of the Puregene® DNA Isolation Kit (Genta 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 tRNAHis, tRNASer, and tRNALeu 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 NH₄₂SO₄, 0.015 M MgCl₂, 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 tRNAHis and to non-coding base positions between genes. Both samples of L. m. thayeri had an additional base between tRNAHis and tRNASer.

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

Table 2
Previously published sequences used in this study

<table>
<thead>
<tr>
<th>Taxon</th>
<th>GenBank no.</th>
<th>Locality</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ptychis catenifer</td>
<td>AF138763</td>
<td>USA: California, San Diego Co.</td>
<td>Rodríguez-Robles and De Jesús-Escobar (1999)</td>
</tr>
<tr>
<td>Pantherophis guttatus</td>
<td>AF138756</td>
<td>USA: Georgia, Fort Benning</td>
<td>Rodríguez-Robles and De Jesús-Escobar (1999)</td>
</tr>
<tr>
<td>Stilosoma externatum</td>
<td>AF138776</td>
<td>USA: Florida, Hillsborough Co.</td>
<td>Rodríguez-Robles and De Jesús-Escobar (1999)</td>
</tr>
<tr>
<td>Lampropeltis getula californiae</td>
<td>AF138759</td>
<td>USA: California, San Benito Co.</td>
<td>Rodríguez-Robles and De Jesús-Escobar (1999)</td>
</tr>
<tr>
<td>L. pyromelana pyromelana</td>
<td>AF138761</td>
<td>USA: Arizona, Cochise Co.</td>
<td>Rodríguez-Robles and De Jesús-Escobar (1999)</td>
</tr>
<tr>
<td>L. zonata multicincta 8</td>
<td>AF136195</td>
<td>USA: California, Mariposa Co.</td>
<td>Rodríguez-Robles et al. (1999)</td>
</tr>
<tr>
<td>L. z. multircincta 9</td>
<td>AF136196</td>
<td>USA: California, Plumas Co.</td>
<td>Rodríguez-Robles et al. (1999)</td>
</tr>
<tr>
<td>L. z. multisulcia 14</td>
<td>AF136200</td>
<td>USA: California, Monterey Co.</td>
<td>Rodríguez-Robles et al. (1999)</td>
</tr>
<tr>
<td>L. z. paracaerulea 21</td>
<td>AF136207</td>
<td>USA: California, Riverside Co.</td>
<td>Rodríguez-Robles et al. (1999)</td>
</tr>
<tr>
<td>L. z. pulchra 24</td>
<td>AF136210</td>
<td>USA: California, San Diego Co.</td>
<td>Rodríguez-Robles et al. (1999)</td>
</tr>
<tr>
<td>Stilosoma alternatum</td>
<td>AF136212</td>
<td>USA: California, San Diego Co.</td>
<td>Rodríguez-Robles et al. (1999)</td>
</tr>
<tr>
<td>Stilosoma terminatus fusekii</td>
<td>AF136213</td>
<td>USA: California, San Diego Co.</td>
<td>Rodríguez-Robles et al. (1999)</td>
</tr>
</tbody>
</table>

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

ARTICLE IN PRESS


ARTICLE IN PRESS

three codon positions in the ND4 gene and the tRNAs using the GTR + $I + I$ 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 + $I + I$). 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 + $I + I$). The third model applied the GTR + $I + I$ 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 $f(\mathcal{X}\mid M_i)$ 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 $2\log B_{10}$, 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 + $I$ model suggested by Modeltest using AIC and BIC. Parameters for this model where $rAC = 1.000$, $rCG = 24.55$, $rAT = 1.000$, $rCG = 1.000$, $rCT = 14.4861$, 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 + $I + I$), was chosen by Bayes factors as the best model with the highest

<table>
<thead>
<tr>
<th>Clade</th>
<th>Southern</th>
<th>Northern</th>
<th>getula</th>
<th>S. extenuatum</th>
<th>pyromelana</th>
<th>zonata</th>
<th>L. calligaster</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td></td>
<td>getula</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southern</td>
<td>10</td>
<td>0.053 (45)</td>
<td>0.014 (45)</td>
<td>0.046 (1)</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.076</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern</td>
<td>10</td>
<td>0.085 (100)</td>
<td>0.069-0.098</td>
<td>0.005-0.069</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.024</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>getula</td>
<td>2</td>
<td>0.096 (20)</td>
<td>0.064 (20)</td>
<td>0.046 (1)</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. extenuatum</td>
<td>1</td>
<td>0.103 (10)</td>
<td>0.068 (10)</td>
<td>0.069 (2)</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.011</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.066-0.071</td>
<td>0.067-0.071</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pyromelana</td>
<td>3</td>
<td>0.088 (30)</td>
<td>0.082 (30)</td>
<td>0.094 (6)</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.003-0.096</td>
<td>0.009-0.096</td>
<td></td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.003-0.099</td>
<td>0.003-0.099</td>
<td></td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.073-0.096</td>
<td>0.078-0.088</td>
<td>0.101-0.110</td>
<td>0.102-0.107</td>
<td>0.107-0.108</td>
<td>0.10-0.050</td>
<td></td>
</tr>
<tr>
<td>zonata</td>
<td>5</td>
<td>0.093 (50)</td>
<td>0.096 (50)</td>
<td>0.105 (10)</td>
<td>0.104 (5)</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.003-0.096</td>
<td>0.009-0.096</td>
<td>0.010-0.107</td>
<td>0.012-0.107</td>
<td>0.013-0.105</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.003-0.099</td>
<td>0.003-0.099</td>
<td>0.003-0.099</td>
<td>0.003-0.099</td>
<td>0.003-0.099</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.073-0.087</td>
<td>0.078-0.088</td>
<td>0.101-0.110</td>
<td>0.102-0.107</td>
<td>0.107-0.108</td>
<td>0.10-0.050</td>
<td></td>
</tr>
<tr>
<td>L. calligaster</td>
<td>1</td>
<td>0.105 (10)</td>
<td>0.091 (10)</td>
<td>0.092 (2)</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.099-0.110</td>
<td>0.089-0.095</td>
<td>0.090-0.094</td>
<td>0.097 (3)</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.103 (5)</td>
<td></td>
<td></td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.097-0.109</td>
<td>0.097-0.109</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
harmonic mean of $-4316.67$. This is in comparison with the
$2(GTR + \Gamma + I)$ and the $GTR + \Gamma + I$ 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, $4(GTR + \Gamma)$,
$2(GTR + \Gamma)$, and $GTR + \Gamma + I$, produced the following
three $\ln L$ scores: $-4366.75$, $-4513.26$, and $-4508.55$. Again,
Bayes factors chose the most parameter rich model,
$4(GTR + \Gamma + I)$, 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 di-

terent clades with similar measures of support. 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, $4(GTR + \Gamma)$,
$2(GTR + \Gamma)$, and $GTR + \Gamma + I$, produced the following
three $\ln L$ scores: $-4366.75$, $-4513.26$, and $-4508.55$. Again,
Bayes factors chose the most parameter rich model,
$4(GTR + \Gamma + I)$, 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 hypothe-
sis. 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. celenops, 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 pyrom-
elana, 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. calligas-
ter, 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 analy-
yses 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. 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.
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*
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; Rodriguez-Robles and De Jesus-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). Gartsa (1982) hypothesized that the L. mexicana group (composed 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. Gartsa (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 Gartsa’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 Cerros-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 homoplasic 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.

Acknowledgments

We thank J. Banda-Leal, G. Ulises de la Rosa-Lozano, D. Hartman, and M.J. Ingrasci for assistance in the field; S. Draper, J.W. Fetzner, Jr., T. Gebhard, D. Hartman, M.J. Ingrasci, A. Kardon, D. Lazcano, K.H. Peterson, G.R. Queen, G. Quintero-Díaz, G.T. Salmon, and J. Vázquez-Díaz for graciously providing tissue samples from animals in their collections; T.D. Hibbits for the photos of *L. alterna* and *L. t. celaenops*; K. de Queiroz and several anonymous reviewers for providing comments that greatly improved the final manuscript; and T. Allen, R. Applegate, J.A. Campbell, J. Cousins, J.D. Dixson, J.W. Fetzner, Jr., R.H. Hibbits, M.J. Ingrasci, D. Lazcano, A. Marquardt, D. McHenry, J.M. Mueller, L. Porras, A.M. Powell, M.S. Price, G.T. Salmon, H.M. Smith, K.L. Sternes, and K. Yaguchi for assistance with various other aspects of this project. Collecting was conducted under permits granted to D. Lazcano and RWB by SEMARNAT.

References


