Phylogenetics, Phylogeography, Historical Demography, and Morphology of Milksnakes (Genus *Lampropeltis*)

by

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A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2013

This manuscript has been read and accepted by the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

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A primary goal for systematists is the discovery and identification of Earth's biodiversity. To meet these goals, coalescent-based phylogenetic and phylogeographic methods have been developed that help quantify diversity and provide a better understanding of processes generating species. Here, I use these methods to examine milksnakes (genus *Lampropeltis*). Milksnakes are among the widest ranging of New World squamates and are frequently used as model organisms in Batesian mimicry studies. Despite this, there has been no research detailing their systematics within a molecular phylogenetic framework. Furthermore, previous molecular studies that include milksnakes have shown they do not form a monophyletic group within the genus and that color pattern, which has been the basis for their previous classification may not be informative with respect to evolutionary history. The study detailed here examines milksnakes across their intercontinental range to determine whether there is unrecognized diversity in this group. To meet this goal, I have developed a multi-locus dataset consisting of 11 nuclear genes and one mitochondrial gene. I use a molecular delimitation method to determine support for putative species, assess migration between species, and, within a species-tree framework, infer a phylogeny for the genus *Lampropeltis*, including all newly elevated milksnakes and all other recognized taxa within the genus. This dataset also provides the opportunity to examine how the inclusion of cryptic taxa and species tree methods provide a more accurate assessment of timing, rates and process of species diversification. After delimiting species, I use both non-genealogical and coalescent-based models to infer the historical demographic patterns of milksnakes. As the milksnake species identified here have representatives in both the temperate and tropical Americas, I am able compare demographic responses with respect to changes in effective population size between the Nearctic and Neotropical taxa. Specifically, I ask, do closely related milksnake species have similar demographic patterns resulting from Pleistocene glacial cycles despite their origination in the Neotropics or Nearctic. Results indicate that milksnakes do not share similar demographic patterns as a taxonomic group or by area and I conclude that authors should use caution in generalizing historical demographic trends based on geography. In addition to examining milksnakes using molecular data, I determine if there are detectable morphological differences independent of color pattern using geometric morphometrics. By taking photographs of 487 specimens, digitizing landmarks on their heads, and using geometric morphometric analyses, I determine that these types of analyses are useful in snake systematics for detecting distinct morphological shape variation among genetically delimited species, although they may not be powerful enough to use as an *a priori* species discovery tool.

Acknowledgements

I would like to thank my dissertation committee members Drs. Michael J. Hickerson, Jason Munshi-South, Eugenia Naro-Maciel, Christopher J. Raxworthy, and especially my dissertation advisor, Dr. Frank T. Burbrink, whose support and advisement made this study possible.

Thank you to all past and present members of the Burbrink Lab (E. Myers, X. Chen., A. McKelvy, L. Jones, H. Yao, J. Burgoon, T. Guiher, A. Pyron), who have helped me in countless ways. A special thanks to E. Myers, whose comments on previous versions of this dissertation substantially improved it.

I would also like to thank the following for providing tissues and support during this project: American Museum of Natural History (D. Frost, C. Raxworthy, D. Kizirian, J. Feinstein) Louisiana State University Museum of Natural Sciences (J. Boundy, D. Dittman, R. Brumfeld, F. Sheldon), Museum of Vertebrate Zoology (J. McGuire, C. Spencer), the University of Texas, Arlington (J. Campbell, E. Smith, C. Franklin, C. Cox, J. Streicher, and R. Jadin), the Texas Cooperative Wildlife Collection, Texas A&M University (T. Hibbitts), the Senckenburg Museum of Natural History (Ulrich Kuch), the United States National Museum (R. McDiarmid and K. de Queiroz), the North Carolina Museum Natural Sciences (J. Beane, C. Fisher), the University of Alabama (L. Rissler), the Peabody Museum, Yale University (G. Watkins-Colwell), the Florida Museum of Natural History (K. Krysko), the Texas Natural History Collection, the University of Texas, Austin (D. Cannatella, T. LaDuc, D. Hall), Southeastern Louisiana Unviersity (B. Crother), the Sternberg Museum, Fort Hays State University (T. Taggart, C. Schmidt, J. Collins), Swaim Biological, Inc. (K. Swaim), La Mica Biological Station (J. Ray), the University of Colorado Museum of Natural History (J. Lemos-Espinal), the Illinois

Natural History Survey (C. Phillips), P. Warny, D. Shepard, L. Vitt, K. Irwin, R. Hansen, B. Bryson, D. Mulcahy, G. Weatherman, J. Harrison, G. Hancock, R. Ruane, B. Ruane, M. Blattberg, L. Clampitt, M. Ryan, D. Finnegan, J. Briggler, R. Highton, D. Heath, C. Stephen, K. Lodrigue, O. Torres Carjaval, S. Ballard, R. King, B. German, U. O. Garcia-Vasquez, the late F. Mendoza-Quijano, I. Solano-Zavaleta, R. Bezy, E. Enderson,, M. Torroco, I. White Murray, W. Howell, G. Salmon, M. Ingrasci, A. Richmond, A. Stengle, C. Wollney, M. Walker, B. Edmond, A. Williams, R. Lovich, R. Gassaway, T. Tyning, J. Iverson, M. Graziano, J. Tucker, C. Jimenez, R. Staub, N. Howe, F. Fontanella, J. Rowell, P. Frank, S. Marshall, and R. Walsh.

Funding for this research was provided in part by the American Museum of Natural History (Theodore Roosevelt Memorial Fund), Graduate Women in Science (Vanessa Notchev Fund), The Explorer’s Club, and CUNY-PSC. This research was also supported, in part, under National Science Foundation Grants CNS-0958379 and CNS-0855217 and the City University of New York High Performance Computing Center, DBI-0905765 to R. Pyron., and for samples granted from the University of Texas at Arlington, support was granted by DEB-0613802 to J. Campbell.

A special thanks to my Grama Bell, Catherine F. Ruane, who never failed to encourage my love of nature and of snakes in particular.

1925-2010

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Introduction to the
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and Morphology of Milksnakes (Genus *Lampropeltis*)

A primary goal for systematists is the discovery and identification of Earth's biodiversity. In the past 20+ years, the molecular revolution has provided systematists with the tools (e.g., PCR, direct sequencing of DNA, and computational methods) necessary to identify cryptic species. Although cryptic organisms may appear morphologically indistinguishable, they have independent evolutionary trajectories marked by deep molecular divergence (Burbrink 2002, Guiher and Burbrink 2008; Pyron and Burbrink 2009a). To meet these goals, coalescent-based phylogeographic methods have helped quantify cryptic diversity and provided a better understanding of processes generating species (Losos and Glor 2003; Leaché and Fujita 2010; Yang and Rannala 2010). Properly quantifying cryptic diversity improves taxonomy, which in turn provides the basis for comparative ecological, behavioral and evolutionary studies (Bickford et al. 2007).

Similarly, coalescent-based methods can also address how population demographics fluctuate over time given historical changes in the environment. In many Holarctic species, Pleistocene glacial cycles are a major factor influencing speciation and changes in population size (Hewitt 1999, 2000, 2004a,b; Schmitt 2007; Shafer et al. 2010). The general dynamic suggests that at glacial maxima population sizes contracted as they were pushed into refugia, while at glacial minima they expanded (Hewitt 2000, 2004a,b). Glaciers may also have isolated formerly contiguous populations resulting in lineage divergence (Haffer 1969; Pielou 1979; Avise and Walker 1998; Avise et al. 1998; Lessa et al. 2003; Guiher and Burbrink 2008). Using coalescent methods, it is possible to extract demographic patterns through time and examine the impact that geographical barriers or environmental changes have had on the distribution of lineages and changes in population sizes (Kingman 1982a,b; Avise et al. 1987, 1998; Hudson 1990; Harding 1996; Neigel 1996; Avise 2000; Edwards and Beerli 2000).

To address both species discovery and historical demography, studies that utilize multiple, independent nuclear loci are becoming the standard for phylogeographic analyses (Brito and Edwards 2009). Many programs are available that incorporate multilocus datasets and a multispecies coalescent model to delimit species, infer phylogeny, and extract demographic trends (e.g., Extended Bayesian Skyline Plots, Heled and Dummond 2008; *BEAST, Heled and Dummond 2010; BPP, Yang and Rannala 2010). For poorly studied organisms that are initially ambiguous with respect to morphology, a molecular approach may be the most efficient and appropriate way to differentiate species and can then be followed by further analyses that include divergence dating, diversification patterns, assessments of migration, and historical demography. Furthermore, while molecular evidence alone is considered sufficient for species differentiation (de Queiroz 2007), once cryptic species have been identified it is possible that morphological differences among taxa that can be detected and that aid in an integrative approach to taxonomy (Padial et al. 2010).

Study System

The milksnake (*Lampropeltis triangulum*) is one of the most well known of all North American (NA) snakes due, in part to their vibrant, striking color patterns (Fig. 1) and resemblance to the dangerously venomous coral snakes (*Micrurus* and *Micruroides*). They are an exemplar for studying the evolution of Batesian mimicry in vertebrates (Brattstrom 1955; Grobman 1978;

Greene and McDiarmid 1981; Brodie 1993; Brodie and Brodie 2004; Pyron and Burbrink 2009b). Despite the popularity of milksnakes in mimicry studies, there has been little work examining the taxonomy of these snakes with respect to phylogeographic structure. Following previous taxonomy, milksnakes consist of 25 subspecies ranging from southeastern Canada, across the United States to the Rocky Mountains, and south to Ecuador; the inter-continental range of the milksnake is among the largest of any NW squamate (Williams 1988). However, recent studies have indicated that milksnakes may not form a monophyletic group with respect to other species in the genus *Lampropeltis*. Additionally mitochondrial DNA (mtDNA) and single copy nuclear gene (scnDNA) trees result in differing phylogenetic placement of milksnakes within *Lampropeltis* (Bryson et al. 2007, Harper and Pfennig 2008; Pyron and Burbrink 2009c). However none of these studies focused on milksnakes specifically and all relied on using gene trees rather than species trees, which may not reflect the evolutionary history of the species (Edwards 2009).

The most recent review of *L. triangulum* is from Williams (1988). This monograph, detailing the systematics and natural history of each subspecies, is the most extensive review to date of milksnakes and expounds upon an earlier edition of the same book (Williams 1978). Previous to this text, the only full treatment of the species was Blanchard’s 1921 monograph of the entire genus *Lampropeltis*. Both of these texts rely on highly variable and unreliable color characters to name subspecies, although it has been more recently demonstrated that color pattern is an unreliable indicator of evolutionary history for Lampropeltini (Rodriguez-Robles et al. 1999; Burbrink et al. 2000). These subspecies are found in a variety of habitats including temperate deciduous forest, long-leaf pine woods, grasslands and prairies, desert, and sub-tropical to tropical habitat in Central and South America. Milksnakes also display a wide variety of color patterns, sizes, and diets across this range. Total adult body size for *L. triangulum* ranges from 500-1900 mm; in turn smaller milksnakes specialize on ectothermic prey, while larger snakes consume a mixed or predominantly endothermic diet (Fitch and Fleet 1970; Dyrcaz 1977; Brown 1979; Williams, 1988; Palmer and Braswell 1995; Rodriguez and Drummond 2000; Pyron and Burbrink 2009b). The morphological and ecological diversity found within *L. triangulum* alone suggests that the milksnake is likely composed of multiple undescribed species.

Goals

The study detailed here consists of three chapters. Chapter 1 examines milksnakes across their range to determine whether there is unrecognized diversity in this group. To meet this goal, I have developed a multi-locus dataset consisting of 11 nuclear loci and one mitochondrial gene. I use a molecular delimitation method to determine support for putative species, assess migration between species, and, within a species-tree framework, infer a phylogeny for the genus *Lampropeltis*, including all newly elevated milksnakes and all other recognized taxa within the genus. This dataset also provides the opportunity to examine how the inclusion of cryptic taxa and species tree methods provide a more accurate assessment of timing, rates and process of species diversification. In Chapter 2, I use both non-genealogical and coalescent-based models to infer the historical demographic patterns of milksnakes. As the milksnake species identified here have representatives in both the temperate and tropical Americas, I am able compare demographic responses with respect to changes in N_e between the Nearctic and Neotropical taxa. Specifically, I ask, do closely related milksnake species have similar demographic patterns resulting from Pleistocene glacial cycles despite their origination in the Neotropical or Nearctic regions. Results indicate that milksnakes do not share similar demographic patterns as a taxonomic group or by area and I conclude that authors should use caution in generalizing historical demographic trends based on geography. Chapter 3 is a geometric morphometrics analysis that determines if there are differences among milksnake species with respect to head shape. By taking photographs of 487 specimens, digitizing landmarks on their heads, and using geometric morphometric analyses, I determine that these types of analyses are useful in snake systematics for detecting distinct morphological shape variation among genetically delimited species, although they may not be powerful enough to use as an *a priori* species discovery tool.

Chapter 1

Gene-tree Discordance and Unrecognized Diversity Mislead Phylogenetic Comparative Analyses in Milksnakes and Kingsnakes (Genus *Lampropeltis*)

Abstract

Both gene-tree discordance and unrecognized diversity are sources of error for accurate estimation of species trees and can affect downstream analyses by obscuring the true number of nodes, their density, and the lengths of the branches subtending them. While the theoretical impact of gene-tree discordance on evolutionary analyses is well-defined, the effect of unsampled or cryptic diversity is not. Here I examine how the inclusion of previously unrecognized species of milksnakes and kingsnakes (genus *Lampropeltis*) and a species-tree approach affects divergence-time estimation for the genus. I delimit six additional species within the group, increasing the diversity of *Lampropeltis* by 40% and find both genealogical and temporal discordance between gene trees and the species tree. In terms of evolutionary inference, the tempo and mode of diversification shifts from a strongly supported early burst of Miocene diversification as inferred using a concatenated gene tree to more even diversification throughout the Pliocene and Pleistocene when inferred using multi-locus species-tree estimation. This study highlights the crucial interaction between phylogeography and systematics, and the importance of species delimitation when inferring species trees for accurate inference of evolutionary patterns of diversity, particularly at recent time-scales.

It is known that properly estimating a phylogeny requires the inclusion of all extant species for the group of interest, but the affect of missing taxa on downstream analyses is less well-studied. Missing species decrease accuracy in determining relationships among taxa, inferring evolutionary models, and estimating node density (reviewed in Heath et al. 2008; Nabhan and Sarkar 2012). Phylogenetic inaccuracy due to missing species can then negatively impact downstream comparative phylogenetic analyses because missing taxa will likely increase the overall divergence time estimates due to an oversampling of deep nodes (Cusimano and Renner 2010). Measures that quantify diversification will then incorrectly indicate that rates of speciation have decreased towards the present (Pybus and Harvey 2000; Cusimano and Renner 2010).

One major source of missing taxa in phylogenies is due to unrecognized and/or cryptic diversity, which causes an underestimation of the true number of nodes in the species tree, as well as their density. This also affects evolutionary inferences, particularly the tempo and mode of diversification, as these analyses are based on the relative timing of speciation events. For many groups, taxonomy is unclear and new species are still being discovered (e.g., vipers, Stümpel and Joger 2009; tree shrews, Roberts et al. 2011; gobies, Vanhove et al. 2012), indicating that all taxa have not been included in previous phylogenies. Therefore, unrecognized species present a real problem when using phylogenies to test diversification timing. As these species are often morphologically cryptic, they depend on molecular methods for discovery and delimitation (e.g., Leaché and Fujita 2010; Burbrink et al. 2011; Camargo et al. 2012).

There are several methods for verifying species delimitation hypotheses using multilocus datasets within a multispecies coalescent framework (reviewed in Fujita et al. 2012). Using parameters such as effective population size and branching times (Rannala and Yang 2003), these delimitation methods do not assume that species are reciprocally monophyletic across all loci, allow for gene-tree discordance due to incomplete lineage sorting, and are able to detect speciation at recent time scales (Fujita et al. 2012). One of the most frequently used multispecies coalescent delimitation programs is Bayesian Phylogenetics and Phylogeography (BPP; Yang and Rannala 2010).This method requires a user-specified guide tree of the proposed species and their topological relationships. Accurate results rely on a phylogenetically meaningful guide tree (Leaché and Fujita 2010), which can come from various external sources, such as gene trees, population structure analyses, subspecies designations, or morphology. Once delimitation is complete, well-supported taxa can be used to estimate a species tree using various methods.

An additional source of error in phylogenies is the use of gene trees rather than species trees. Gene-tree discordance and the misleading effects of concatenation have well-known effects on species-tree analyses (Edwards 2009), but the use of gene trees is also likely to add to the problem of divergence-time overestimation and subsequently influence downstream analyses. Since genes diverge earlier than species/populations, gene-tree methods will incorrectly estimate older branching times, which are proportionally greater than the species divergence at the youngest nodes (Edwards and Beerli 2000; Carstens and Knowles 2007; Burbrink and Pyron 2011). This has been demonstrated empirically in *Aphelocoma* jays where dates from gene trees are older than those from species trees; while the gene trees result in pre-Pleistocene diversification for this group, the species tree indicates that speciation occurred mostly in the Pleistocene (McCormack et al. 2011). Missing taxa and the failure to use species-tree methods can therefore yield overall older divergence time estimates producing a decrease in rates of speciation (Rabosky and Lovette 2008; Burbrink and Pyron 2011; McCormack et al. 2011).

Here, I examine how missing taxa and gene-tree approaches confound the inference of evolutionary processes in New World kingsnakes and milksnakes (*Lampropeltis*). Recent studies have demonstrated that many wide-ranging species within the tribe Lampropeltini consist of multiple distinct species (Burbrink et al. 2000; Rodríguez-Robles and De Jesús-Escobar 2000; Burbrink 2002; Bryson et al. 2007; Pyron and Burbrink 2009a,b) and that unlike many other North American taxa (e.g., Rand 1948; Avise 2000; Johnson and Cicero 2004), diversification in this tribe occurred mostly during the Miocene and Pliocene, with few species originating during the Pleistocene (Pyron and Burbrink 2009c; Bubrink and Pyron 2010). The genus *Lampropeltis* is the most diverse within the tribe and five species of *Lampropeltis* have recently been re-elevated (Pyron and Burbrink 2009b; Bubrink et al. 2011).

Surprisingly, the milksnake (*L. triangulum*), which is the widest-ranging and most morphologically diverse species in Lampropeltini, has never been the focus of a molecular phylogenetic study, although previous work indicates milksnakes may not be a monophyletic group with respect to other species in the genus *Lampropeltis* (Bryson et al. 2007; Harper and Pfennig 2008; Pyron and Burbrink 2009c). Milksnakes consist of 25 subspecies ranging from southeastern Canada, across the United States to the Rocky Mountains, and south to Ecuador (Williams 1988). They are found in many habitat types, including temperate forests, grasslands, and tropical thorn-scrub, and display a wide variety of color patterns, body sizes, and diets across this range (Brown 1979; Dyrkacz 1977; Fitch and Fleet 1970; Williams 1988; Palmer and Braswell 1995; Rodríguez and Drummond 2000; Pyron and Burbrink 2009a). This extensive geographic range, as well as the morphological and ecological diversity found within *L. triangulum* alone, suggests the milksnake is composed of multiple unrecognized species.

To examine species diversity and timing of diversification in *Lampropeltis*, I use multiple loci and individuals spanning the geographic range of *L. triangulum* to perform Bayesian species delimitation. I also include representatives from all taxa in *Lampropeltis* to then infer a species tree for the genus. Specifically, I delimit taxa using coalescent methods based on guide trees from both a mitochondrial gene tree and from a population structure analysis. I find genealogical discordance in the mitochondrial dataset, which misleads inference of the *Lampropeltis* phylogeny. Proper delimitation in *Lampropeltis* reveals 7 taxa within the former *L. triangulum* in three unrelated species groups. The species tree for *Lampropeltis* reveals recent Pliocene and Pleistocene diversification, in stark contrast to the early burst of Miocene diversification inferred using concatenated datasets. This study demonstrates the importance of delimiting cryptic or unrecognized taxa for use with species tree methods to properly understand the tempo of diversification and other downstream comparative analyses.

Materials and Methods

Data Collection

I obtained genetic samples for 276 milksnakes ranging across North America, Central America, and South America, including 22 of the 25 subspecies (Fig. 2), as well as 49 samples representing the remaining 14 species in the genus *Lampropeltis* (≥ 2 individuals for each species, following Pyron and Burbrink 2009a,c; Burbrink et al. 2011) and two samples each for the outgroup taxa, *Arizona elegans* and *Cemophora coccinea* (Pyron and Burbrink 2009c; Appendix 1). *Lampropeltis elapsoides* (formerly *L. triangulum elapsoides*) is included as a milksnake here due to its historical placement as a subspecies and to further clarify its position in the genus. DNA was extracted using Qiagen® DNEasy kits (tissue protocol) to obtain genomic DNA from samples of shed skin, liver, muscle tissue, or whole blood. I optimized the amplification and sequencing protocols for one mtDNA gene (Cytochrome b; Cytb) and eleven single-copy nuclear markers (scnDNA), including five anonymous loci developed using the protocol from Noonan and Yoder (2009). Details for loci, primers, and PCR protocols are listed in Appendix 2. For Cytb I amplified and sequenced all individuals. For the scnDNA loci, I amplified and sequenced a subset of individuals (n = 124). These individuals were chosen to represent a wide geographic range within the US, and included all samples from Mexico, Central, and South America, as well as ≥ two individuals from all *Lampropeltis* species (Appendix 1). All sequences were aligned by eye using SEQUENCHER 4.5 (Genecodes 2000) and no gaps were found in any of the protein coding genes. The phase of heterozygous genotypes was resolved using PHASE v2.1.1 (Stephens and Donnelly 2003) and the most probable pair of alleles was used for each heterozygous individual. For all genes and subsequent analyses, I determined the most appropriate substitution model using Bayesian Information Criterion in the program jModeltest (Table 1; Posada, 2008); where applicable, protein coding genes were partitioned by codon, allowing each base pair position to have a unique substitution rate.

Migration

I examined rates of migration in order to have a better understanding of how much gene flow occurs among the resulting geographically adjacent milksnake species using migrate-n v.3.2.16 (Beerli 2008). The delimitation program BPP only assesses error due to incomplete lineage sorting, meaning migration between lineages may violate assumptions (Zhang et al. 2011). Results also provide evidence as to whether lineages should be treated as independent species.

Migrate-n was run using the complete scnDNA dataset. Each migrate-n analysis was run for $1x10^4$ generations with four Markov-chains, sampled every100th generation, and with the first 25% of samples discarded as burnin. A mean generation time for *Lampropeltis* of 2.5 years was used for analyses (Ernst and Ernst 2003; Werler and Dixon 2000). Each run was repeated four times with different starting seeds to ensure consistency between runs.

In addition to the migrate analyses, I constructed a scnDNA network for resulting lineages using Splitstree v.4.12.3 (Huson and Bryant 2006) with the neighbor net algorithm. Networks can further help visualize reticulate relationships among taxa and possible mixed ancestry or hybrid individuals.

Species Delimitation

To address species delimitation, I used BPP (Yang and Rannala 2010). BPP is a genealogical method that uses multiple independent loci and operates under a coalescent framework using a reversible-jump Markov chain Monte Carlo (rjMCMC) method to estimate Θ_a (effective population N_e * mutation rate μ for each species), τ_a (the time of origin for each species) and τ_d (the timing of diversification into two descendent species). Results in the form of posterior probability distributions (Pp) indicate whether two or more pre-defined lineages can be differentiated from each other. This method has already been used to delimit a number of species of squamate (Leaché and Fujita 2010; Burbrink et al. 2011; Cox et al. 2012) and has been found to be robust in simulation studies when migration levels are low (Zhang et al. 2011).

BPP was run using the algorithm 0 and I adjusted the fine-tuning parameters to ensure swapping rates ranged between 0.30 and 0.70 for each parameter, allowing the rjMCMC to mix properly among species-delimitation models. Following previous studies, (Leaché and Fujita, 2010; Burbrink et al. 2011; Cox et al. 2012), I parameterized both ancestral population size (Θ) and root age (τ_o) using a gamma (Γ) distribution (a, b) for large populations and deep divergences, Γ (1, 10), small ancestral populations and shallow divergences, Γ (2, 2000), and large ancestral populations Γ (1, 10), and shallow divergences Γ (2, 2000). A large ancestral population with shallow divergences is considered the most conservative model with respect to favoring speciation events (Leaché and Fujita, 2010). For each of these, I ran a minimum of three analyses using different starting seeds for $5x10^5$ generations with a burnin of $1.5x10^4$, and thinning every 5 generations. I ran all of these analyses using the different guide trees (see below). Following Burbrink et al. (2011), I also randomized the individuals in each clade in order to ensure that BPP results were robust.

Guide Trees

The guide trees for BPP were constructed two ways. First, using the mtDNA + scnDNA dataset (the mtDNA dataset was reduced to the same individuals used in the scnDNA dataset for all subsequent analyses, see Appendix 1), I used the program Structurama (Huelsenbeck and Andolfatto 2007) to infer groups and assign individuals to these groups. Structurama uses a Dirichlet process prior for a random number of *k* populations with the initial concentration parameter of populations set to 5 to estimate the number of groups and assignment of individuals with the highest probability. Using MCMC, I ran Structurama four times for $1x10^6$ generations, taking every 100th sample, with the first 25% of samples discarded as burnin. The appropriate value of *k* was chosen as the one with the highest posterior probability value. To generate the guide tree, the resulting populations were then treated as terminal taxa for analysis in *BEAST (Heled and Drummond 2010), with the resulting species tree used as a guide tree for the BPP analyses. *BEAST was run using both the mtDNA + scnDNA dataset and using the scnDNA dataset alone.

Second, I inferred a gene tree for Cytb using all samples as the basis for a second guide tree. The mtDNA tree was inferred in BEAST v.1.7.1, chains were run for $5x10^7$ generations, and sampled every 1000th generation. The resulting milksnake lineages from the mtDNA tree were treated as terminal taxa and run in *BEAST using the scnDNA dataset, with the resulting tree used as the guide tree in BPP analyses. All *BEAST analyses to generate guide trees were run for $1.5x10^6$ generations, sampled every 1000th generation, with a burnin of 25%.

Species Tree Estimation

To estimate the species tree I used *BEAST (Heled and Drummond 2010) implemented in BEAST v.1.7.2. This method uses a multispecies coalescent model to estimate the species tree from multiple genes and multiple individuals per species, while taking into account incomplete lineage sorting. In addition, *BEAST can use a relaxed clock model (Drummond et al. 2006) to estimate divergence times for the species tree. For this analysis, I used the scnDNA dataset consisting of the milksnake lineages recovered in the BPP analyses and all other currently recognized species in the genus *Lampropeltis*. The locus 2CL3 was not used in the *BEAST analysis because it did not amplify for all species. Due to the possibility of mtDNA introgression, which violates assumptions of admixture, I ran the species tree analysis without the Cytb dataset. Individuals were assigned to species based on the results from the BPP analyses. For the species tree and divergence time estimation, I used an uncorrelated lognormal tree prior with a Yule speciation process prior and calibrated the tree using two appropriate fossils constraints. Following Pyron and Burbrink (2009c), I enforced these soft constraints using lognormal distributions, with the age of the fossil as the mean time for the divergence at the node and a 95% prior distribution around the mean as soft bounds for the timing of bifurcation at that node. From Holman (2000) the two calibrations are as follows: 1) The divergence time between the genera *Lampropeltis* and *Cemophora* was given a mean date of 13.75 million years ago (Ma) (95% HPD: 8.4–24.4 Ma) based on the oldest known kingsnake, *L. similis*, from the medial Barstovian of the Miocene; 2) The divergence between the *L. getula* complex and *L. extenuata* was given a mean date of 6.8 Ma (95% HPD: 4.75–9.94 Ma) based on the oldest known fossils of *L. getula* and *Stilosoma* (= *Lampropeltis*) *venustum* from the middle Hemphillian of the Miocene. I ran *BEAST for 200-300 million generations, sampled every 5000 generations, and assessed burnin using Tracer v.1.5 (Drummond and Rambaut 2007). The analysis was run four times to ensure consistency among results.

Concatenated scnDNA Tree

To explore the difference in divergence times between gene trees and species trees I ran BEAST v1.7.2 using the concatenated scnDNA dataset with one representative for the same terminal taxa used in the species tree. Calibrations were the same as those used for the species tree and I ran the concatenated dataset for $5x10^7$ generations, sampling every 1000th generation. I also calculated the Robinson-Foulds distance (Robinson and Foulds 1981) between the two trees using R v2.13.1 (R Development Core Team 2009) in the package PHANGORN (Schliep 2011); this metric measures the number of bipartitions found in one tree but not the other, indicating the amount of topological discordance between trees.

Timing and Models of Species Diversification

I used the Wilcoxon signed rank test in R to determine if the concatenated tree results in significantly different mean divergence times when compared to the species tree. Following Burbrink and Pyron (2011), I calculated the scaled branching-time error by taking the difference between the mean gene-tree branching times and the mean species-tree branching times, divided by the depth of the corresponding nodes of the species tree. I regressed the scaled branching-time error against the branching times of the species tree in R. A significantly negative slope would indicate branching-time error decreases as nodes get older. To compare the temporal discordance of the concatenated tree and the species tree, I ran Pybus and Harvey's γ in the R package LASER (Rabosky 2007), which tests for early, late, or constant diversification (Pybus and Harvey 2000).

I also tested preferred models of species diversification for the concatenated tree and species tree. Using the function Misfits developed by FTB and utilizing the packages APE (Paradis et al. 2004) and LASER (Rabosky 2007) in R, I compared the nine approximate likelihood coalescent models described in Morlon et al. (2010); these models include both time-constant or time-variable rates of speciation (λ) and extinction (μ) and time-constant or time-variable diversity. I also assessed full likelihood standard models that had constant rates of speciation (Yule, Birth-Death), variable rates of speciation due to diversity dependence (DDL, DDX), variable rates of speciation due to non-density dependent factors (SPVAR, Yule2, Monotonic Decay, Hyberbolic Decay), variable extinction rates (EXVAR), or both variable speciation and extinction rates (BOTHVAR). Because the standard density dependent models do not account for extinction, I also tested maximum likelihood models that include extinction as an additional parameter for Diversity Dependence (DD+E) against Birth-Death (BD), and Shift-

Point (which allows for a shift in the parameters at a time point and is equivalent to Yule2 with extinction) in the R package DDD (Etienne et al. 2012). The best fitting model for each of the standard models, the coalescent models, and the models with extinction was determined by calculating corrected AIC values for small sample sizes (AICc; Burnham and Anderson 2002).

Results

Loci

Cytochrome b was sequenced for 329 individuals (full or partial coverage; 448 variable sites for *Lampropeltis* and 341 variable sites for milksnakes). The scnDNA loci for the subset of 124 samples resulted in 100 to 124 sequences for each gene, with a maximum of 69 and a minimum of 20 variable sites for *Lampropeltis* and a maximum of 56 and a minimum of 15 variable sites for milksnakes (Table 1; Appendices 1, 3). All sequences are deposited on GenBank (Accession numbers: XXX) and all trees are available from TreeBASE (XXX).

Migration, Guide Trees, and Delimitation

Migrate-n analyses indicated low migration between resulting species of milksnakes and so BPP should give robust results with respect to delimitation (Zhang et al. 2011); results were nearly identical between the independent runs and so results from one run are shown (Table 2). Although still low (0.45 individuals/generation, HPD = 0.06-1.22), migration from the Eastern lineage into the Western lineage was highest, followed by migration from the Tamaulipas lineage into the Western lineage (0.31 individuals/generation, HPD = 0.01-0.88). The lowest migration rates were found among the Mexico (MX), Tamaulipas, Central America (CA), and South

America (SA) lineages (≤ 0.06 individuals/generation, HPDs = 0.00-0.30). There is also little migration between *L. alterna* and the Western lineage (≤ 0.17 individuals/generation, HPDs = 0.00-0.55).

The Cytb tree generated by BEAST recovered 11 well-supported milksnake clades (Fig. 2a, c); three mainly in the US (Eastern, Western, *L. elapsoides*), five in Mexico (Colima, Guerrero, Oaxaca, Puebla, Sonora), two mainly in Central America (Honduras, Nicaragua), and one mainly in South America (South America). Many of these clades are not sister taxa (Fig. 2a) and do not form a monophyletic group with respect to other species in the genus *Lampropeltis*. *Lampropeltis alterna*, a species not traditionally allied with milksnakes, was found to have mtDNA haplotypes very similar (<0.2% divergence between sequences) to those of milksnakes in western Texas. This resulted in all *L. alterna* samples (both from Texas and Mexico) being included within the Western lineage (Fig. 2a). One sample identified as *L. mexicana* (RB4) from Nuevo Leon, Mexico was also recovered as a member of the same Western milksnake clade. Because it is the only individual showing this pattern available to us I did not include it in further analyses (but see Bryson et al. 2007).

When BPP was run using a guide tree consisting of the 11 Cytb milksnake lineages (and *L. alterna*), all 11 lineages + *L. alterna* were recovered as distinct species with high support values (Pp ≥ 95%) for the models with less conservative speciation priors (Table 3). However, the combination of a large ancestral population and shallow divergences resulted in lower support values for the divergences within some of the Mexican and Central American Cytb lineages (Table 3). Randomizations of individuals into the clades resulted in high support (Pp ≥ 98%) of all nodes collapsed, indicating that BPP is identifying legitimate speciation events in the analyses.

Structurama results indicated the highest Pp (58%) for seven populations: six populations of milksnake (*L. elapsoides*, Eastern/Western, Tamaulipas, Mexico, Central America, South America) plus *L. alterna*. The populations recovered by Structurama had individuals assigned to them with high support, (Pp ≥ 98%; see Appendix 1) and resulted in a lower number of milksnake groups than identified in the Cytb tree. *Lampropeltis alterna* was found to be a distinct population by Structurama and was not included within any groups of milksnakes. Additionally, Structurama recovered a group of milksnakes that were part of the Western lineage in the Cytb tree. This group, (the Tamaulipas lineage), was also found to be distinct in the BPP analyses (Pp = 100%), despite not being a separate lineage in the Cytb gene tree. Results from all BPP analyses based on the Structurama groupings indicate high support for the six milksnake groups and *L. alterna* as separate species (Table 3).

I ran three additional BPP runs, using the same parameters previously specified to explicitly test if 1) *L. alterna* could be delimited from the Western milksnake lineage and 2) if the Tamaulipas milksnake lineage could be delimited from the Western milksnake lineage, and 3) if the Eastern and Western milksnake lineages could be delimited from one another. All of these runs resulted in Pp = 100% for each as a separate species (Table 3).

The network constructed from the scnDNA dataset resulted in similar groups as the Structurama results (Fig. 3). An individual from Guerrero, MX (AMNH21940) and one from Veracruz, MX (AMNH22617) that were classified in the CA group by Structurama were found mixed in among MX lineage individuals. The MX clade contained more variation than did the others and showed some mixing of alleles between the MX and CA clades, although may be an artifact of the MX lineage having the most representatives in the network (Fig. 3). The Eastern and Western clades formed two groups within the network, but with one Eastern individual (FTB442) among the Western individuals (Fig. 3).

Species Tree

Burnin for the species tree was determined using Tracer v1.5 (Rambaut and Drummond 2007) and the first 25% of samples were discarded for all replicates of the analysis. The effective sample sizes for many parameters were high (>200) and nearly identical topologies and dates between the four runs suggests stability with respect to the species relationships and timing of divergence events; as results were extremely similar between runs, I show the resulting species tree chronogram from one *BEAST analysis (Figs. 2, 4). Results from the *BEAST analysis, using the delimited species (Table 4), indicate that milksnakes do not form a monophyletic species complex and the species tree topology is incongruent with both the Cytb gene tree (Fig. 2) and the scnDNA concatenated tree (Fig. 4). Despite being recovered by delimitation analysis, I declined to use the lineages recovered by only the Cytb gene tree in the species tree analysis due to the lower support values under the more conservative BPP speciation model, the small sample sizes for several of the clades (~3 individuals in some clades, see Appendix 1), and limited geographic coverage of these clades (see discussion). Unlike the Cytb gene tree, the species tree placed *L. alterna* in a more traditional arrangement (based on morphological data) as sister to *L. mexicana* (Gehlbach and Baker 1962), not sister to any milksnake lineage (Fig. 2b). The species tree supports (≥95% Pp) two main clades that diverged ~8 Ma; one clade contains *L. calligaster*, *L. extenuata*, and the *L. getula* complex, while the other contains all the milksnake lineages and a well supported (≥95% Pp) monophyletic clade of mountain and Mexican kingsnakes (Fig. 2b). Speciation within *Lampropeltis* took place mostly during the Pleistocene

(15 extant species), with five species originating in the Pliocene and one in the late Miocene (Fig. 2b).

Concatenated scnDNA Tree and Timing of Diversification

Burnin for the concatenated tree was determined using Tracer v1.5 (Drummond and Rambaut 2007) and the first 25% of samples were discarded. Both the topology and divergence times differed between the concatenated and species trees. Comparing the species tree to the concatenated tree resulted in a Robinson-Foulds distance = 14, indicating that a total of 14 bipartitions are unique to one tree and not found in the other, although this score may be amplified by taxa whose placement is poorly supported in both trees (e.g., *L. elapsoides*). Divergence dates estimated using the scnDNA concatenated tree (Fig. 4) resulted in mean divergence times that were significantly older when compared to the species tree (Wilcoxon signed rank test, $Z = 3.883$, d.f. = 20, $P < 0.001$), with the linear regression of branching-time error against species-tree branching time indicating significantly higher error at younger nodes ($t = -4.298$, d.f. = 18, $P < 0.001$, $r = -0.5064$; Fig. 5). The divergence times from the concatenated tree show a similar number of extant species diverging during the Pleistocene (9 species) and the Pliocence (10 species), and two species originating in the Miocene (Fig. 4). The species tree resulted in a non-significant γ (-1.11; $P = 0.13$), indicating that diversification has been constant through time (Pybus and Harvey 2000). In contrast, the scnDNA concatenated tree resulted in a significantly negative γ (-2.44; $P < 0.01$), meaning that most divergence events took place further in the past and diversification has slowed down towards the present.

The best-fitting diversification models for the species tree differed from those for the concatenated tree, with the exception of the extinction models, where DD+E had the lowest

AICc values for both trees and indicates that diversification is diversity dependent, although the estimated speciation rates, extinction rates, and carrying capacity differed between the two (Table 5). The best standard model for the species tree was Yule2, which indicated a shift in speciation rates at ~1 Ma from $\lambda = 0.440$ to $\lambda = 0.090$ and the best coalescent model was Model 6, indicating expanding diversity through time with no extinction and an exponentially declining speciation rate (Table 5). For the concatenated tree, the best fitting standard model was DDL, which indicates that diversification is density dependent, and the best-fitting coalescent model was Model 5, which is equivalent to a Yule process with a constant birth-rate through time (Table 5).

Discussion

Accurately inferring a species tree and estimating divergence times relies on the inclusion of all extant species. In particular, missing taxa may cause an overestimation of deep nodes, which results in a bias towards slowdowns in diversification rates (Cusimano and Renner 2010). Furthermore, divergence time-estimation using gene trees rather than species trees results in older divergence date estimates at all nodes due to gene divergence predating speciation events (Edwards and Beerli 2000; McCormack et al. 2011). Here, I find delineating unrecognized species of milksnakes and then using a species-tree approach to estimate divergence times for *Lampropeltis* results in younger speciation times when compared to gene-tree methods or studies with higher levels of incomplete taxon sampling (Pyron and Burbrink 2009c; Burbrink and Pyron 2010). In contrast to these previous studies, the mean divergence dates estimated using the species tree suggests that diversification was at its highest during the Pleistocene and Pliocene for the genus *Lampropeltis*. In addition, I detect no slowdown in diversification for this genus using the γ statistic, although best-fitting diversification models do indicate that speciation rates have declined over time. My results underscore the necessity to include all extant taxa in species tree analyses and the importance of using multispecies coalescent-based methods to infer phylogenies, not gene trees.

Species Delimitation and Phylogeny

The Cytb tree and Structurama reveal previously unrecognized diversity within *L. triangulum*, with the Cytb tree indicating 11 milksnake lineages and Structurama finding six groups. Because of the small number of individuals in some of the Cytb clades (Appendix 1) and their corresponding low geographic coverage, it seems premature to describe all the Cytb lineages as species. Simulations using BPP show that the number of samples from a population affects the accuracy of the program and that analyses with lower numbers of individuals give less robust results (Zhang et al. 2011). The lower support values from BPP when using the large ancestral population size and shallow divergence speciation model for some lineages also suggest that it is premature to consider these independent species (Table 3).

While I acknowledge that there may be additional species of milksnake, particularly from Mexico, that should be delimited after more intensive sampling efforts are made, I recommend that seven milksnake species be recognized at this time (Table 4). These include the six milksnake groups found by Structurama that were also supported in the BPP analyses (Table 3), with distinction between the Eastern and Western lineages found in the Cytb tree. The network also indicates seven milksnake groups distinct from *L. alterna*, although there is some mixing of individuals from the CA and MX lineages (AMNH21940 and AMNH22617) and Eastern and

Western lineages (FTB442; Fig. 3); this may be due to incomplete lineage sorting within the nuclear loci, or low levels of migration that were not detectable using migrate-n analyses (Table 2).

The seven species that I propose as distinct have all been described as full species before being synonimized with *L. triangulum* and are as follows: *L. triangulum* (Lacépède 1788), *L. gentilis* (Baird and Girard 1853), *L. elapsoides* (Holbrook 1838), *L. annulata* Kennicot 1861, *L. polyzona* Cope 1861, *L. abnorma* (Bocourt 1866), and *L. micropholis* Cope 1861 (species designations and distributions in Table 4 and Fig. 2d; see Appendix 3 details). I refer to the seven milksnake species by their species name throughout the rest of the discussion.

These species of milksnakes do not form a monophyletic clade in the species-tree analysis (Fig. 2). Not only have milksnakes been incorrectly considered a monophyletic taxon, but the relationships found within *Lampropeltis* using concatenation methods and/or mtDNA to infer phylogeny are in likely in error (Bryson et al. 2007; Harper and Pfennig 2008; Pyron and Burbrink 2009c), as they are not supported by my species tree analysis. The species tree indicates a well-supported clade that consists of *L. calligaster,* the *L. getula* complex, and *L. extenuata*, which in turn is the sister group to a well-supported milksnake, Mexican kingsnake, and mountain kingsnake clade (Fig. 2b). Similar to the species tree, Blanchard's revision of *Lampropeltis* (1921) based on morphology also indicates close relationships among the tri-colored *Lampropeltis* species (milksnakes, Mexican kingsnakes, and mountain kingsnakes) and *L. calligaster*, the *L. getula* complex, and *L. extenuata*.

Although mtDNA is frequently used to infer pattern within a phylogeographic context, this study adds to the mounting evidence that for many major groups of vertebrates mtDNA is misleading (e.g., Brumfield et al. 2003; Bossu and Near 2009; Spinks and Shaffer 2009; Bryson et al. 2010; Leaché 2010; Waters et al. 2010; Fontenot et al. 2011; Pasachnik et al. 2010; Roos et al. 2011; Yu et al. 2011; Lee et al. 2012; Veith et al. 2012). Relationships within *Lampropeltis* inferred using mtDNA (Cytb, Fig. 2a; ND4, Bryson et al. 2007) result in well-supported topologies that conflict with the probable species tree (Fig. 2b). *Lampropeltis alterna* is typically allied with *L. mexicana* based on morphology (Gehlbach and Baker 1962), yet the individuals used in these analyses have Cytb haplotypes similar or identical to those of *L. gentilis* in Texas with which they are adjacent/sympatric. These haplotypes are also found in *L. annulata* and a single *L. mexicana* included in this study. Two likely reasons are incomplete lineage sorting or hybridization/introgression between species. It is expected that the failure of lineages to sort is unlikely to be found for mtDNA, but not nuclear DNA as well, due to mtDNA's faster sorting time has (Moore 1995). It is possible that infrequent hybridization between *L. gentilis* and *L. alterna*, *L. annulata*, and some *L. mexicana* has lead to mitochondrial introgression. Migrate-n analyses shows little migration of scnDNA among *L. gentilis* , *L. alterna*, and *L. annulata* (Table 2).

Despite using the same genes and terminal taxa to construct the concatenated scnDNA tree and the species tree, there is still discordance between the two and the Robinson-Foulds distance (= 14) indicates the two trees are topologically incongruent. This further underscores that even when the same species are delimited and included in analyses, gene trees are not equal to multispecies coalescent-based species trees with respect to either temporal or topological congruence.

Timing and Processes of Diversification

The time-calibrated species-tree indicates that *Lampropeltis* originated in the Miocene, but differs from previous studies (Pyron and Burbrink 2009c) in that my expanded species tree shows the majority of divergences for milksnakes, as well as other extant *Lampropeltis* species, occurred during the Pleistocene and Pliocene (Fig. 2b). This is likely for two reasons; one, my tree has more than twice as many terminal taxa for the genus, with 21 species of *Lampropeltis* compared to the previous study that had 10. The study here includes five newly delineated milksnake species and five species elevated in two recent phylogeographic studies (*L. getula* complex species, Pyron and Burbrink 2009b, *L. knoblochi*, Burbrink et al. 2011) not included by Pyron and Burbrink (2009c). The inclusion of these species result in nine additional divergence events occurring mostly in the Pleistocene (78% of new nodes). I recognize that there may still be cryptic diversity to be discovered within *Lampropeltis* (e.g., the wide-ranging *L. calligaster*), but additional cryptic taxa would still likely result in the accumulation of more shallow divergence times.

Second, divergence time estimates from species trees should result in younger dates than concatenation/gene-tree methods due to gene divergences predating the timing of speciation events, at least in the absence of gene flow (Edwards and Beerli 2000; Carstens and Knowles 2007; McCormack et al. 2011; Burbrink and Pyron 2011). My results support this, with the scnDNA concatenated tree showing most diversification during the Pliocene (55% of nodes) and Miocene (25% of nodes), and reduced speciation during the Pleistocene (20% of nodes). In contrast, the species tree indicates the majority of speciation events occur more recently during the Pleistocene (45% of nodes) and the Pliocene (45% of nodes), with lower diversification during the Miocene (10%). There is a significant difference in the mean divergence times between two trees and this difference becomes larger as nodes become younger (Figs. 4, 5). The older divergences from the scnDNA concatenated tree also result in a misleading significantly negative γ statistic indicating early diversification for *Lampropeltis*, while the species tree yields a non-significant γ value, further illustrating the problems that can result from relying on gene trees in diversification analyses (Burbrink and Pyron 2011, 2012). In contrast to recent studies (Pyron and Burbrink 2009c; Burbrink and Pyron 2010), which found diversification for *Lampropeltis* only occurred during the Miocene and Pliocene, my results are similar to studies of vertebrates that reveal elevated diversification during the Pleistocene (Rand 1948; Avise 2000; Hewitt 2000, 2004a,b; Johnson and Cicero 2004; Rull 2006; Beheregaray 2008; Koscinski et al. 2008; Zarza et al. 2008; Daza 2009) suggesting the potential of a strong role for Pliocene/Pleistocene glacial cycles on the diversification of *Lampropeltis*.

Although the γ statistic showed node density to be consistent through time for the species tree, the best-fitting species diversification models for the species tree indicate that speciation rates for *Lampropeltis* have decreased through time (Yule2, coalescent model 6, DD+E; Table 5). Moreover, the best-fitting models for the species tree are similar to those from the concatenated tree of Burbrink and Pyron (2010), who found that density-dependent and Yule2 models explained diversification of Lampropeltini equally well. It may be that speciation rates declined as the number of species increased and saturated available niches or, alternately, that some extrinsic factor during the Pleistocene, such as reduced habitat availability due to climatic change, could have caused a decline in speciation rates (Zink and Slowinski 1995; Burbrink and Pyron 2010). The concatenated tree gives mixed results, with both constant speciation rate (coalescent model 6) and variable-rate density-dependent models being supported (DDL, DD+E; Table 5). Additionally, the best-fitting model that includes extinction (DD+E) for the two trees is the same, but parameter estimates differed between the two (Table 5). Overall, the diversification models indicate a slowdown in diversification rates for *Lampropeltis*, consistent with previous studies (Burbrink and Pyron 2010), despite the contrasting results from the γ statistic.

Conclusions

Using a gene-tree approach rather than a species tree approach influences branching times, gives misleading results regarding the timing and tempo of diversification and this error increases as divergence times become younger. Orthogonal to this but still a crucial consideration, species delimitation is a necessary but often overlooked first step for inferring a species tree. Coalescent-based delimitation may be especially important to test species hypotheses from phylogenetic studies prior to species tree inference because this provides statistical support as to what lineages should be considered species. The repercussions of missing taxa extend past taxonomic relationships and also affect divergence time estimation, with speciation events occurring significantly earlier in time when species are excluded. My results suggest that making an effort to delimit species whenever possible prior to species-tree inference or analyses that use phylogenies will provide more robust results with respect to topology, timing, and tempo of diversification compared to studies that are missing taxa and/or rely on gene-tree approaches. This intersection between phylogeography and systematics has not been considered by most previous researchers, but is demonstrated here to have a crucial impact on the primary goals of both fields, including species delimitation, species-tree estimation, and the inference of evolutionary processes of speciation using phylogenetic comparative methods.

By including all milksnake species as well as other recently recognized species within the genus, I provide both a well-supported species tree and diversification time estimates for

Lampropeltis. I find that speciation of *Lampropeltis* was highest during the Pleistocene and Pliocene, indicating recent glacial cycles may have been important for diversification in this group. In addition, the recognition of seven milksnake species provides a better estimate of the diversity not only within the Lampropeltini, but for NW squamate diversity as well. More generally, I show that the omission of unrecognized species diversity and the use of concatenated gene-trees has serious and far-reaching repercussions for the correct inference of relationships within the group, and the dynamics of recent speciation events. Even for groups which are fully sampled in terms of currently described species, the common inference of early bursts of speciation may be driven in many cases by inadequate taxon sampling at the phylogeographic level, and the use of gene-tree based divergence-time estimates.

Chapter 2

Historical Demography of Temperate and Tropical Milksnakes (Genus *Lampropeltis*)

Abstract

While the effects of Late Quaternary climate change have been examined for many temperate New World taxa, the impact of Pleistocene glacial cycles on Neotropical taxa is not as well understood, specifically with respect to changes in population demography. Here, I examine historical demographic trends for six species of milksnake with representatives in both the temperate and tropical Americas. Using a multilocus dataset, I test for the demographic signature of population expansion and decline using non-genealogical summary statistics such as Tajima’s D, as well as coalescent-based methods in the form of extended Bayesian skyline plots. In addition, I determine whether range sizes are correlated with effective population sizes for milksnakes. My results indicate that there are no identifiable trends with respect to demographic response based on location, and that species responded to changing climates individually. There is also no correlation between range size and effective population size, with the largest population size belonging to the milksnake with the smallest geographic extent. My results highlight the importance of not generalizing the demographic histories of taxa by region and further illustrate that the New World tropics were not necessarily a stable refuge for species during periods Pleistocene climate change.

Populations of all extant species that originated prior to the Holocene have experienced climate change. For those found in temperate regions of North America and Europe, populations likely responded directly to climate changes induced by the glacial and interglacial cycles of the Quaternary (Zachos 2001). Species affected by changing environments can have different responses to these alterations, including population decreases, stability, or expansion. Some taxa, such as mammoths and other large mammals, were unable to survive climate change, declined in abundance, and ultimately went extinct (Nogués-Bravos et al. 2008; Nogués-Bravos et al. 2010). Others persisted by adapting to the new environment (Davis and Shaw 2001; Davis et al. 2005) or, for instance during the Last Glacial Maxima (LGM) migrating to more suitable areas (Hewitt 2000; Davis and Shaw 2001). However, even though some species persisted through periods of climatic change, population sizes may have been drastically reduced (e.g., Mapelli et al. 2012). In contrast for some taxa, it has been demonstrated that when conditions become favorable, population sizes for many species will increase rapidly (e.g., Mila et al. 2000; Lessa et al. 2003; Fontanella et al. 2008; Guiher and Burbrink 2008; reviewed in Hewitt 1996, 2000, 2004a,b).

The signature of these demographic changes can be detected using genetic data with coalescent-based models (reviewed in Ho and Shapiro 2011). Trends with respect to changes in effective population size (N_e) through time can be associated with climatic or geological phenomena which may have altered population sizes or structure (Kingman 1982a,b; Avise et al. 1987; Hudson 1990; Harding 1996; Neigel 1996; Avise et al. 1998, 2000; Edwards and Beerli 2000). Demographic studies have until recently relied heavily on single locus datasets. Unfortunately, the results from a single locus may not track the demographic history of the species or population under examination, especially if there has been selection on that particular gene (Brito and Edwards 2009). For example, balancing selection results in an excess of intermediate-frequency alleles for a locus, giving a genetic signature that is indistinguishable from population contraction (Maruyama and Fuerst 1985; Schmidt and Pool 2002). Conversely, it may not be possible to separate the effects of purifying selection from population expansion, as both yield an excess of rare alleles (Maruyama and Fuerst 1984; Schmidt and Pool 2002). These confounding effects of selection can be avoided by using multiple, unlinked markers (e.g., Galtier et al. 2000).

For temperate North American species, Pleistocene glacial cycles may have directly altered distributions and populations sizes, where cycles of decline and growth are expected to occur with the contraction and expansion of favorable habitat, respectively (Rand 1948; Beecher 1955; Mengel 1964; Avise and Walker 1998; Avise et al. 1998). The most recent glacial event in North America, the Wisconsin, began ~120-80 ka and reached its maximum at ~20 ka (Andrews and Barry 1978), with its retreat at ~10 ka marking the end of the Pleistocene and beginning of the Holocene epoch. During the LGM, North America was covered by ice sheets as far south as the 38° north latitude (Dyke and Prest 1987). Conditions were cooler throughout North America at this time and boreal forest dominated much of the eastern U.S., with hardwood forests pushed southwards (Jackson et al. 2000). Open pine-forest habitat was predominant across the western U.S. and the deserts of the southwest were cooler and wetter compared to current climates (Thompson and Anderson 2000). Several studies have found that after the LGM, temperate North American species expanded as more suitable habitat became available (e.g., pitvipers, Guiher and Burbrink 2008; rodents, Light et al. 2012; woodpeckers, Pulgarin and Burg 2012). These climate changes may have also contributed to the diversification of species by isolating once contiguous populations into separate refugia (reviewed in Hewitt 2000, 2004a,b; Johnson and Cicero 2004; Beheregaray 2008).

The same glacial cycles may also be important for speciation and population size changes in the Neotropics (Haffer 1969; Veulleumier 1971; Simpson and Haffer 1978)**,** yet studies examining historical demography in the species-rich tropical biota of the New World (NW) are lacking (reviewed in Beheregaray 2008). In contrast to the impacts that glacial cycles had on temperate species, the effects on Neotropical taxa may not have been as severe (Lessa et al. 2003) given the lack of extensive ice sheet formation in the tropics. Earlier studies suggested that the Neotropics were a region of climatic stability during the Pleistocene (CLIMAP project 1981; Broecker 1986; Lyle et al. 1992), but this has been contradicted by evidence of significantly lower temperatures of ~4-9° C throughout the NW tropics during the LGM (Rind and Peteet 1985; Clapperton 1993; Guilderson et al. 1994; Stute et al.1995; Thompson et al., 1995; Colinvaux et al., 1996; Webb et al., 1997; Bush and Philander 1999; van der Hammen and Hooghiemstra 2000; Mora and Pratt 2001; Piperno and Jones 2003; Bush et al. 2004). It has been demonstrated that glaciers existed in high altitude regions of Mexico, Guatemala, Costa Rica, and in the Andes Mountains of South America during this time (Horn 1990; Metcalfe et al. 2000; Thompson et al. 2000; Lachniet and Seltzer 2002), and there is evidence of not only Pleistocene cooling in both Central and South America but also of increased aridity that persisted until the start of the Holocene (Leyden 1984; Hewitt 2000; Piperno and Jones 2003). The climate alterations subsequently affected plant distributions (Piperno and Jones 2003), causing high altitude vegetation to shift to lower clines and reducing the amount of tropical rain forest while increasing grasslands in Central and South America (reviewed in Hewitt 2000; Piperno and Jones 2003). These habitat changes may have impacted population dynamics of species inhabiting tropical rainforests, but this has not been extensively explored. Studies that include NW tropical species indicate that diversification took place during the Pleistocene for some taxa

(Rull 2006; Koscinski et al. 2008; Peterson and Nyari 2008; Zarza et al. 2008; Daza 2009; Ruane Chapter 1), but geological events throughout the Miocene and Pliocene also contributed to speciation in both Meso and South America (Klicka and Zink 1999; Moritz et al. 2000; Kronauer et al. 2005; Devitt 2006; Zarza et al. 2008; Daza 2009). In addition to contributing to diversification for some tropical species, Quaternary climatic events may have impacted the population demographics of taxa, as they did with temperate species.

The snake genus *Lampropeltis*, commonly known as the kingsnakes and milksnakes, has closely related species in both temperate North America and tropical Central and South America. Most of these taxa originated during the early Pleistocene and Pliocene (Ruane Chapter 1). Therefore, the effects of Quaternary climate change for closely related and biologically similar taxa can be examined in both Nearctic and Neotropical regions. Using a multilocus dataset, I test whether temperate North American species of milksnake had similar demographic responses compared to tropical Central/South American species. Specifically, I examine changes in population size through time for each taxon to determine if temperate and tropical species show similar demographic trends throughout the Pleistocene. I expect that temperate species would have experienced range expansion after the LGM due to their proximity to the Laurentide ice sheet, with subsequently more habitat available as the glacier retreated, resulting in an increase in population sizes. This may be especially true for the most northerly distributed milksnakes, as previous studies of temperate snakes have found that closely related species/lineages occurring farther north have greater population size increases at the end of the LGM compared to more southerly distributed taxa (Fontanella et al. 2008). Given the temperature and habitat changes that would have occurred during the Pleistocene throughout the Central and South American ranges of the three tropical species, I also expect to see increases in tropical milksnake population sizes around the beginning of the Holocene associated with the recovery of favorable environmental conditions present today. Although a previous study that compared demographic responses of Amazonian small mammals to those of temperate North America found that the tropical mammals had no changes in population sizes at the end of the last glacial, while the temperate species experienced expansion (Lessa et al. 2003), these results may not be applicable to reptiles. As ectotherms, snakes are dependent upon their environment for maintaining body temperatures and thus populations are sensitive to changes in climate (Araújo et al. 2006).

I estimate the mean N_e for each species and calculate geographic extent to determine if species with larger range sizes have larger effective population sizes. Previous work has determined that tropical species tend to have smaller geographic ranges compared to temperate species and that with smaller ranges come smaller population sizes (reviewed in Lawton 1993; Gaston 1996; Gaston et al. 1998). Some studies have also shown that for snake lineages, those with larger ranges generally have a higher N_e (e.g., Fontanella et al. 2008), but this is not consistent (e.g., Burbrink et al. 2008) and has not been explicitly tested.

By examining milksnakes across their extensive range in a historical demographic context, I address how population structure has been altered by Pleistocene climate change in closely related Nearctic and Neotropical taxa. First, I ask if there are general unified responses to climate change for closely related species? If so, this suggests that closely related taxa experience similar population increases or decreases regardless of location given changing climates. In contrast to this, I might find that specific historical demographic trends are reflective of climate change that is associated with a specific region. My results suggest that effective population sizes among the six species of milksnake examined here remain stable, increase, or decrease given each taxon and there is no clear trend found across the temperate or tropical species.

Methods

Sampling and genes

I used six milksnake species for my analyses of demographic change; *L. triangulum*, *L. gentilis*, *L. elapsoides*, *L. polyzona*, *L. abnorma*, and *L. micropholis* (Ruane Chapter 1). These six taxa were chosen because of a high number of samples available for multiple genes and their distributions (Figs. 6, 7), with *L. triangulum*, *L. gentilis*, and *L. elapsoides* (referred to as temperate species) occurring in the USA/Canada and *L. polyzona*, *L. abnorma*, and *L. micropholis* ranging from Western/Central Mexico to Ecuador (referred to as the tropical species; see Appendix 3 for details). These six species originated between 1.1 and 3.6 Ma (Ruane Chapter 1) and so would have experienced Late Pleistocene climate change. Genetic samples were taken from across the ranges of the species whenever possible (Appendix 4). Sequencing and PCR protocols for all loci used in analyses follow Ruane Chapter 1. All nuclear loci heterozygosities were resolved with PHASE v2.1.1 (Stephens and Donnelly 2003) using the most probable pair of alleles. To determine the most appropriate model of nucleotide substitution I utilized Bayesian Information Criterion in the program jModeltest (Posada, 2008). For *L. triangulum*, *L. elapsoides*, and *L. gentilis*, three genes were used, the mitochondrial gene cytochrome b (Cytb) and two anonymous nuclear loci, 2CL8 and CL4. For *L. polyzona*, *L. abnorma*, and *L. micropholis*, the same three genes were used, as well as the nuclear protein coding genes NT3 and PRLR, the introns SPTBN intron 1, Vimenton intron 5, NAV intron 5, and Z-chromosome GAD intron 15, and the anonymous loci 2CL3, 2CL4, and LATCL (see Appendix 5 for details).

Historical Demographic Analyses

To determine if temperate and tropical milksnakes show similar or disparate trends with respect to demographic histories, I examined population size changes through time using BEAST v.1.7.2 (Drummond and Rambaut 2007) to generate Extended Bayesian Skyline Plots (EBSP; Heled and Drummond 2008) for each species. This method uses a multi-gene coalescent approach to estimate population size through time and does not require a pre-specified demographic model. I used the mean substitution rate of Cytb estimated for *Lampropeltis* of $1\text{x}10^{-8}$ to scale the time axis, following Pyron and Burbrink (2009c). The EBSP for each species was run between $2\text{x}10^{8}$ to $5\text{x}10^{8}$ generations to achieve high effective sample size (ESS) values. Burnin was assessed using Tracer v.1.5 (Drummond and Rambaut, 2007). I also examined the demographic.populationSizeChange parameter in Tracer, which indicates the most probable number of population size changes for each species. To determine if higher N_e was correlated with range size, I calculated the approximate range size in square kilometers in Google Earth Pro using the polygon function (googleearth.com) for each species, with the ranges based on Ruane Chapter 1. I then used a Spearman rank correlation test to determine the relationship between range size and mean N_e. I also used this test to determine if the net population size change (the difference between the N_e at start of the EBSP and the N_e at time zero) and range were correlated. Spearman rank correlations were performed in the program STATISTICA v.6 (StatSoft, Inc., Tulsa, Oklahoma).

I tested for population size changes using non-genealogical coalescent methods as well to compare estimates for independent loci. This helps address the confounding effects of changes on N_e versus selection upon a particular locus. I used the program DNAsp 5.0 (Librado and Rozas 2009) to examine population size changes using several statistics. The R_2 statistic (Ramos-Onsins and Rozas 2002) was used to test for population expansion and is based on the difference in the number of singleton mutations and the average number of pairwise differences between samples. Coalescent simulations implemented in DNAsp were then used to assess significance and confidence intervals. I also generated mismatch distributions; resulting distributions indicate changes in population size or selection (Rogers et al. 1996). Harpending's raggedness index (r_g, Harpending 1994) was then used to assess the statistical significance of these distributions. Additionally, Tajima's D (Tajima 1989) and Fu and Li's D* and F* statistics (Fu and Li 1993) were used to test for population growth or decline versus constant population size. For these three statistics, an overabundance of rare polymorphisms, resulting in negative values indicates there has been population expansion or positive selection, while positive values, resulting from an overabundance of intermediate-frequency polymorphism, indicate population decline or balancing selection.

Results

Sampling and Genes

All resulting sequences were used for the EBSP analyses, with 3-11 polymorphic loci for each species and 11-34 individuals per locus (Appendix 5). For DNAsp analyses, sequences missing a significant amount of data for a gene were removed from analyses, as DNAsp excludes all sites with missing data (Appendix 5).

Demographic Analyses

High ESS values (>200) were achieved in each analysis, with the exception of *L. micropholis*. Despite increasing the chain length for *L. micropholis*, the ESS values for some parameters (e.g., the prior) did not greatly improve and so multiple runs were used to ensure consistency among results. The EBSPs showed stable populations through time for *L. triangulum* and *L. gentilis*, but for *L. elapsoides*, there was an increase in population size at ~50 ka (Fig. 8). In contrast, the two most southerly distributed species, *L. abnorma* and *L. micropholis*, show a slight population increase at ~70 ka and then a decline starting at ~45 ka continuing to the present (Fig. 8). The third tropical species, *L. polyzona*, showed a long period of population decline starting at least 1 Ma, followed by a slight increase and population stability beginning ~80 ka (Fig. 8). Mean N_e ranged from ~1,100,000 (*L. micropholis*) to ~10,000,000 (*L. polyzona*; Table 6). The temperate species had fewer population size changes compared to the tropical species (Table 6). Generally, the temperate species also had larger population sizes and larger ranges than did the tropical species, with the exception of *L. polyzona*, which had the largest population size and the smallest range (Table 6). However, the Spearman rank correlations for both range size versus mean N_e ($r_s = -0.029$) and range size vs. net N_e ($r_s = 0.086$) were not significant (d.f. = 4; $P > 0.05$).

The results from tests performed in DNAsp indicated no selective or demographic forces operating on *L. triangulum*. For the other five species, no clear trend was detected across any of the loci for any species (Table 7). Although several loci resulted in positively and negatively significant test results, this was not consistent across loci for a given species and in several cases, different loci for the same species indicated different patterns (discussed below; Table 7).

Discussion

Whether glacial cycles have had similar effects on population sizes in both temperate and tropical regions is of interest for two reasons. First, it provides insight into the factors that have shaped the current demographic patterns and distributions of organisms in disparate environments and helps determine whether closely related taxa respond similarly regardless of location. Second, from a conservation perspective, understanding how past climate change affected populations may be applicable to determining what threats species face when confronted with anthropogenic climatic alteration and whether certain taxa are likely to be more vulnerable. Here, I find that for milksnakes in the genus *Lampropeltis*, there is no clear demographic pattern with respect to temperate versus tropical species. Based on the EBSPs, populations of temperate species have either remained stable or increased in size during the recent Pleistocene/Holocene (Fig. 8). This is surprising, considering that the LGM would have had a direct impact on milksnakes living in temperate North America, with the Laurentide Ice Sheet covering a proportion of the ranges of two species (Fig. 6; Delcourt and Delcourt 1987; Dyke and Prest 1987; Pielou 1991). As glaciers receded at the end of the Pleistocene, habitat availability for the temperate species should have increased and allowed populations to expand, but I found no evidence for this among all three temperate taxa; populations remained constant or expanded prior to glacial decline (Fig. 8). The three tropical taxa also had dissimilar responses, with both population expansion as well as declines (Fig. 8); this supports the hypothesis that the tropics did not provide stable environments during the last glacial. Despite finding no significant correlation between range size and population size, the temperate species had greater ranges and larger mean effective population sizes. The exception to this was the tropical, *L. polyzona* which had the largest mean population size despite having the smallest range (Table 6; Figs. 6, 7). There is the possibility that individuals with the greatest amounts of genetic variation were inadvertently sampled, which would result in signal that indicates large population sizes (Kuhner 2009). This potential bias could be corrected by increasing sample sizes, which would better reflect the true allelic variation within *L. polyzona* and result in a more accurate estimate of population size. However, if *L. polyzona* represents a single, panmictic population, the large N_e is likely an accurate estimate, as the sample size for this species ($n = 31$) should be sufficient for detecting population size changes (Heled and Drummond 2008).

EBSP Results and Summary Statistics

I generally find a lack of concordance between the EBSPs and the non-genealogical summary statistic results. The summary statistics for many loci did not indicate the same demographic signal as the EBSP trends did, resulting in conflicting signals or a lack of significance (Table 7). The exception to this is *L. triangulum*, which showed no significant N_e changes for any non-genealogical test across loci and had an EBSP showing a stable population size with no evidence for expansion or decline (Fig. 8) or population size change (Table 6). For the remaining species, the degree to which the summary statistics disagreed with the EBSP trends was variable. For example, *L. gentilis* showed no evidence of population changes through time based on the EBSP (Table 6; Fig. 8), and two out of the three loci sequenced supported this result as they were not significantly different from the null (Table 7). Similarly, the EBSP of *L. elapsoides* showed a population expansion (Table 6; Fig. 8) and two out of three loci indicated the same demographic signal for several of the summary statistics I examined (Fu and Li's F*, Fu and Li's D*, R_2; Table 7). For the remaining species, while there was some agreement with the EBSPs, such as the signal of expansion for five of the eleven loci sequenced for *L. polyzona* (Table 7), most loci were not significantly different from neutral expectations or conflicted with one another when significant. For example, one locus for *L. abnorma* was significantly negative and indicated expansion (PRLR, R_2; Table 7), another was significantly positive for population decline (SPTBN1, Fu and Li's D*; Table 7) and the remaining nine loci were neutral for all tests (Table 7). In contrast, the EBSP showed a population decline beginning ~45 ka. These results may indicate there is either balancing or purifying selection acting on some of the loci as the signal cannot be detected across all or most of the loci and would explain the conflicting results. However, for the majority of loci that are not significantly different from neutral, it is also possible the summary statistics are not be powerful enough to detect population size changes. Summary statistics may not provide as realistic an estimate of historical population size changes compared to methods that are based on a coalescent genealogy (Kuhner 2009). Previous studies have recognized that N_e estimates that rely on the calculation of pairwise differences and segregating sites are inefficient at estimating population demographics when compared to methods that take the phylogeny of the sample into account (Felsenstein 1992). EBSPs in contrast account for the demographic signal and coalescent history across all loci simultaneously and provide an estimate of the phylogenetic error associated with the data (Ho and Shapiro 2011). Ideally, all loci should retain demographic signature, but events such as extreme bottlenecks can erase signal at a given locus; when using multiple loci given varying substitution rates, there is a chance that some loci are preserving signal lost by other genes (Ho and Shapiro

2011). Furthermore, in cases where growth rates are slow and/or size changes are small, genealogical methods should outperform pairwise methods (Slatkin and Hudson 1991; Kunher et al. 1998); this may be applicable to My data, as the EBSPs for several milksnakes show low levels of population size change (Table 6; Fig 3). Considering the robustness associated with genealogical methods using coalescent models and specifically those using multiple loci (Kuhner et al. 1998; Kuhner 2009), I base my discussion on the EBSP results rather than the summary statistics.

Temperate Milksnakes

For two of the temperate species, *L. triangulum* and *L. gentilis*, I found that populations were stable, with no changes in population size having the highest support in the EBSP analyses (Table 6; Fig. 8). *Lampropeltis triangulum* occurs in the eastern U.S., southeastern Canada and as far west as Iowa, with the Mississippi River Embayment defining the southwestern extent of their range (Fig. 6). This milksnake inhabits deciduous forests of eastern North America (Ernst and Ernst 2000) and the transition from deciduous hardwood forest to prairies in the west coincides with the limit of their distribution. In the western U.S., *L. gentilis* is found west of the Mississippi River from Arizona to Montana, (Fig. 6), often in grassland or prairie habitat (Ernst and Ernst 2000; Werler and Dixon 2000). Despite these two species having the largest ranges and reaching the greatest latitudes in the north, I was unable to detect changes in population size (Fig. 8; Table 6). It is possible these species expanded into suitable habitat that was pushed further south by the Laurentide Ice Sheet and population sizes remained constant. Other studies of snakes of the U.S. have found a similar pattern with stable population sizes throughout the Pleistocene (e.g., Fontanella 2008; Myers et al. in press). In addition, while *L. gentilis* is often associated with prairies and grasslands, it can be found in a wide variety of habitats, including desert lowlands, pine and hardwood forest, and stream valleys, (Ernst and Ernst 2000; Werler and Dixon 2000). The ability of *L. gentilis* to inhabit many distinct environments may indicate it was able to readily adapt to climatic changes of the Pleistocene and thus population sizes remained stable (King et al. 2009).

Unlike the two most northern temperate milksnakes, I found that the southeastern milksnake *L. elapsoides* underwent a population expansion at ~50 ka. *Lampropeltis elapsoides* is found in the pine forests of the southeastern coastal plain, throughout the Florida peninsula, and west to eastern Louisiana (Fig. 6). The onset of the population increase occurred during the middle of the Wisconsin glacial interval (Fig. 8; Duplessy et al. 2007), and continued to the present. A “thermal enclave” from warm ocean winds throughout the southeastern U.S. during the Pleistocene may have facilitated this population growth (Russell et al. 2009). It is also possible that *L. elapsoides* shifted its distribution and populations subsequently expanded, as species inhabiting the eastern U.S. may have moved south into both peninsular Florida and Mexico during periods of glacial cooling and returned north once climates became more favorable (Martin and Harrell 1957).

Tropical Milksnakes

I found that one of the tropical milksnake species increased in population size during the last 100 ka, while the other taxa both experienced population declines. At ~80 ka populations of the Mexican milksnake *L. polyzona* began increasing (Fig. 8). Despite having the smallest geographic range, *L. polyzona* has the largest mean population size of all six species (Figs. 6, 7; Table 6). This milksnake is found along the western versant of the Sierra Madre Occidental

(SMOc), north of the Sierra Madre del Sur (SMS), along the eastern versant of the Sierra Madre Oriental (SMOr), and throughout the Trans-Mexican Volcanic Belt region (TMVB) (Figs. 6, 7). While the physical formation of these geological features predates the origination of *L. polyzona* (SMS, Nieto-Samaniego et al. 1999; SMOr, SMOc, McDowell and Keizer 1977; Ferrusquia-Villafranca and Gonzalez-Guzman 2005; TMVB, Ferrusquia-Villafranca and Gonzalez-Guzman 2005), the presence of glaciers in these montane regions during the Wisconsin glacial could have influenced demography (Metcalfe et al. 2000). However, *L. polyzona* populations showed no decline during the late Quaternary as might be expected, and increase in population size from ~80 ka to the present. It has been proposed that despite glacial presence in northern and central Mexico, climate did not change significantly (Deevey 1944) and has been consistent during the last 30 ka (Bradbury 1989), although arguments against stability have been made (reviewed in Metcalfe et al. 2000). The range of *L. polyzona* also appears tightly correlated with that of tropical dry forest (Becerra 2005). Dry tropical forest in Mexico appeared prior to the origin of this species of milksnake (Becerra 2005) and if this habitat remained stable during the Pleistocene, the *L. polzona* population may have been able to persist and expand.

In contrast to *L. polyzona*, both *L. abnorma* and *L. micropholis* have experienced population declines beginning at ~45 ka (Fig. 8). The areas occupied by both species are thought to have undergone significant cooling and increased aridity during the Pleistocene. *Lampropeltis abnorma* inhabits much of Mesoamerica, from south of the SMS in Mexico, and throughout Central America as far south as Costa Rica, while *L. micropholis* occurs in eastern Costa Rica, Panama, and Columbia, Ecuador, and Venezuela north/west of the Andes (Fig. 7). Glaciation of the Talamanca cordillera of Costa Rica and the highlands of Guatemala and Mexico during the Wisconsin contributed to the cooling of Central America (Horn 1990; Lachniet and Seltzer

2002), which may have started as early as 45 ka and could have resulted in the decline of *L. abnorma* populations (Bush 2002; Bush et al. 2009). While it is suggested that deglaciation occurred ~11 ka, aridity throughout Central America was at its maximum at this point in time (Bush et al. 2009). Snakes are sensitive not only to changes in temperature but also moisture, with drier conditions potentially limiting the distribution of a species (Araújo et al. 2006). Like *L. abnorma*, *L. micropholis* populations may have been influenced by the glaciers in Costa Rica as well as the changing climate of lower Central America, which was cooler and drier throughout much of the last 100 ka (Bush and Colinveaux 1990). The extent of their range also means *L. micropholis* could have been impacted by Andean glaciations during the Pleistocene. As glaciers expanded across the Andes (Thompson et al. 2000) vegetation moved with respect to elevation (Pielou 1979; Bush 2002) and similar to Central America, climates became drier and tropical forests decreased (Clapperton 1993). This may have restricted the size of habitat available for *L. micropholis* and caused the population to decline during the last 45 ka.

Conclusions

My study illustrates that glacial cycles have impacted both Nearctic and Neotropical milksnakes but that species did not share demographic responses by area. Most surprising, I found no evidence for demographic changes in the two most northerly distributed species, which were closest to the southern extent of the glacier during the LGM. In addition, results showed population declines for two of the tropical taxa. While it is likely that climatic events have contributed to the historical demographic patterns of these snakes, I cannot discount the possibility that unknown abiotic or biotic factors have altered population. However, My examination of milksnake demography demonstrates that I cannot assume all temperate NW taxa have had similar responses to the last Ice Age, as two species show constant population sizes during the Late Pleistocene and Early Holocene, with neither a decline nor recovery corresponding to the LGM or glacial retreat. Furthermore, My study adds to the mounting evidence that the tropics were not an area of stability during the Pleistocene and that populations underwent both expansions and declines. Based on these results I recommend using caution in generalizing demographic responses of taxa by region. Studies in a comparative phylogeographic framework examining multiple taxonomic groups would be especially useful in determining whether species respond individually or if there are discernible patterns with respect to both taxonomy and biotic community.

Chapter 3

Using Geometric Morphometrics to Examine Head Shape Variation in Milksnakes (Genus *Lampropeltis*)

Abstract

Species discovery and identification has long relied on traditional morphometric analyses, however molecular methods for delimiting species are becoming increasing popular and important. Although many studies rely solely on molecular data to differentiate between species, additional evidence that supports genealogically-based species delimitation is desirable at least for field and museum identification of taxa and should be a major part of an integrative approach to taxonomy. Here, I use geometric morphometric (GM) analyses to examine six species of milksnake (genus *Lampropeltis*) that have recently been delimited based on multi-locus data in a coalescent framework. I plot landmarks onto the dorsal view of 487 specimens and use canonical variates analysis (CVA) to determine if the differences in head shape of these six species can be used to correctly classify specimens. I find that for five of the six species, CVA accurately classifies individuals > 70% of the time. Cross-validation of the results using a jackknife procedure on the dataset gives similar accuracy, with unknown specimens correctly classified at a rate of 73%. This study illustrates that although GM-based analyses may not correctly differentiate between species 100% of the time, GM-methods can be useful for detecting shape differences between species.

Morphological analyses have long been the standard for the discovery and description of species. For many taxa, morphological characters provide information pertaining to taxonomic identity and evolutionary relationships among taxa. However for some species, the number of morphological characters may be limited or not useful with respect to phylogenetic inference; relying solely on morphology to define species has long been recognized as problematic (Mayr 1942). In particular, within species complexes or among cryptic and pseudo-cryptic taxa (Saez and Lozano 2004), molecular methods are often used to disentangle phylogeny (reviewed in Bickford et al. 2007). However, including morphological data is useful because it can often provide additional information that aids in the understanding of ecology, as certain morphological characters are often associated with specific diet or microhabitat use (Lillywhite and Henderson 1993; Losos et al. 2009). For taxa that have a limited number of informative or discretely different characters, traditional morphological methods that qualify or quantify characters (e.g., number of digits, limb length, ornamentation), may not be able to detect differences and alternative approaches are necessary. One alternative approach to standard morphological methods is geometric morphometrics (GM). Geometric morphometrics is a collection of shape-analysis techniques that assess the relative spatial distribution of a set of pre-determined landmarks, such as points where the sutures of a skull come into contact with one another; the resulting set of coordinates represent a shape that is scaled to be independent of size and is typically analyzed using multivariate statistics (Zelditch et al. 2004). Geometric morphometric methods have been used to describe variation in many taxonomic groups, including turtles (Claude et al. 2003), lizards (Stayton 2005; Kaliontzopoulou et al. 2008; Leaché et al. 2009) and mammals (Cardini et al. 2009). These methods have also been found to be more powerful when compared to traditional morphological analyses based on linear mensural data in discriminating between species and populations of “morphologically ambiguous” taxa (e.g., moths, Mutanen and Pretorious 2007; bats, Evin et al. 2008; cichlids, Maderbacher et al. 2008).

For snakes, which have a limited number of categorical morphological traits due to their lack of appendages and generalized elongate body form, GM should be useful for capturing shape variation with respect to head shape, which for snakes may be closely tied with feeding ecology (Lillywhite and Henderson 1993; Shine et al. 2002; Vincent et al. 2004). Additionally, there are already a few GM-based studies that have found significant differences in head shape between sexes and subspecies of snake (Vincent et al. 2004; Gentilli et al. 2009). Morphological features that are more commonly used in snake systematics include color pattern and scale count. Both of these characters may be misleading with respect to taxonomy and evolutionary history. Color pattern is often found to be variable within species and thus is not a reliable indicator of evolutionary relationships (Burbrink et al. 2000), while scale counts can be influenced by both biotic (e.g., diet; Fabien et al. 2004) and abiotic (e.g., temperature; Fox 1948) factors throughout the range of a species. This lack of suitable characters is also confounded by cryptic diversity, as revealed by the number of phylogeographic studies demonstrating the existence of numerous independently evolving lineages within wide-ranging taxa (e.g., *Agkistrodon contortrix*, Guiher and Burbrink 2008; *Lampropeltis pyromelana*, Burbrink et al. 2011; *L. triangulum,* Ruane Chapter 1). Not only have phylogeographic studies uncovered previously unknown diversity, but new multi-locus coalescent-based methods are able to further delineate between these lineages as distinct species (e.g., *Lampropeltis pyromelana*, Burbrink et al. 2011; *L. triangulum*, Ruane Chapter 1), although classification based solely on DNA is considered undesirable by some authors (see Bauer et al. 2011). While molecular data alone is sufficient evidence of speciation under the general species concept (de Queiroz 2007), determining whether there are identifiable morphological features inherent to a species may provide additional information with respect to ecological factors, such as diet (Lillywhite and Henderson 1993; Shine et al. 2002; Vincent et al. 2004). There is a paucity of studies that have used GM to examine shape variation in snakes (e.g., Manier et al. 2004; Vincent et al. 2004; Gentilli et al. 2009) but GM techniques show promise for adding support to molecular taxonomic hypotheses; a study on European vipers found that subspecies that were distinct clades based on a molecular phylogeny was also distinct morphologically (Gentilli et al. 2009).

Geometric morphometrics may be particularly useful for groups or complexes where morphology has been misleading. One such group is the milksnakes (genus *Lampropeltis*). Milksnakes have been until recently been considered a single taxon (formerly *L. triangulum*) with 25 subspecies ranging from southeastern Canada to Ecuador. These subspecies designations were based almost entirely on color pattern (Williams 1988). Color patterns within the former milksnake subspecies are highly variable and not always diagnostic if locality information is unavailable for an individual (Williams 1988). Subsequent molecular studies using multiple loci and a variety of methods have shown that milksnakes from different geographic locations are not monophyletic within *Lampropeltis* (Bryson et al. 2007; Pyron and Burbrink 2009c; Ruane Chapter 1). The most recent molecular study on milksnakes (Ruane Chapter 1) determined that there are seven species that form three distinct clades within *Lampropeltis* that generally do not correspond to the initial subspecies classifications of Williams (1988) based on color pattern. Here I examine six of these taxa that have specimens readily available within a GM framework to determine if there are distinct head shape differences between the species. Considering the disparity in diet and habitat use among milksnakes (Williams 1988; Ernst and Ernst 2000; Werler and Dixon 2000), head shape may be a good starting point for determining if morphological differences exist among these species. Specifically I plot landmarks on six milksnake species and then use GM multivariate analyses to determine whether head shape is distinct among species. My results illustrate that GM is useful in determining whether morphological differences can be detected among species, as well as providing integrative taxonomic support to the species delimitated using molecular data for snakes, but may not be powerful enough to discriminate between species *a priori* or as a stand-alone species discovery tool due to elevated variation within some taxa.

Materials and Methods

Samples

I photographed the dorsal view of the head of 487 individuals representing six species of milksnake (Fig. 9; Appendix 6). Although seven species have been recently elevated (Ruane Chapter 1), I was unable to obtain samples from Mexico that were definitively *L. annulata* and so this species was excluded. Individuals were assigned to species using the available locality information for each specimen and following the ranges for each species from Ruane Chapter 1. I used tpsUtil v.1.52 (Rohlf 2012) to build a tps file from the photographs. The tps format is the standard file format used in geometric morphometric analyses. This dataset included individuals considered to be juveniles based on minimum adult snout-vent lengths (see below for details) and so I ran a second series of analyses that included only adult snakes to account for potential allometric changes within species (*n* = 344; Appendix 6). Minimum adult body sizes were obtained from Ernst and Ernst (2000) for *L. elapsoides*, *L. gentilis*, and *L. triangulum*. For *L. abnorma*, *L. micropholis*, and *L. polyzona*, specific information regarding minimum mature size was unavailable and thus the minimum mature size for *L. annulata* was used for these three taxa as *L. annulata* is comparable in adult body size (Williams 1988). The smallest sexually mature size for any subspecies that has been synonymized by the species elevation of Ruane Chapter 1 was used as the minimum size for each species.

Geometric Morphometric Analyses

I used tpsDig2 v.2.16 (Rohlf 2010) to digitize 11 landmarks composed of the junctions of scales on the dorsal view of the head for each snake (Fig. 10a). These landmarks were chosen because they were clear on all specimens and have been found to be useful in detecting shape differences between snake taxa (S. Green, per. comm.; Gentilli et al. 2009; Fig. 10a) and may correspond to differences in ecology (Lillywhite and Henderson 1993; Shine et al. 2002; Vincent et al. 2004). Statistical analyses were conducted in the IMP software package (www3.canisius.edu/sheets/morphsoft.) for geometric morphometrics. I first conducted a Procrustes alignment in CoordGen6f (Sheets et al. 2004) to remove the differences in location and orientation from the photographs of each specimen. I then used a canonical variates analysis (CVA) in CVAGen6j (Sheets et al. 2004) with an assignment test to determine if individuals could be correctly classified to their pre-assigned species. The CVA determines the set of CV axes there are in the dataset at a *P*-value of 0.05 that maximize the variation among the pre-determined groups (species); significant axes indicate that at least one group can be distinguished along that CV, although it does not explicitly reveal which group(s) (Webster and Sheets 2010). Simultaneously, it calculates a canonical variates score for each individual in the dataset, which can plotted along the CV axes to visualize the differences among all individuals in multidimensional space (Webster and Sheets 2010). The assignment test is a distance-based method that then determines the probability that an individual has a mean canonical variates score closer to the species to which it was assigned than to any other species. To cross-validate the results, a jackknife consisting of 1000 replicates with 20% of samples considered as "unknown" was conducted, also in CoordGen. A Wilcoxon signed rank test was done in the software package STATISTICA v.6 (StatSoft, Inc., Tulsa, Oklahoma) to determine if the CVA results were significantly different between the dataset including all specimens versus the dataset of only adult specimens. In addition, I generated a Procrustes consensus alignment of all adult specimens (Fig. 10b) in tpsSuper v.1.14 (Rohlf 2004) and then used tpsSplin (Rohlf 2004) to generate a thin-plate spline deformation for a representative of each species in to help visualize how the head shape varied among the six species. A Spearman rank correlation, also performed in STATISTICA, between species age (from Ruane Chapter 1) and the bending-energies from the thin-plate splines was conducted to determine if there was a relationship between species age and the degree of morphological differentiation of species. Thin-plate spline bending energies are based on how much energy it takes to deform a thin metal plate from the Procrustes consensus alignment of landmarks to the landmark positions of a particular species (Bookstein 1989).

Results

The CVA detected four significant canonical variates axes for the dataset of all specimens, meaning that at least one species differed significantly from the others along each axis (Table 8; Fig. 11) but assignment tests were not considered robust for any species when using the entire dataset, with all species having classification rates <90% (Tabachnik and Fidell 2001). The assignment test from CVAGen identified *Lampropeltis polyzona* correctly less often than any other species (56.6%; Table 9) while *L. elapsoides* was correctly identified most frequently (87.5%; Table 9). Generally, most misidentifications were individuals of *L. triangulum* and *L. polyzona* being assigned to the wrong species (Table 10). The jackknife sampling found that "unknown" specimens could be assigned to the correct species 71.6% of the time. For the dataset using only adult specimens results were similar to the entire dataset and the CVA detected four significant canonical variates axes (Table 8). *Lampropeltis polyzona* was also identified correctly least often in the assignment analysis (59.2%) and *L. elapsoides*, followed closely by *L. gentilis* were identified correctly most often and were well supported at values >90% (Tables 9, 10). The jackknife sampling results using the adult dataset was slightly better than the entire dataset, with 73.4% of "unknown" individuals correctly classified to species. The Wilcoxon signed rank test indicated that there were no significant differences between the dataset consisting of all specimens and that of adults only (d.f. = 6; $P > 0.05$). However, the adult specimen dataset had a higher mean percentage of correct identifications compared to the dataset of all specimens (78.1% versus 74.9%). Therefore, I used the adult dataset results as the basis for further the discussion and presentation of the canonical variates plots (Fig. 11).

Thin-plate spline visualizations illustrate the shape differences between the species (Fig. 12); most notably, the thin-plate splines show *L. elapsoides* has the smallest relative distances between most landmarks, with the smallest eyes of all six species based on landmarks 6 and 7 and an overall narrower head shape (Fig. 12). In contrast, *L. polyzona* had a broad head and large eyes relative to the other species. The thin-plate splines also show that *Lampropeltis polyzona* and *L. abnorma* have eyes that are positioned closer to their rostrum and that they, along with *L. micropholis* have a wider and more rounded head based on landmarks 1,4,5,6,7, and 11 compared to the head shapes of *L. triangulum*, *L. gentilis*, and *L. elapsoides* (Fig. 12). The head shapes of *L. triangulum* and *L. gentilis* were similar and intermediate in overall shape between the wider, rounded heads of *L. polyzona*, *L. abnorma*, and *L. micropholis* and that of *L. elapsoides*, with a more narrow head. The Spearman rank correlation showed no significant relationship between species age and thin-plate spline bending energies (d.f. = 4; $r_s = -0.073$; $P>0.05$).

Discussion

Geometric morphometric techniques have not been frequently used to examine shape variation in snakes, despite the prevalence of GM-based analyses for many other taxonomic groups (e.g., fish, Kerschbaumer and Sturmbauer 2011; insects, Francoy et al. 2011; primates, Bienvenu et al. 2011). Here, I find that GM generally detects differences in the head shape of milksnakes previously identified using molecular species delimitation, albeit with a relatively low jackknife support value of 73.4% for assigning individuals to the correct species. For the six species I examined, the CVA based on the GM alignments correctly classified individual specimens to species ~59-91% of the time (Table 9); these results are robust for only two species (*L. elapsoides* and *L. gentilis*; Table 9) as values < 90% are not considered well-supported for classification analyses (Tabachnick and Fidell 2001). Analyses based on mature snakes gave more accurate results overall, with a greater percentage of correct classifications in the CVA, perhaps indicating ontogenetic changes with respect to allometry, although no significant difference was found between the dataset using both adults and juveniles and that using only adult specimens.

Accuracy of GM Among Species

Among the six taxa examined, *L. elapsoides and L. gentilis* were correctly identified more frequently than any other species (Table 9) and were the only two species classified with high support (> 90%; Table 9). *Lampropeltis elapsoides*, found in the southeastern United States, is the most distinct milksnake examined with respect to head shape and consistently forms discrete clusters along the CV axes (Fig. 11). This species has a relatively narrow snout and a head that is less distinct from the body compared to other milksnake species (Fig. 9; Williams 1988). *Lampropeltis elapsoides* was the only species examined that has a semi-fossorial lifestyle and its narrow head shape likely reflects this (Williams 1988), as it has been shown that fossorial snakes tend to have a reduced head-width (Savitzky 1983). The other species identified correctly most often, *L. gentilis*, is more of a habitat generalist (Ernst and Ernst 2000; Werler and Dixon 2000). However, the samples of *L. gentilis* available were limited almost exclusively to Texas, Louisiana, and Kansas (Appendix 6), although the species ranges from Louisiana as far west as Arizona and north to Montana (Ruane Chapter 1). The distribution of available specimens may have limited the extent of the variation among samples, resulting in fewer mistakes regarding classification than the other species with similarly broad ranges (Table 9).

In contrast to *L. elapsoides* and *L. gentilis*, the Mexican species *L. polyzona* was correctly identified only ~59% of the time (Table 9). This species has been shown to have large population sizes with the greatest amount of genetic variation relative to the other species examined here (Ruane Chapter 2). Those two factors may correspond to high levels of morphological variation, making the GM methods used here less useful in identifying *L. polyzona* specimens. As sample sizes for *L. polyzona* were second only to *L. triangulum* (Appendix 6), it is unlikely that low sample sizes were a problem in identifying this species. However, it is also possible there are additional unidentified cryptic taxa within *L. polyzona* that account for this variation; intensive sampling within a molecular framework would be beneficial for testing this hypothesis. The three remaining species, *L. triangulum*, *L. abnorma*, and *L. micropholis* were all correctly identified ~75% of the time in the CVA (Table 9). *Lampropeltis triangulum* ranges across the eastern United States as far west as Iowa (Ruane Chapter 1) and sampling covered the majority of its range and was extensive with respect to sample sizes (Appendix 6). Therefore, it is likely that much of the morphological variation present among populations was captured and the GM identification rate of ~79% is realistic. The Central American *Lampropeltis abnorma* and lower Central/ northern South American *L. micropholis* had similar numbers of correctly identified specimens (~73% and ~78% respectively). These two species had the lowest sample sizes, although samples covered a smaller portion of their presumed ranges (based on Ruane Chapter 1). Being that sample sizes were low, it is difficult to determine whether the identification rates for *L. abnorma* and *L. micropholis* are reliable or whether additional samples would increase accuracy as canonical variates analysis has been shown to perform better with increasing sample sizes relative to the number of variables (James and McCulloch 1990; Mitteroecker and Gunz 2009). Interestingly, *L. abnorma* and *L. micropholis* are sister species (Ruane Chapter 1) found in similar, tropical rainforest habitat (Williams 1988; Campbell 1998; Savage 2002; Kohler 2008) and CVA misidentifications were mostly the classification of *L. abnorma* as *L. micropholis* and vice versa (Table 10). *Lampropeltis abnorma* was also frequently misclassified as *L. polyzona*, which is the sister taxon to *L. abnorma* + *L. micropholis* (Ruane Chapter 1).

Utility of Geometric Morphometrics in Species Delimitation

My results indicate GM methods are generally useful for detecting head shape variation among milksnakes, but may fail when species have elevated intraspecific variation, as is indicated by the visual examinations of the CVA plots, where *L. polyzona* does not form discrete clusters of points and subsequently is classified correctly least often. In cases where GM-based methods are unable to correctly classify species it has been suggested that environmental constraints may limit differentiation of species morphology or that not enough time has passed to allow the accumulation of detectable amounts of morphological divergence (Dobigny et al. 2002). Both of these factors are likely relevant to milksnakes, particularly with regard to *L. abnorma* and *L. micropholis*. As previously stated, these two taxa are found in similar tropical rainforest habitats (Williams 1988; Campbell 1998; Savage 2002; Kohler 2008). It is possible that niche is conserved between them, resulting in similar morphology; this has been demonstrated between many sister-taxon pairs (Peterson et al. 1999). Contributing to the lack of morphological differentiation could also be the amount of time that has passed since speciation; *L. abnorma* and *L. micropholis* are the youngest sister-species pair included here (~1.1 Ma, Ruane Chapter 1) and studies on similarly aged sister-taxa often show a lack of morphological differentiation (e.g., Mayer and von Helversen 2001; Berman et al. 2005). However, the correlation between species age and the thin-plate spline deformation from the consensus alignment was not significant, indicating that younger milksnake species do not have less morphological change than do older taxa. In addition, sampling throughout the entire range of a species is necessary to determine how well GM performs. By limiting the distribution of samples to a few populations, it is possible that some intraspecific variation in morphology is remains unexamined. The CVA plots (Fig. 11) show that many of the species overlap with respect to geometric morphometric space.

Therefore GM may be, at least in the case of milksnakes, better for detecting differences between species post delimitation and would be less useful as an exploratory analysis in differentiating between taxa. Similar conclusions have been reached by previous studies that have used GM, as well as traditional morphometrics, to identify differences between species (Mutanen and Pretorius 2007).

Conclusions

Despite their ability to provide insight into shape variation and enhance studies pertaining to ontogeny, phylogeny, and taxonomy, geometric morphometric techniques have rarely been used for examining snakes. Although results indicate that GM is not 100% accurate in classifying the six milksnake species examined here and only two of them are classified with high support (*L. elapsoides* and *L. genetilis*; Table 9), the results do indicate that the species identified by the molecular delimitation of Ruane Chapter 1 have detectable shape differences. Furthermore, the overall accuracy of species differentiation in this study is similar to that found in other studies for closely related taxa (Dobigny et al. 2002; Francuski et al. 2008). Using GM-methods has also been found to be more accurate than visual morphological evaluations of species (Mutanen and Pretorius 2007) and, therefore, it is likely that the differences between the six species described here are more reliable than those based on polymorphic traits such as the degree of mottling on scales, which has been previously used in milksnake taxonomy (Williams 1988). More accurate results might be obtained if larger sample sizes with greater geographic extent were available, especially for *L. gentilis, L. abnorma,* and *L. micropholis*. Furthermore, the inclusion of the missing species, *L. annulata* would allow for a more thorough examination of whether the seven species identified by Ruane Chapter 1 through molecular delimitation all have detectable shape differences. Additional GM analyses that incorporate other aspects of species morphology could enhance the accuracy of results (e.g, side-views of the head), as might semi-landmark methods that capture head shape though the use of outlines. Despite GM analyses not resulting in perfect species classifications for milksnakes, my study illustrates that GM is able to provide additional information with respect to shape differentiation among taxa that might otherwise be overlooked.

Table 1. Loci amplified for *Lampropeltis*. The length in base pairs (bp), the model of evolution, number of variable sites, and total number of sequences for each locus is listed. Additional details for each locus and PCR protocols are listed in Appendix 2.Variable sites (V.S.) for all Lampropeltis species and milksnakes are included.

Locus	**Length (bp)**	**Model**	**V.S. *Lampropeltis***	**V.S. Milksnakes**	**Number of Sequences**
CYTB	1117	TPM+Γ+I	448	341	329
NT3	481	GTR+ Γ	30	26	105
PRLR	552	TRN+Γ+I	30	21	115
GAD2 intron 15	541	HKY+Γ+I	36	24	111
NAV intron 5	561	HKY+Γ	30	22	115
SPTBN1 intron 1	839	TPM+Γ+I	59	44	124
VIM introns 5	584	HKY+I	43	25	118
CL4	373	HKY+Γ+I	25	17	102
LAT clone	705	TRN+ Γ	35	25	100
2CL3	429	F81+ Γ	20	17	106
2CL4	376	TRN+ Γ	25	15	103
2CL8	466	HKY+Γ+I	69	56	120

Table 2. Mean number of migrants per generation between geographically adjacent milksnake lineages + *L. alterna* using Migrate v.3.2.16. Results are based on eleven nuclear loci and a mean generation time for *Lampropeltis* of 2.5 years. The 95% highest posterior density is shown in parentheses.

From Lineage	**To Lineage**	**Migrants Per Generation**
Eastern	Western	0.45 (0.06-1.22)
Western	Eastern	0.27 (0.02-0.82)
Eastern	*L. elapsoides*	0.03 (0.00-0.17)
L. elapsoides	Eastern	0.13 (0.00-0.43)
Western	*L. elapsoides*	0.03 (0.00-0.17)
L. elapsoides	Western	0.22 (0.01-0.70)
Western	*L. alterna*	0.02 (0.00-0.14)
L. alterna	Western	0.17 (0.01-0.55)
Western	Tamaulipas	0.15 (0.00-0.53)
Tamaulipas	Western	0.30 (0.01-0.88)
Tamaulipas	MX	0.03 (0.00-0.15)
MX	Tamaulipas	0.04 (0.00-0.09)
MX	CA	0.06 (0.00-0.22)
CA	MX	0.06 (0.00-0.30)
CA	SA	0.03 (0.00-0.26)
SA	CA	0.04 (0.00-0.21)

Table 3. Posterior probabilities from BPP analyses based on the Cytb guide tree, the Structurama-based guide tree, and additional BPP runs to verify support for several taxa as delimitable species. Results are based on three runs from each parameterization. The parameters for ancestral population size and root age are as follow: BPP1 = large populations and deep divergences, BPP2 = small ancestral populations and shallow divergences, and BPP3 = large ancestral populations, and shallow divergences.

	Cytb Guide Tree				**Structurama Guide Tree**				**Additional Tests**		
	BPP1	**BPP2**	**BPP3**		**BPP1**	**BPP2**	**BPP3**		**BPP1**	**BPP2**	**BPP3**
L. alterna	100%	100%	100%	*L. alterna*	100%	100%	100%	*L. alterna*	100%	100%	100%
Colima	<90%	≥95%	100%	CA	100%	100%	100%	Eastern	100%	100%	100%
Eastern	100%	100%	100%	Eastern/Western	100%	100%	100%	Tamaulipas	100%	100%	100%
L. elapsoides	100%	100%	100%	*L. elapsoides*	100%	100%	100%	Western	100%	100%	100%
Guerrero	≥95%	≥95%	100%	MX	100%	100%	100%	N/A	—	—	—
Honduras	<95%	≥95%	100%	SA	100%	100%	100%	N/A	—	—	—
Nicaragua	<95%	≥95%	100%	Tamaulipas	100%	100%	100%	N/A	—	—	—
Oaxaca	100%	≥95%	100%	N/A	—	—	—	N/A	—	—	—
Puebla	<95%	100%	100%	N/A	—	—	—	N/A	—	—	—
SA	100%	100%	100%	N/A	—	—	—	N/A	—	—	—
Sonora	<90%	≥95%	100%	N/A	—	—	—	N/A	—	—	—
Western	100%	100%	100%	N/A	—	—	—	N/A	—	—	—

Table 4. Lineages of milksnake and their corresponding species designation and general geographic area. More detailed ranges are shown in Fig. 2. Species names are taken from the oldest known subspecies name within the range of that lineage as reported by Williams (1988).

Lineage	**Species Name**	**Main Geographic Extent**
Eastern	*L. triangulum* (Lacépède, 1788)	Eastern USA, Southeastern Canada
Western	*L. gentilis* (Baird and Girard, 1853)	Western USA
L. elapsoides	*L. elapsoides* (Holbrook, 1838)	Southeastern USA
Tamaulipas	*L. annulata* Kennicot, 1861	Eastern Mexico
Mexico (MX)	*L. polyzona* Cope, 1861	Western and Central Mexico
Central America (CA)	*L. abnorma* (Bocourt, 1866)	South-central Mexico to Eastern Costa Rica
South America (SA)	*L. micropholis* Cope, 1861	Western Costa Rica south to Ecuador

Table 5. Best diversification model and its corresponding AICc value for both the scnDNA concatenated tree (Concat.) and the species tree (Species) for full likelihood standard models, approximate likelihood coalescent models, and models that include extinction. The applicable parameter estimates for each model are included; λ = speciation rate, μ = extinction rate, K = carrying capacity, r1 = speciation rate 1, r2 = speciation rate 2, sp = shift point. Non-applicable parameters are indicated for the models (NA). *For the species tree, the next best AICc value was 3.099 for BD, less than 1 from the DD+E model.

Models	**Concat.**	**AICc**	**λ**	**μ**	**K**	**Species**	**AICc**	**λ**	**μ**	**K**	**sp**	**r1**	**r2**
Standard Model	DDL	4.815	0.68	NA	22	Yule2	-1.555	NA	NA	NA	1.080	0.440	0.090
Coalescent Model	Model 6	-6.542	NA	NA	NA	Model 5	-16.590	NA	NA	NA	NA	NA	NA
Extinction Model	DD+E	7.56	0.68	0.000003	22	DD+E	2.888*	0.57	0.05	28	NA	NA	NA

Table 6**.** Approximate range size, mean and median effective population size and number of size changes for six species of *Lampropeltis*. The 95% highest posterior density is shown for each in parentheses.

Species	**Range Size**	**Population Size Mean, Median**	**Population Size Change Mean, Median**
L. triangulum	1,800,400 km^2	5.122^{6}, 1.558^{6} (6.385^{4}- 1.564^{7})	0.5, 0.0 (0-2)
L. gentilis	1,700,000 km^2	9.547^{6}, 2.389^{6} (1.104^{5}- 2.559^{7})	0.6, 0.0 (0-2)
L. elapsoides	900,000 km^2	7.950^{6}, 3.463^{6} (1.168^{5}- 2.260^{7})	1.6, 2.0 (0-3)
L. polyzona	400,400 km^2	9.777^{6}, 7.057^{6} (1.509^{6}- 2.428^{7})	2.6, 2.0 (2-4)
L. abnorma	750,400 km^2	1.929^{6}, 1.379^{6} (2.060^{5}- 5.034^{6})	2.0, 2.0 (2-2)
L. micropholis	560,200 km^2	1.108^{6}, 7.638^{5} (1.044^{5}- 3.128^{6})	2.0, 2.0 (2-2)

Table 7. DNAsp results for each species (Sp.) and locus, including the number of haplotypes (h), haplotype diversity (Hd), number of segregating sites (S), nucleotide diversity (Pi), average number of nucleotide differences (k), Tajima's D (D), Fu and Li's D* (D*), Fu and Li's F* (F*), Ramos-Onsins and Rozas'S R_2, and Harpending's raggedness index (*rg*). For all tests, the P-value is included and for the R2 and rg results, the coalescent simulation results are shown, including the average R_2 and *rg*. Significant results ($P < 0.05$) are indicated (*).

Species	**Locus**	**h**	**Hd**	**S**	**D**	**Pi**	**k**	**D***	**F***	R_2	**Avg.** R_2	***rg***	**Avg. *rg***
L. abnorma	2CL3	4	0.477	4	-0.623	0.002	0.922	1.123	0.744	0.115	0.164	0.345	0.270
L. abnorma	2CL4	4	0.611	2	-0.543	0.002	0.674	1.006	0.670	0.168	0.164	0.263	0.306
L. abnorma	2CL8	7	0.900	6	1.120	0.006	2.400	1.272	1.412	0.200	0.154	0.061	0.167
L. abnorma	CL4	5	0.502	3	-0.600	0.002	0.632	-0.159	-0.324	0.114	0.162	0.110	0.299
L. abnorma	CYTB	9	0.845	64	0.132	0.027	24.050	-0.352	-0.247	0.157	0.156	0.046	0.060
L. abnorma	GAD2	7	0.844	5	0.283	0.003	1.500	1.176	1.067	0.150	0.141	0.103	0.211
L. abnorma	LATCL	2	0.337	1	0.352	0.001	0.337	0.650	0.653	0.168	0.178	0.220	0.361
L. abnorma	NT3	11	0.937	7	0.904	0.006	2.520	1.299	1.373	0.180	0.139	0.041	0.146
L. abnorma	PRLR	2	0.209	1	-0.529	0.000	0.209	0.667	0.405	0.105*	0.188	0.382	0.351
L. abnorma	SPTBN1	6	0.846	8	0.594	0.004	2.910	1.367*	1.327	0.182	0.155	0.176	0.151
L. abnorma	VIM56	3	0.358	3	-0.410	0.001	0.716	1.006	0.712	0.119	0.164	0.296	0.297
L. elapsoides	2CL8	3	0.579	6	2.968*	0.012	3.090	1.214	2.024	0.258	0.120	0.487	0.111
L. elapsoides	CL4	2	0.094	1	-0.714	0.000	0.094	0.526	0.190	0.047*	0.099	0.067	0.030
L. elapsoides	CYTB	17	0.831	41	-1.760	0.005	5.270	-2.840*	-2.930*	0.074	0.119	0.047	0.067
L. gentilis	2CL8	9	0.911	13	0.050	0.017	3.990	1.534*	1.262	0.153	0.125	0.113	0.094
L. gentilis	CL4	11	0.874	10	0.984	0.009	3.020	1.399	1.488	0.151	0.107	0.027	0.104
L. gentilis	CYTB	19	0.938	38	-0.610	0.009	8.133	-1.583	-1.491	0.095	0.116	0.026	0.045
L. micropholis	2CL8	5	0.496	6	-0.931	0.003	1.110	0.497	0.097	0.062	0.113	0.216	0.244
L. micropholis	CYTB	7	0.657	43	-1.734	0.008	7.870	-1.730	-1.994	0.092*	0.143	0.103*	0.079
L. micropholis	GAD2	2	0.492	1	1.437	0.001	0.492	0.612	0.961	0.246	0.152	0.243	0.335
L. micropholis	NT3	3	0.234	2	-0.606	0.001	0.347	0.798	0.461	0.087	0.146	0.429	0.375
L. micropholis	PRLR	3	0.420	2	0.062	0.001	0.551	0.837	0.718	0.138	0.154	0.126	0.322
L. micropholis	SPTBN1	3	0.331	2	0.176	0.001	0.540	0.798	0.718	0.135	0.144	0.353	0.334
L. polyzona	2CL3	3	0.477	2	0.203	0.001	0.470	-0.914	-0.676	0.132	0.122	0.208	0.367
L. polyzona	2CL8	6	0.306	8	-1.837*	0.001	0.491	-0.301	-0.926	0.041*	0.125	0.323	0.370
L. polyzona	CL4	7	0.625	5	-0.068	0.003	1.106	1.104	0.867	0.111	0.115	0.033*	0.238
L. polyzona	CYTB	18	0.968	129	0.345	0.049	39.570	0.579	0.599	0.146	0.123	0.043	0.017

Table 7. (Continued)

Species	**Locus**	**h**	**Hd**	**S**	**D**	**Pi**	**k**	**D***	**F***	**R_2**	**Avg. R_2**	**rg**	**Avg. rg**
L. polyzona	GAD2	3	0.513	3	0.424	0.001	0.566	0.761	0.768	0.141	0.127	0.169	0.328
L. polyzona	LATCL	3	0.284	3	-0.660	0.001	0.295	0.762	0.400	0.074	0.139	0.265	0.390
L. polyzona	NAV56	8	0.854	9	-0.455	0.005	1.870	1.364	0.935	0.104	0.123	0.048	0.164
L. polyzona	NT3	19	0.947	16	1.312	0.015	5.510	1.613*	1.788*	0.172	0.109	0.019*	0.060
L. polyzona	PRLR	8	0.438	8	-1.658	0.002	0.749	0.612	-0.130	0.048*	0.120	0.148	0.298
L. polyzona	SPTBN1	8	0.730	8	-1.102	0.002	1.176	-0.030	-0.428	0.078	0.123	0.064	0.238
L. polyzona	VIM56	4	0.243	4	-1.448	0.001	0.340	1.013	0.307	0.043*	0.131	0.373	0.392
L. triangulum	2CL8	8	0.751	10	0.153	0.017	2.470	1.402	1.179	0.124	0.114	0.072	0.132
L. triangulum	CL4	7	0.419	7	-0.475	0.004	1.233	1.232	0.799	0.088	0.109	0.280	0.217
L. triangulum	CYTB	18	0.943	31	-0.500	0.007	6.512	-0.720	-0.764	0.098	0.114	0.030	0.053

Table 8. Results from canonical variates analysis showing the lambda, *P*-value, and degrees of freedom (D. F.) for each significant axis.

		All Specimens	**Adult Specimens**
Axis 1	*P*-value	$P < 0.001$	$P < 0.001$
	Lambda	0.0153	0.112
	D. F.	90	90
Axis 2	*P*-value	$P < 0.001$	$P < 0.001$
	Lambda	0.414	0.366
	D. F.	68	68
Axis 3	*P*-value	$P < 0.001$	$P < 0.001$
	Lambda	0.745	0.670
	D. F.	48	48
Axis 4	*P*-value	$P = 0.001$	$P = 0.006$
	Lambda	0.884	0.812
	D. F.	30	30

Table 9. Canonical variates analyses results summary. The number of correctly identified and misidentified individual for each species is shown for both the entire dataset and the dataset consisting of only mature, adult specimens.

Species	**Misidentified (All, Adults)**	**Percentage Correct (All, Adults)**
L. triangulum	77/340, 52/243	77.4%, 78.6%
L. gentilis	7/ 31, 3/ 31	77.4%, 90.3%
L. elapsoides	4/ 33, 2/ 23	87.9%, 91.0%
L. polyzona	23/ 52, 11/ 27	56.6%, 59.2%
L. abnorma	4/ 17, 3/ 11	76.4%, 72.6%
L. micropholis	4/ 15, 2/ 9	73.3%, 77.8%

Table 10. Groupings from the canonical variates analyses for all specimens and adults only showing how many individuals from a species were identified to each species.

	L. triangulum	***L. gentilis***	***L. elapsoides***	***L. polyzona***	***L. abnorma***	***L.micropholis***
L. triangulum	263, 191	20, 14	2, 0	49, 36	4, 1	1, 1
L. gentilis	4, 1	24, 29	1, 0	2, 1	0, 0	0, 0
L. elapsoides	1, 0	2, 1	29, 21	1, 1	0, 0	0, 0
L. polyzona	8, 4	4, 1	0, 0	29, 16	10, 5	1, 1
L. abnorma	0, 0	0, 0	0, 0	2, 2	13, 8	2, 1
L. micropholis	0, 0	0, 0	0, 0	0, 0	3, 2	12, 7

Figure Legends

Figure 1. Photographs of milksnakes from across their range. Localities are as follows (a) Alabama, USA, (b) Montana, USA (c) Colima, Mexico, (d) Indiana, USA, (e) Veracruz, Mexico, and (f) Pinchincha, Ecuador . Photos by S. Ruane (a, d), D. Shepard (b), J. Streicher (c, e), and D. Salazar (f).

Figure 2. Cytochrome b gene tree (a) and species tree chronogram (b) for *Lampropeltis* + two outgroups (*n* = 329, Cytb tree; *n* = 124, species tree) inferred using Bayesian inference in the program BEAST/*BEAST. Time for the species tree chronogram is in millions of years ago (Ma). All species/clades include ≥2 individuals (see Appendix 1 for specimen details). Milksnake clades are named and indicated by colored lineages; names and colors correspond with the Cytb lineage map (c) and species tree map (d). Posterior probabilities ≥ 95% indicated by ●; ≥ 85% < 95% indicated by ■. Note that all *L. alterna* (*n* = 13) and one *L. mexicana* are included within the Western *L. triangulum* clade in the Cytb tree.

Figure 3. Nuclear gene network from Splitstree showing relationships for milksnakes (*n* = 82) and *L. alterna* (*n* = 7). Lineage names and colors correspond to those used in the species tree and are as follows: A) MX lineage, red, B) *L. alterna*, grey, C) *L. elapsoides*, purple, D) Tamaulipas lineage, teal, E) Western lineage, green, F) Eastern lineage, blue, G) Central America lineage, yellow, H) South America lineage, pink. Photos by S. Ruane (A, C), R. Hansen (B, D), D. Shepard (E), M. Graziano (F), L. Porras (G), J. Streicher (H).

Figure 4. Species tree chronogram (a) from *BEAST and concatenated scnDNA chronogram (b) from BEAST showing mean divergence times (Ma) and including error bars indicating the 95% highest posterior density. Support values <95% are indicate by (*).

Figure 5. Plots showing a) the species tree branching times vs. the scnDNA concatenated tree branching times for *Lampropeltis*, with a line through the origin illustrating that the mean times of the concatenated tree are generally older and b) a linear regression of the scaled-branching time error against the species-tree branching times, including the R^2 and *P*-values.

Figure 6. Map showing geographic features discussed in text and the samples and ranges of three temperate milksnakes, *Lampropeltis triangulum* (blue), *L. gentilis* (green), *L. elapsoides* (purple), and one tropical species, *L. polyzona* (red). The extent of the Laurentide Ice Sheet during the last glacial maximum is shown in grey (adapted from Pielou 1991). Ranges of all species are approximate and based on Ruane Chapter 1.

Figure 7. Map showing geographic features discussed in text and the samples and ranges of three tropical milksnakes, *Lampropeltis polyzona* (red), *L. abnorma* (yellow), and *L. micropholis* (pink). Ranges of all species are approximate and based on Ruane Chapter 1.

Figure 8. Extended Bayesian Skyline Plots for each species for the last one million years before present (YBP) showing the median (black) and 95% highest posterior density changes in effective population size (N_e) over time on a log-transformed scale.

Figure 9. Representatives of the six milksnake species used in the geometric morphometric study; a) *L. triangulum*, b) *L. gentilis,* c) *L. elapsoides,* d) *L. polyzona*, e) *L. abnorma*, d) *L. micropholis*.

Figure 10. The eleven landmarks used for geometric morphometric analyses (a) and the Procrustes superimposition consensus of the landmarks averaged across all specimens (b).

Figure 11. Canonical variates analysis plots of the first axis plotted against the second (a), third (b), and fourth (c) axes. Species symbols are as follows:○ = *L. triangulum*, **X** = *L. gentilis*,★ = *L. polyzona*,■ = *L. abnorma*, + = *L. micropholis*, and ✳ = *L. elapsoides*.

Figure 12. Thin-plate splines of each species deformed from the Procrustes consensus alignment of all species.

Figure 1

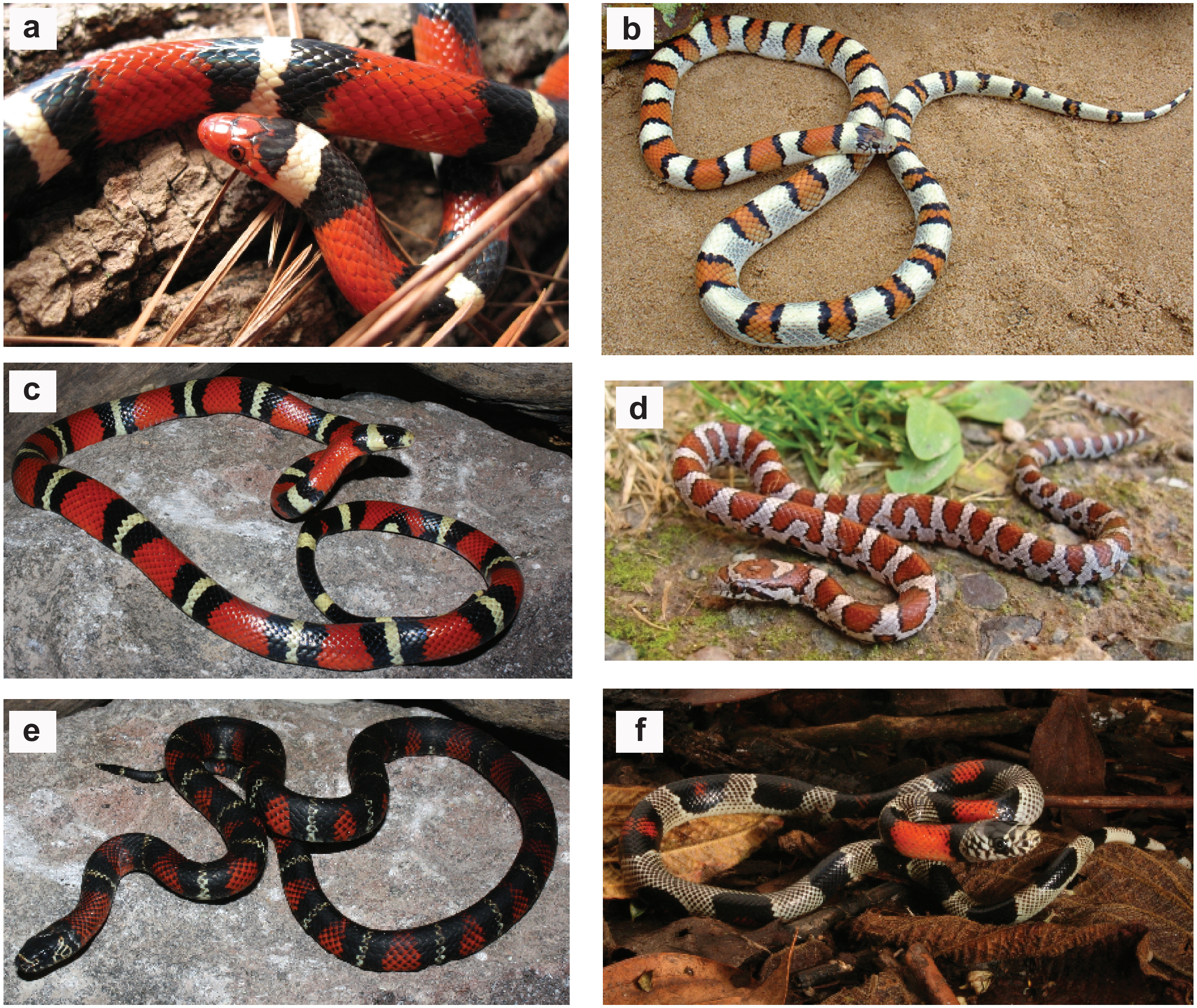


Figure 2 (a, b)

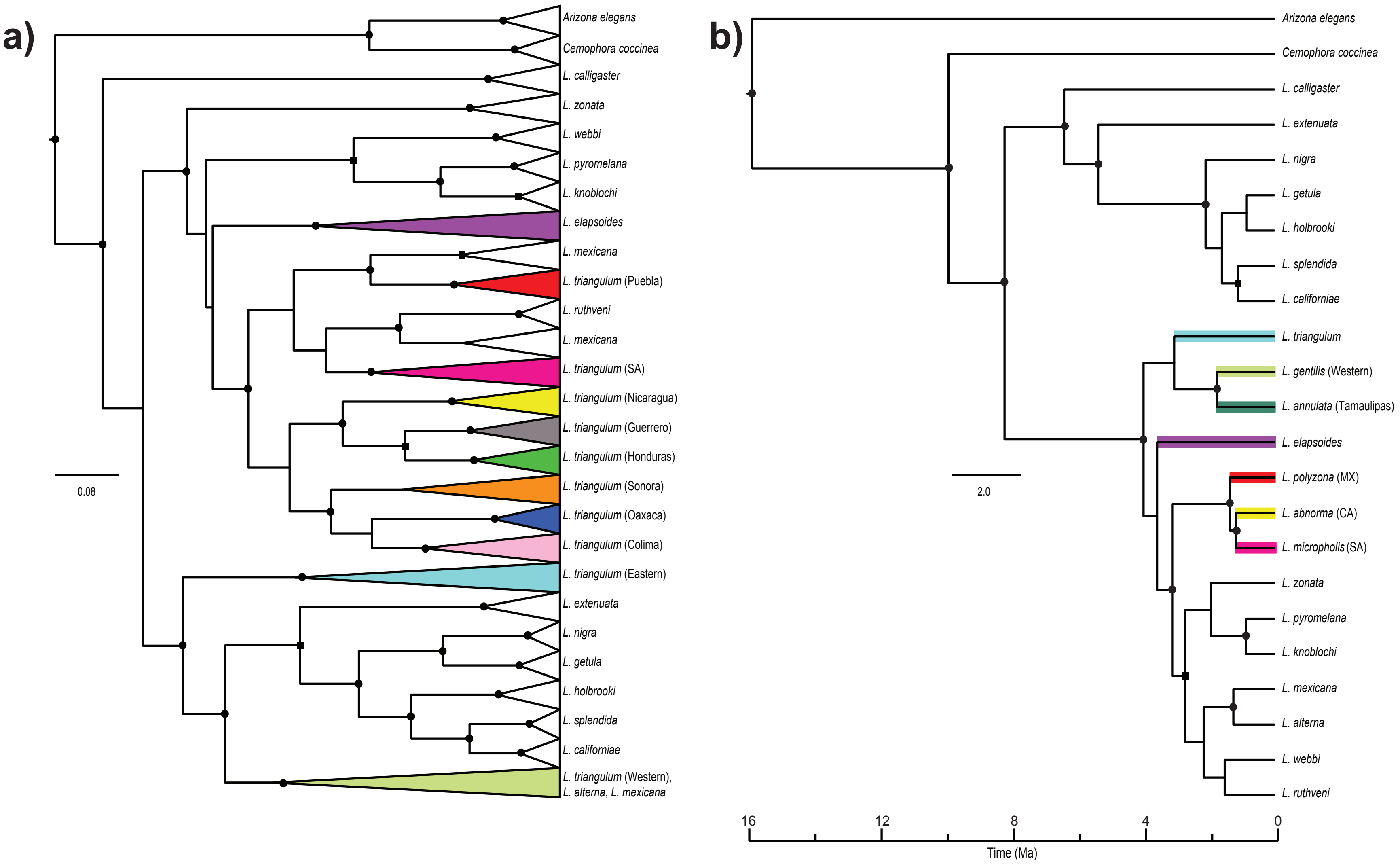

a)
Arizona elegans
Cemophora coccinea
L. calligaster
L. zonata
L. webbi
L. pyromelana
L. knoblochi
L. elapsoides
L. mexicana
L. triangulum (Puebla)
L. ruthveni
L. mexicana
L. triangulum (SA)
L. triangulum (Nicaragua)
L. triangulum (Guerrero)
L. triangulum (Honduras)
L. triangulum (Sonora)
L. triangulum (Oaxaca)
L. triangulum (Colima)
L. triangulum (Eastern)
L. extenuata
L. nigra
L. getula
L. holbrooki
L. splendida
L. californiae
L. triangulum (Western),
L. alterna, L. mexicana
0.08
b)
Arizona elegans
Cemophora coccinea
L. calligaster
L. extenuata
L. nigra
L. getula
L. holbrooki
L. splendida
L. californiae
L. triangulum
L. gentilis (Western)
L. annulata (Tamaulipas)
L. elapsoides
L. polyzona (MX)
L. abnorma (CA)
L. micropholis (SA)
L. zonata
L. pyromelana
L. knoblochi
L. mexicana
L. alterna
L. webbi
L. ruthveni
2.0
16
12
8
4
0
Time (Ma)

Figure 2 (c,d)

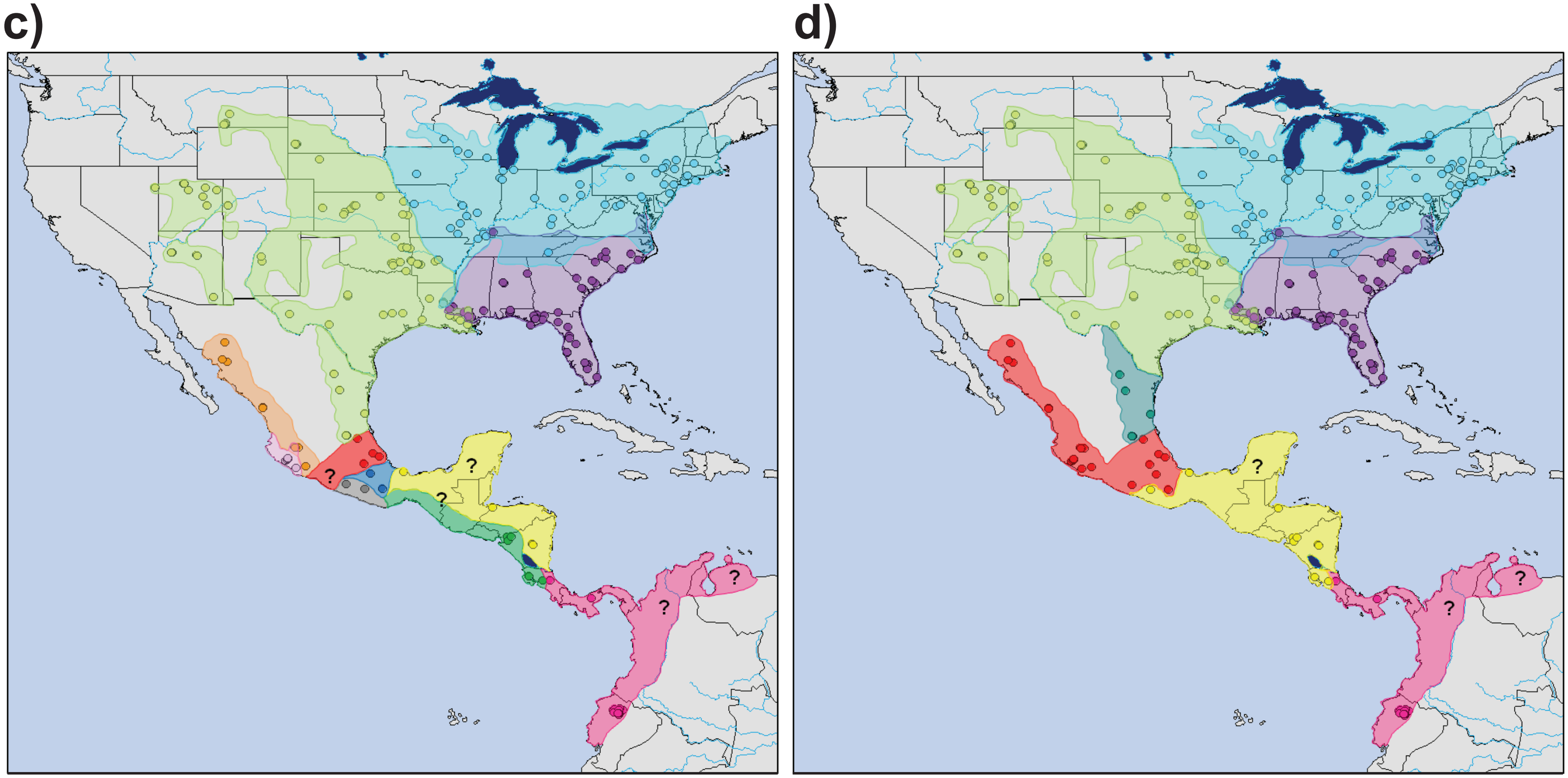


Figure 3

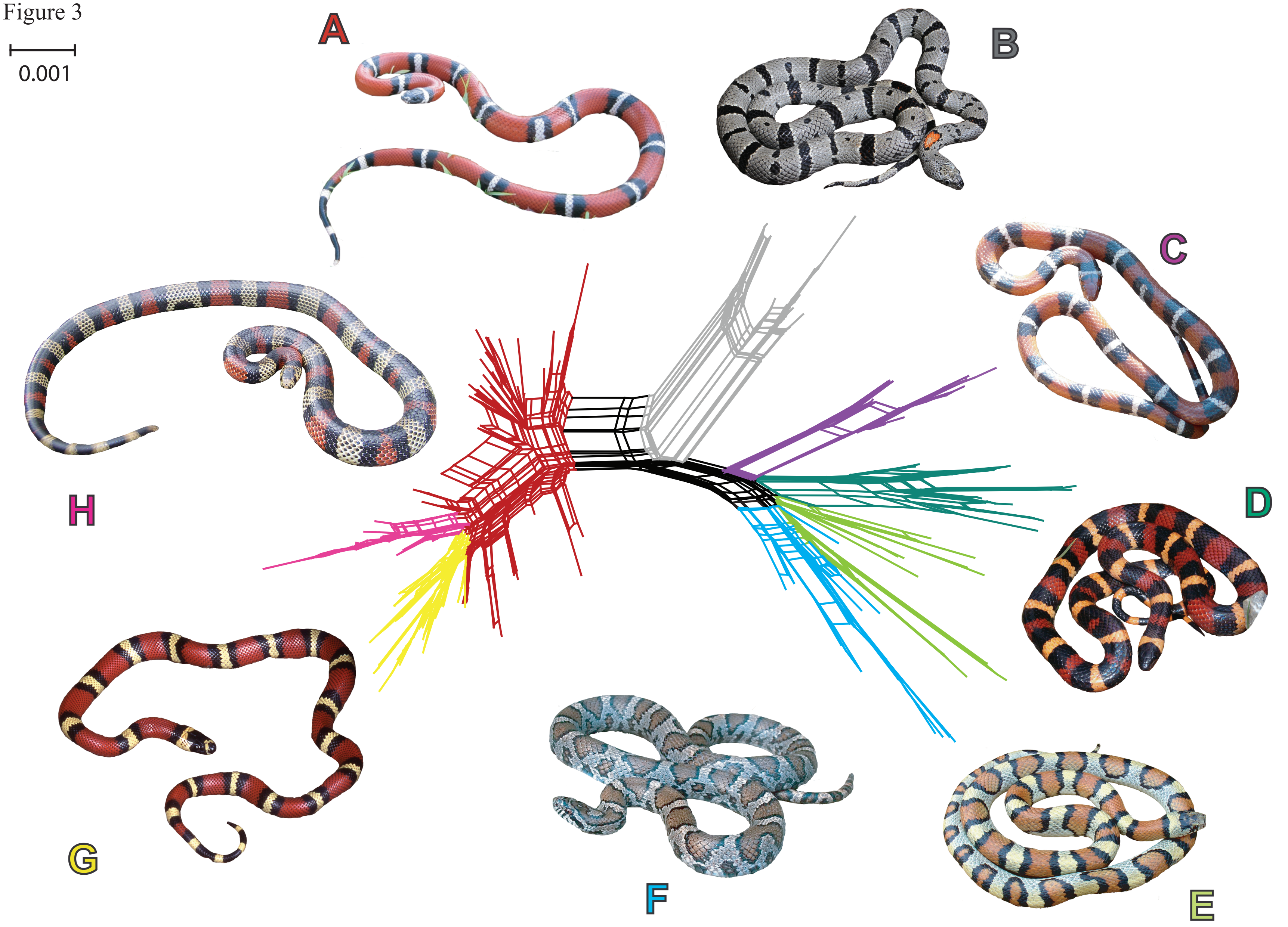


Figure 4

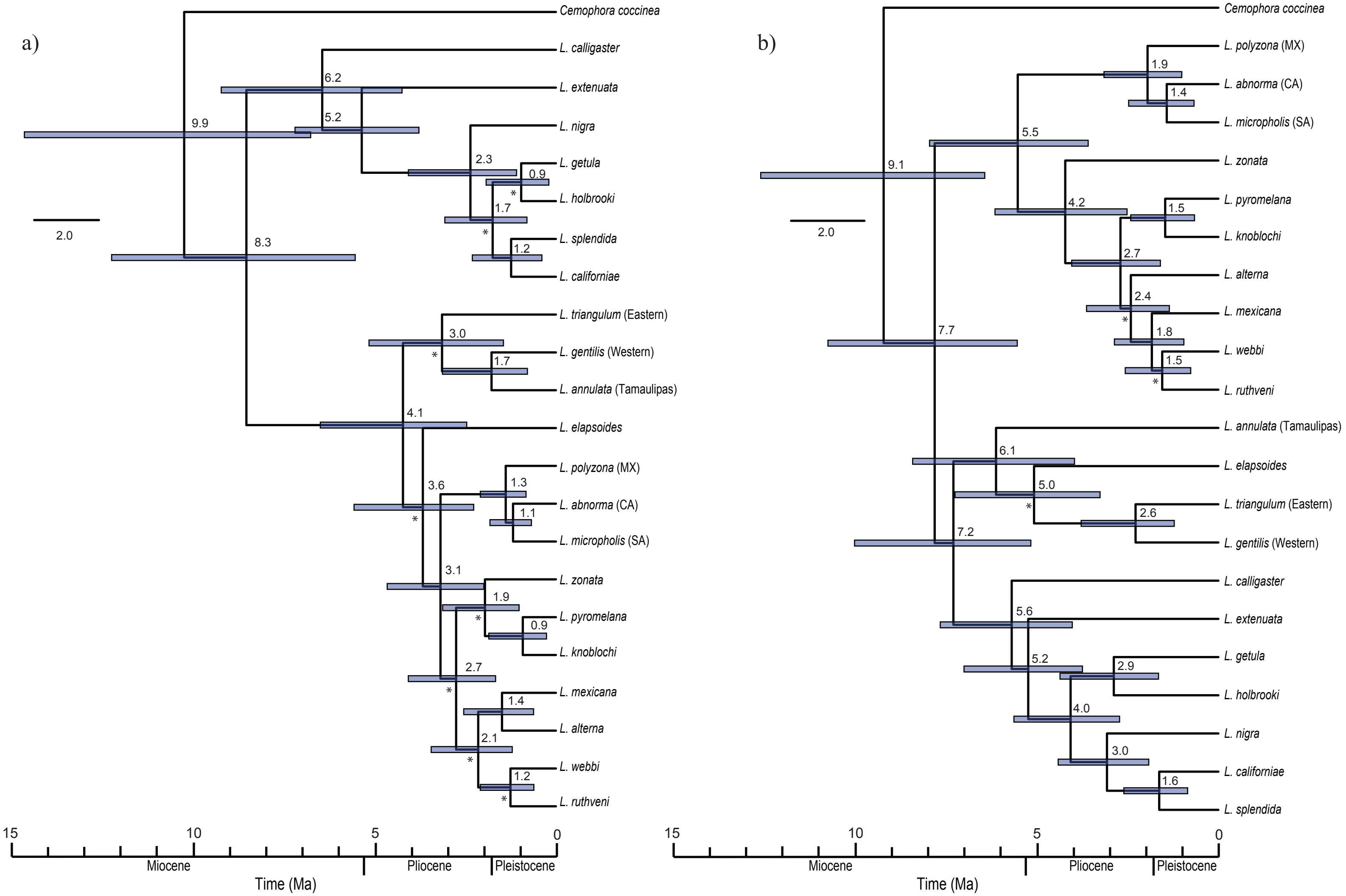

a)
Cemophora coccinea
L. calligaster
L. extenuata
L. nigra
L. getula
L. holbrooki
L. splendida
L. californiae
L. triangulum (Eastern)
L. gentilis (Western)
L. annulata (Tamaulipas)
L. elapsoides
L. polyzona (MX)
L. abnorma (CA)
L. micropholis (SA)
L. zonata
L. pyromelana
L. knoblochi
L. mexicana
L. alterna
L. webbi
L. ruthveni
9.9
8.3
6.2
5.2
2.3
0.9
1.7
1.2
3.0
1.7
4.1
3.6
1.3
1.1
3.1
1.9
0.9
2.7
1.4
2.1
1.2
*
2.0
15
10
5
0
Miocene
Pliocene
Pleistocene
Time (Ma)
b)
Cemophora coccinea
L. polyzona (MX)
L. abnorma (CA)
L. micropholis (SA)
L. zonata
L. pyromelana
L. knoblochi
L. alterna
L. mexicana
L. webbi
L. ruthveni
L. annulata (Tamaulipas)
L. elapsoides
L. triangulum (Eastern)
L. gentilis (Western)
L. calligaster
L. extenuata
L. getula
L. holbrooki
L. nigra
L. californiae
L. splendida
9.1
7.7
7.2
5.5
1.9
1.4
4.2
1.5
2.7
2.4
1.8
1.5
6.1
5.0
2.6
5.6
5.2
2.9
4.0
3.0
1.6
*
2.0
15
10
5
0
Miocene
Pliocene
Pleistocene
Time (Ma)

Figure 5

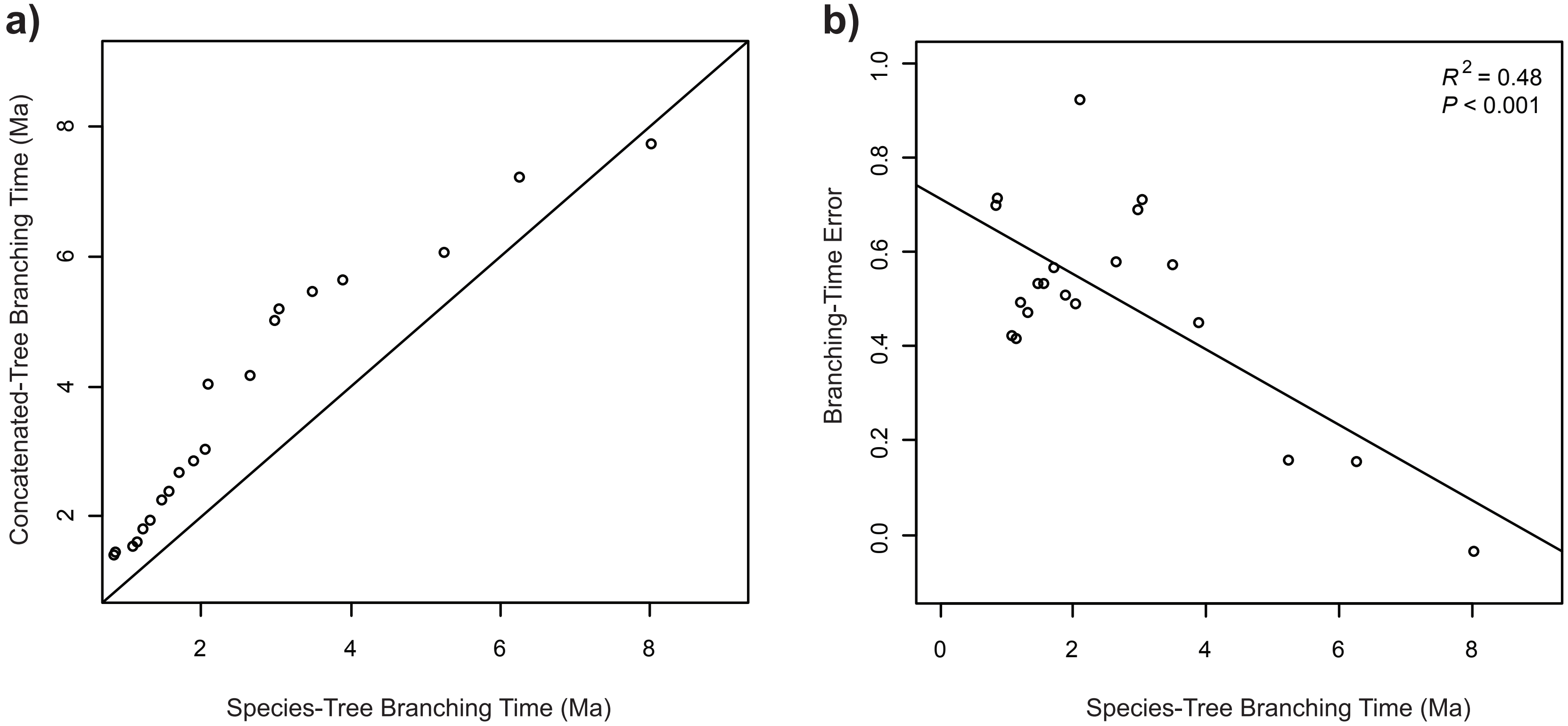

a)
Concatenated-Tree Branching Time (Ma)
2
4
6
8
2
4
6
8
Species-Tree Branching Time (Ma)
b)
Branching-Time Error
0.0
0.2
0.4
0.6
0.8
1.0
$R^2 = 0.48$
$P < 0.001$
0
2
4
6
8
Species-Tree Branching Time (Ma)

Figure 6

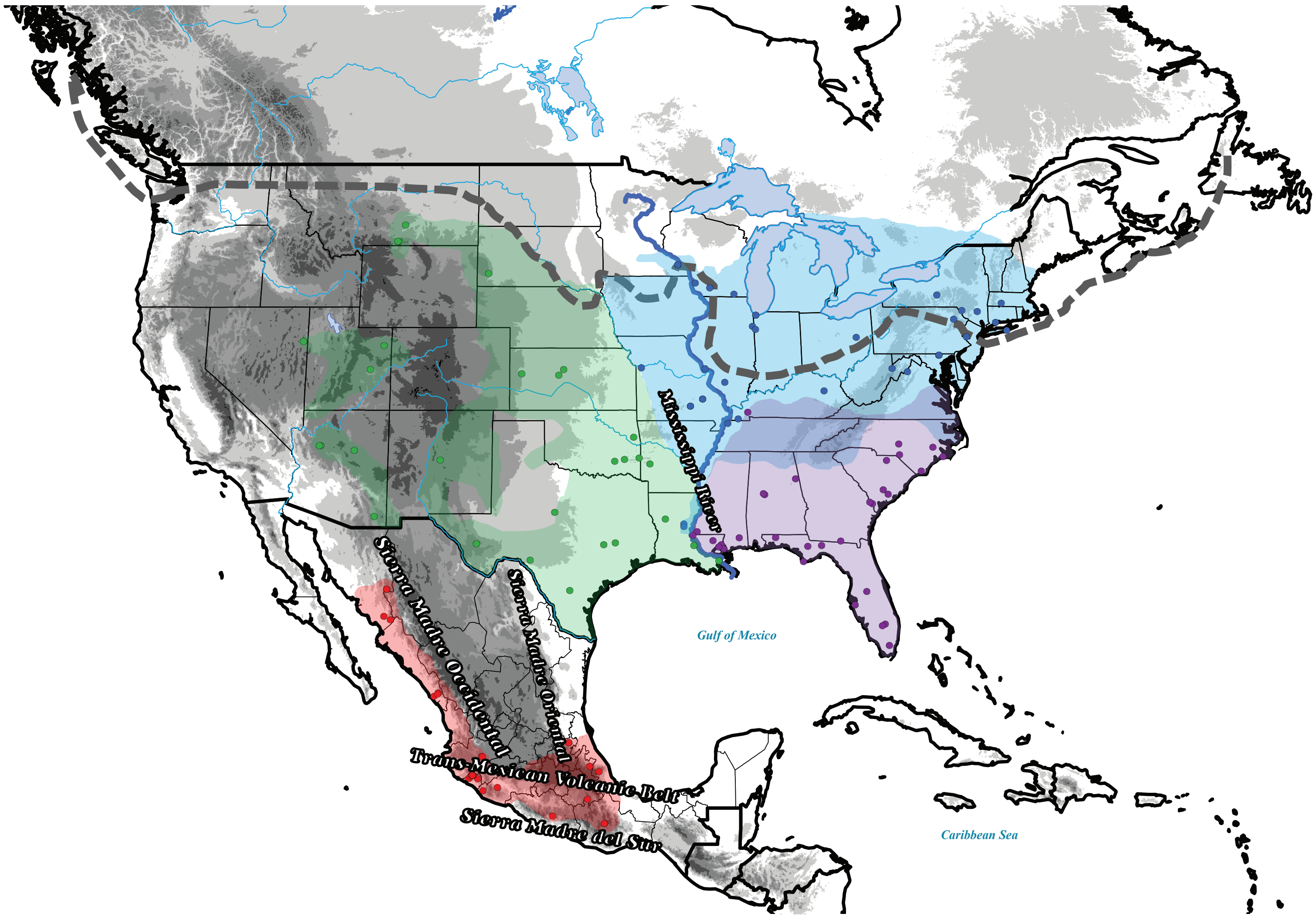

Mississippi River
Sierra Madre Occidental
Sierra Madre Oriental
Trans-Mexican Volcanic Belt
Sierra Madre del Sur
Gulf of Mexico
Caribbean Sea

Figure 7

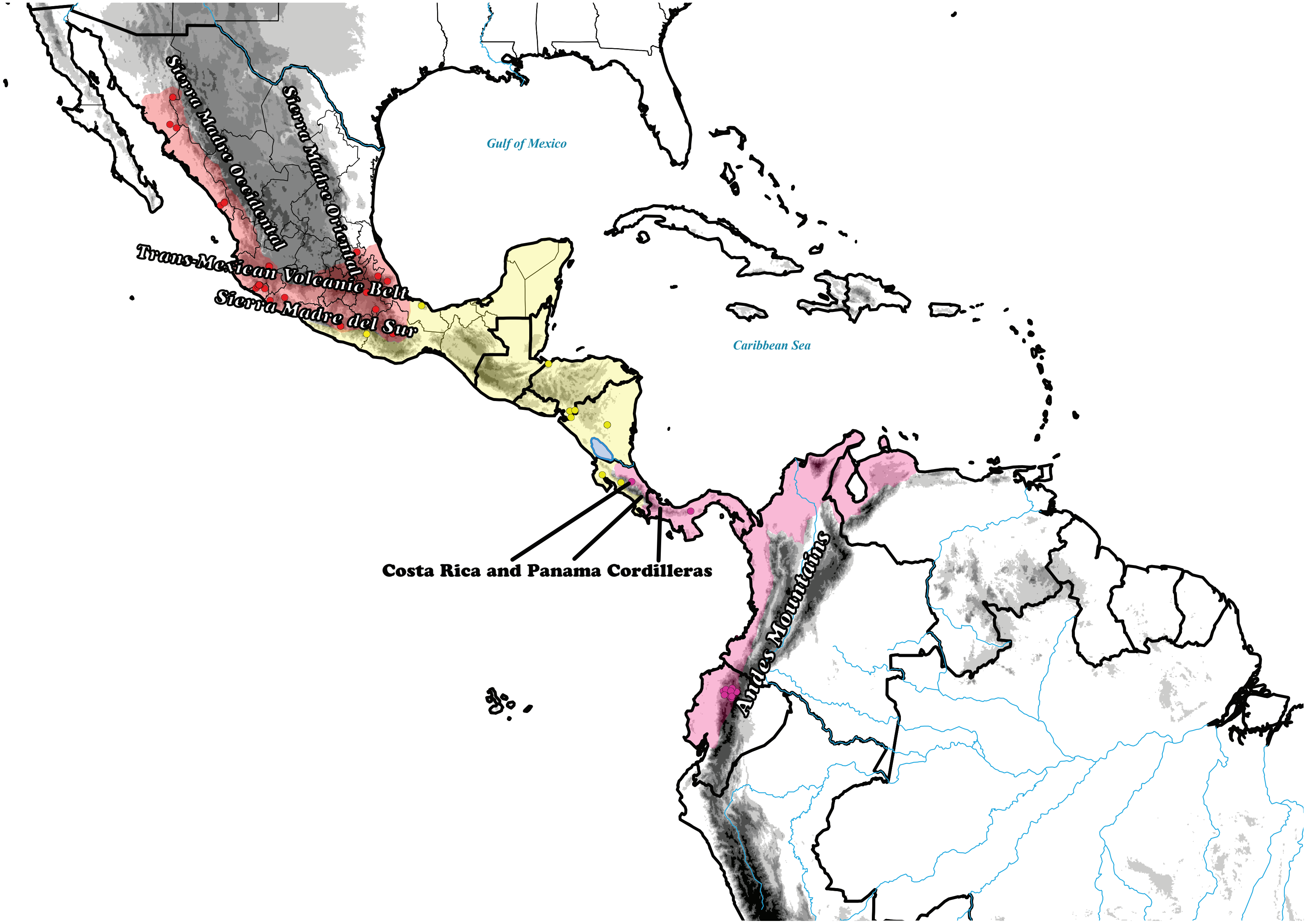

Sierra Madre Occidental
Sierra Madre Oriental
Gulf of Mexico
Trans-Mexican Volcanic Belt
Sierra Madre del Sur
Caribbean Sea
Costa Rica and Panama Cordilleras
Andes Mountains

Figure 8

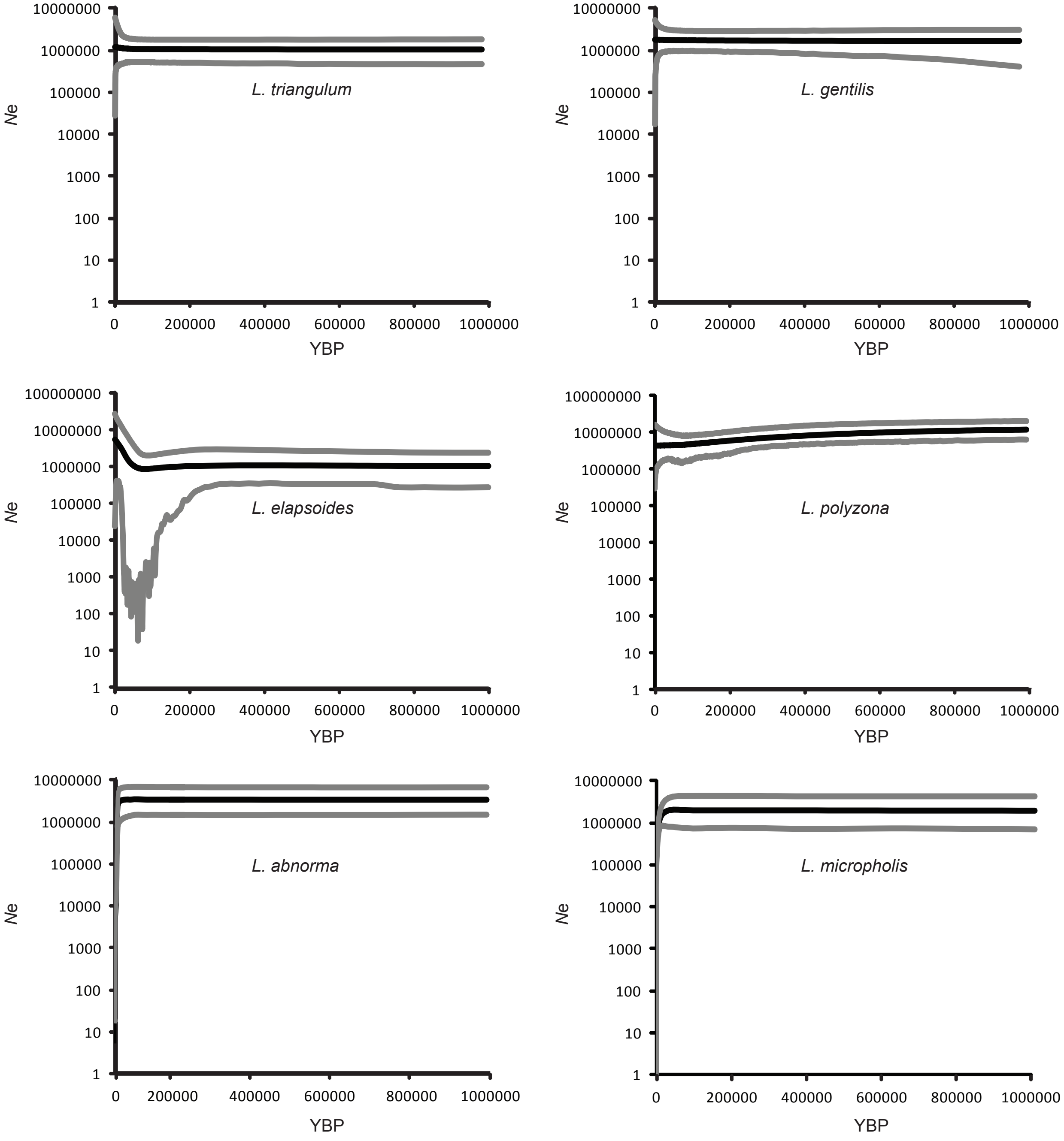

10000000
1000000
100000
10000
1000
100
10
1
Ne
L. triangulum
0
200000
400000
600000
800000
1000000
YBP
L. gentilis
100000000
L. elapsoides
L. polyzona
L. abnorma
L. micropholis

Figure 9

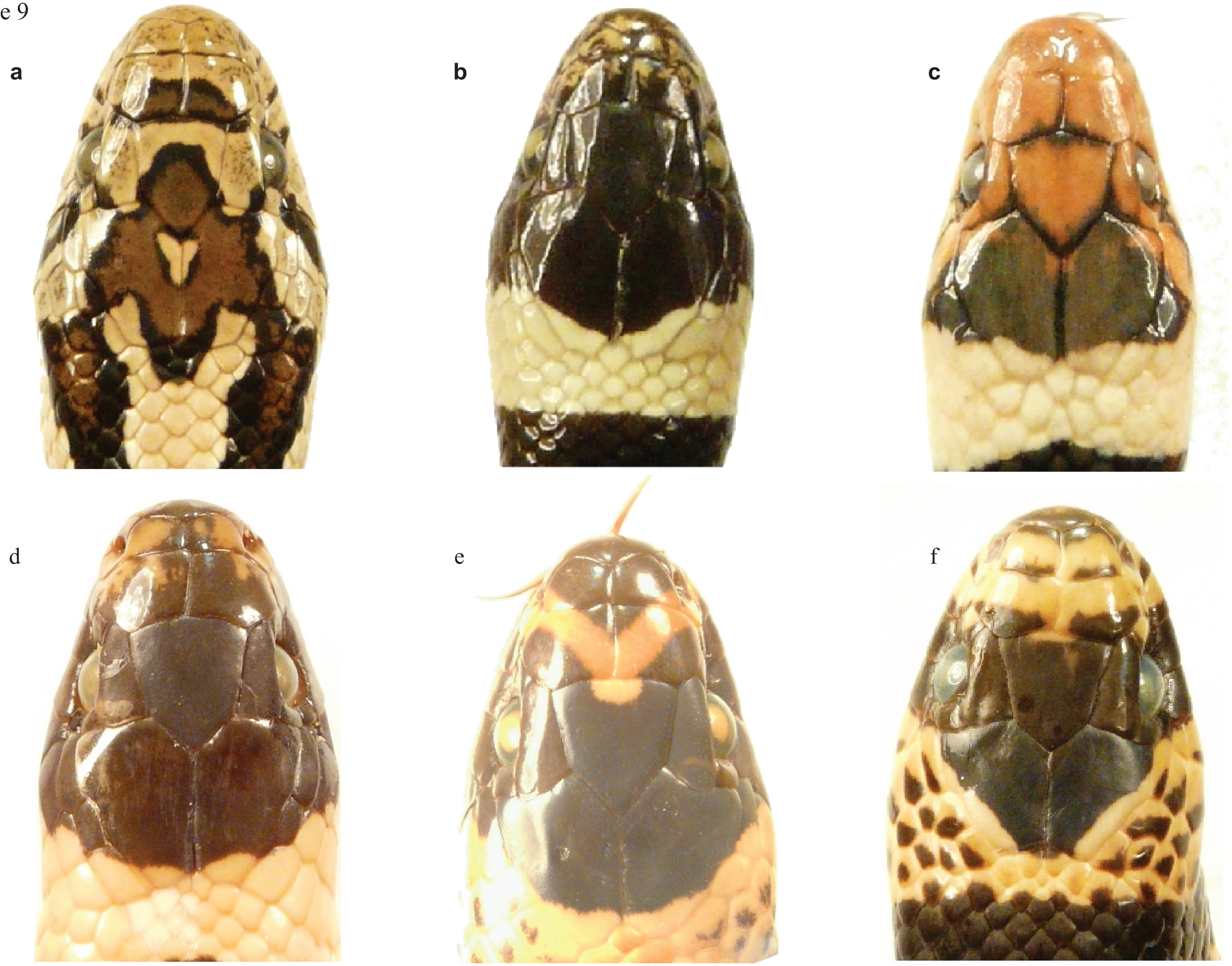


Figure 10

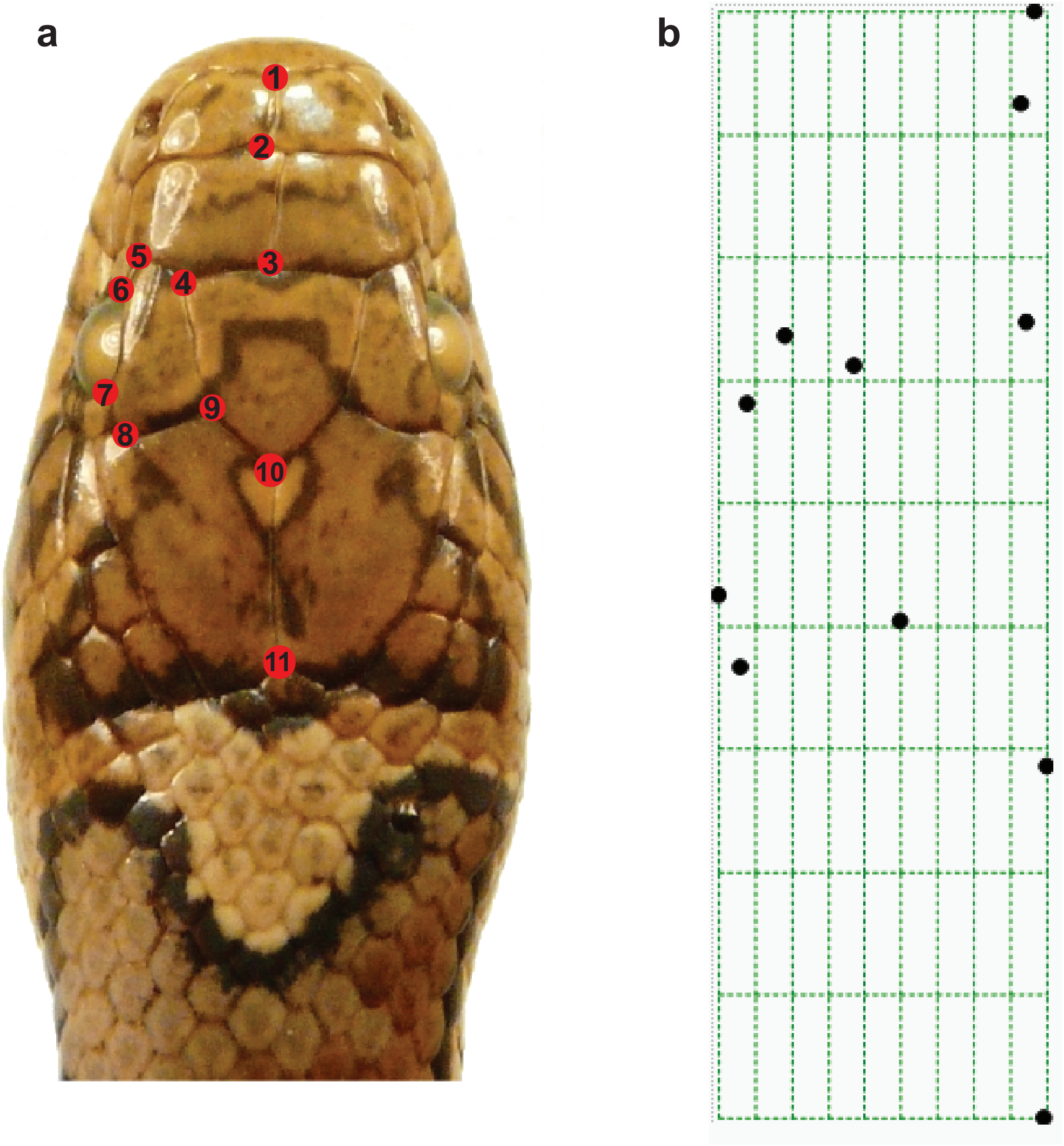


Figure 11

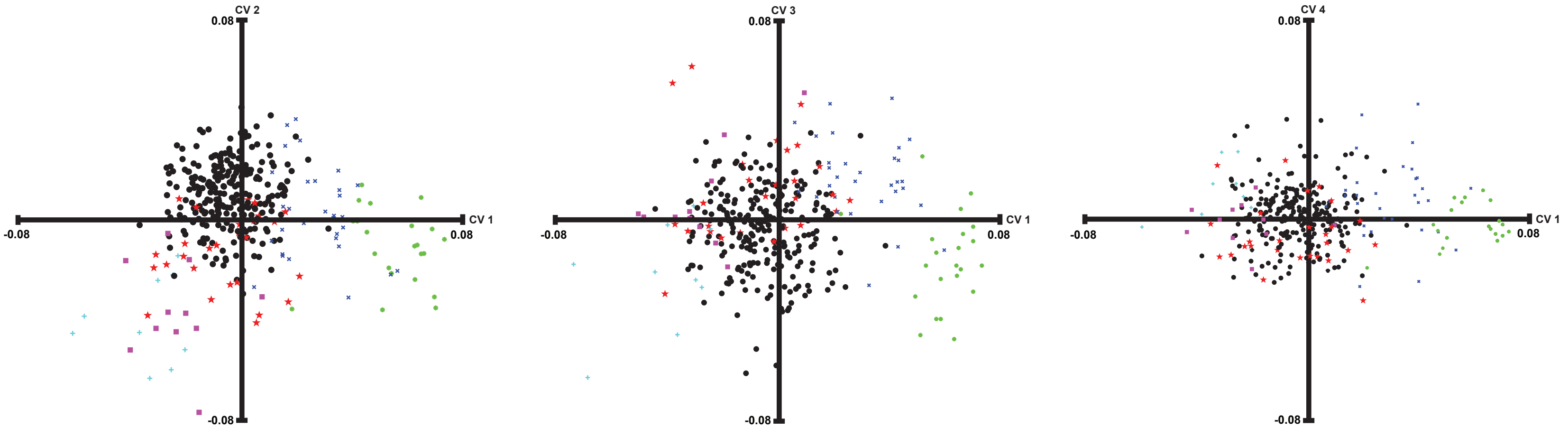
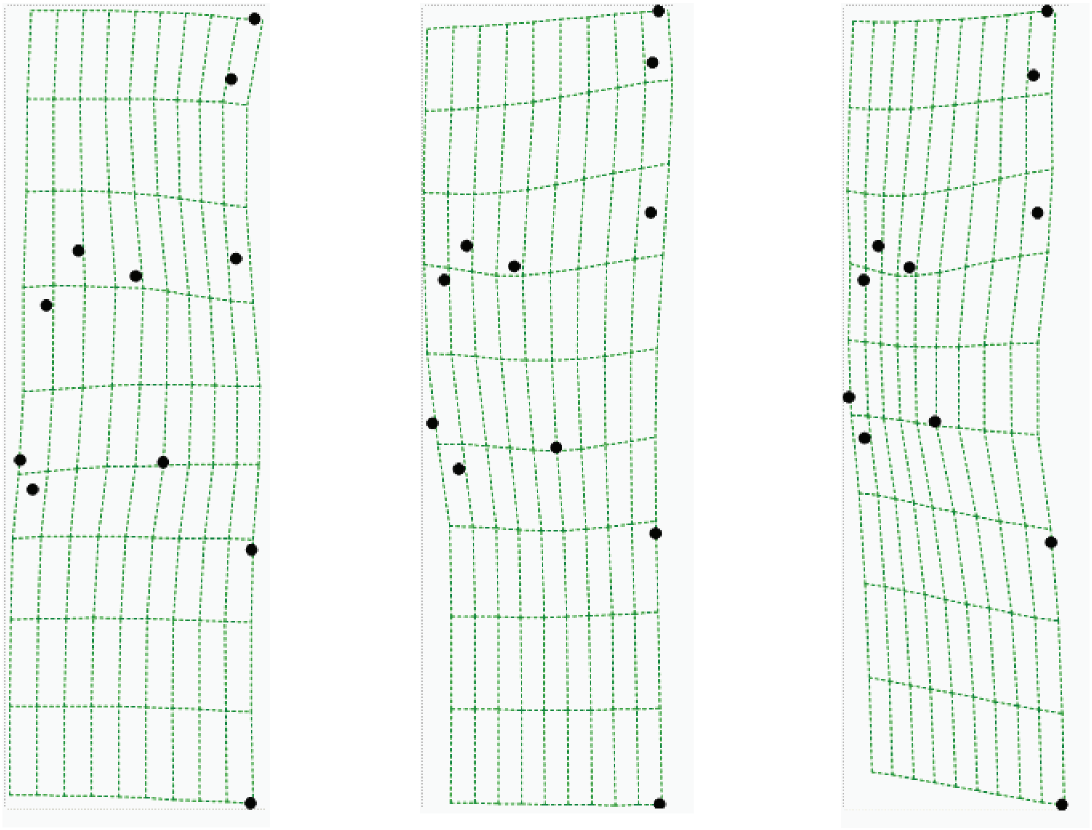
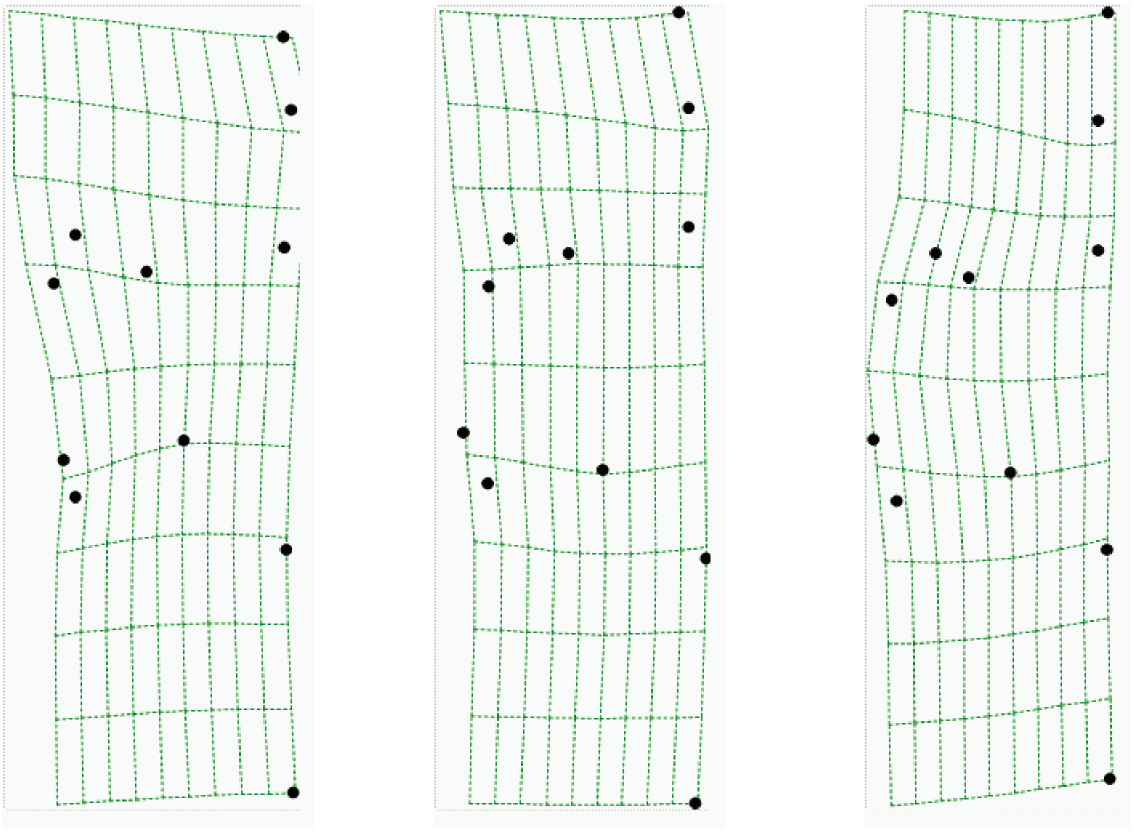

CV 2
0.08
-0.08
CV 1
-0.08
0.08
CV 3
0.08
-0.08
CV 1
-0.08
0.08
CV 4
0.08
-0.08
CV 1
-0.08
0.08

Figure 12



L. triangulum *L. gentilis* *L. elapsoides*



L. polyzona *L. abnorma* *L. micropholis*

Abbreviations for Appendix 1:

AMNH=American Museum of Natural History
RB=Robert Bryson, Jr.
CLC=Christian L. Cox
DBS=Donald B. Shepard
DGM=Daniel G. Mulcahy
FHGO= Fundación Herpetológica G. ORCÉS, Quito, Ecuador
FMNH=Florida Museum of Natural History
FMQ=Fernando Mendoza-Quijano
FTB=Frank T. Burbrink Collection, City University of New York
INHS=Illinois Natural History Survey
ISZ= Israel Solano-Zavaleta, Universidad Nacional Autónoma de México
JAC=Jonathan A. Campbell Collection, University of Texas Arlington
LJV= Laurie J. Vitt
MHP=Sternberg Museum of Natural History
MVZ=Museum of Vertebrate Zoology
NCSM=North Carolina Science Museum
PPC/L177306=Paulino Ponce-Campos
QCAZR=Museo de Zoología, Pontificia Universidad Católica del Ecuador
SRSU=Sul Ross State University
TJC=Timothy J. Colston
TNHC=Texas Natural History Collections
UANL= Universidad Autónoma de Nuevo León
UCM=University of Colorado Museum of Natural History
UF=University of Florida
UOGV=Uri Omar Garcia-Vasquez, Universidad Nacional Autónoma de México
USNM=United States National Museum
YPM=Yale Peabody Museum

Appendix 1. Samples used in analyses. Whether the samples were sequenced for only the mtDNA tree (Mt) or for the analyses using nuclear genes (Scn) is indicated (X), as is the clade/species they are in (milksnakes and *L. alterna* only) for the Cytb gene tree, the Structurama groups and the species tree. The putative subspecies designations for each milksnake sample, based on the locality and the subspecies ranges from Williams (1988) are included. Abbreviations are as follows: MX= Mexico, CA =Central America, SA = South America.

ID #	**Genus**	**Species**	**State/ Country**	**County/ State**	**Subspecies**	**Mt**	**Scn**	**CytbTree**	**Structurama**	**Species Tree**
AMNH21940	*Lampropeltis*	*triangulum*	Mexico	Guerrero	*L. t. conanti*	X	X	Guerrero	CA	*L. abnorma*
FTB1986	*Lampropeltis*	*triangulum*	Costa Rica	Alajuela	*L. t. stuarti*	X	X	Honduras	CA	*L. abnorma*
FTB2411	*Lampropeltis*	*triangulum*	Costa Rica	Guanacaste	*L. t. stuarti*	X	X	Honduras	CA	*L. abnorma*
FTB2386	*Lampropeltis*	*triangulum*	Honduras	Choluteca	*L. t. stuarti*	X	X	Honduras	CA	*L. abnorma*
FTB2387	*Lampropeltis*	*triangulum*	Honduras	Choluteca	*L. t. stuarti*	X	X	Honduras	CA	*L. abnorma*
FTB2322	*Lampropeltis*	*triangulum*	Honduras	S Honduras	*L. t. stuarti*	X	X	Honduras	CA	*L. abnorma*
FTB2323	*Lampropeltis*	*triangulum*	Honduras	S Honduras	*L. t. stuarti*	X	X	Honduras	CA	*L. abnorma*
FTB2321	*Lampropeltis*	*triangulum*	Honduras	NW Honduras	*L. t. abnorma*	X	X	Nicaragua	CA	*L. abnorma*
AMNH22617	*Lampropeltis*	*triangulum*	Mexico	Veracruz	*L. t. polyzona*	X	X	Nicaragua	CA	*L. abnorma*
FTB2317	*Lampropeltis*	*triangulum*	Nicaragua	C Nicaragua	*L. t. hondurensis*	X	X	Nicaragua	CA	*L. abnorma*
FTB2320	*Lampropeltis*	*triangulum*	Nicaragua	C Nicaragua	*L. t. hondurensis*	X	X	Nicaragua	CA	*L. abnorma*
UANL6337	*Lampropeltis*	*triangulum*	Mexico	Nuevo Leon	*L. t. annulata*	X	X	Western	Tamaulipas	*L. annulata*
RH011	*Lampropeltis*	*triangulum*	Mexico	Queretaro	*L. t. dixoni*	X	X	Western	Tamaulipas	*L. annulata*
RH013	*Lampropeltis*	*triangulum*	Mexico	Queretaro	*L. t. dixoni*	X	X	Western	Tamaulipas	*L. annulata*
UANL6780	*Lampropeltis*	*triangulum*	Mexico	Queretaro	*L. t. dixoni*	X	X	Western	Tamaulipas	*L. annulata*
FTB1819	*Lampropeltis*	*triangulum*	Mexico	Tamaulipas	*L. t. annulata*	X	X	Western	Tamaulipas	*L. annulata*
UANL Unc.43	*Lampropeltis*	*triangulum*	Mexico	Tamaulipas	*L. t. annulata*	X	X	Western	Tamaulipas	*L. annulata*
FTB1794	*Lampropeltis*	*elapsoides*	AL	Bibb	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FTB1795	*Lampropeltis*	*elapsoides*	AL	Bibb	*L. t. elapsoides*	X	X	*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FTB1756	*Lampropeltis*	*elapsoides*	AL	Cleburne	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH128356	*Lampropeltis*	*elapsoides*	FL	Alachua	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH151339	*Lampropeltis*	*elapsoides*	FL	Calhoun	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FTB1309	*Lampropeltis*	*elapsoides*	FL	Citrus	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FTB1310	*Lampropeltis*	*elapsoides*	FL	Gadsden	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH150369	*Lampropeltis*	*elapsoides*	FL	Glades	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*

ID #	Genus	Species	State/ Country	County/ State	Subspecies	Mt	Scn	CytbTree	Structurama	Species Tree
FLMNH152378	*Lampropeltis*	*elapsoides*	FL	Glades	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FTB797	*Lampropeltis*	*elapsoides*	FL	Gulf	*L. t. elapsoides*	X	X	*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH152369	*Lampropeltis*	*elapsoides*	FL	Hendry	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH151493	*Lampropeltis*	*elapsoides*	FL	Hendry	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH142914	*Lampropeltis*	*elapsoides*	FL	Lafayette	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH140549	*Lampropeltis*	*elapsoides*	FL	Leon	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH138988	*Lampropeltis*	*elapsoides*	FL	Liberty	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH128357	*Lampropeltis*	*elapsoides*	FL	Liberty	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH114326	*Lampropeltis*	*elapsoides*	FL	Liberty	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH141137	*Lampropeltis*	*elapsoides*	FL	Manatee	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH141134	*Lampropeltis*	*elapsoides*	FL	Manatee	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH141133	*Lampropeltis*	*elapsoides*	FL	Manatee	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH138396	*Lampropeltis*	*elapsoides*	FL	Manatee	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH141138	*Lampropeltis*	*elapsoides*	FL	Manatee	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH141131	*Lampropeltis*	*elapsoides*	FL	Manatee	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH141139	*Lampropeltis*	*elapsoides*	FL	Manatee	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH141135	*Lampropeltis*	*elapsoides*	FL	Manatee	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH144257	*Lampropeltis*	*elapsoides*	FL	Miami-Dade	*L. t. elapsoides*	X	X	*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH144256	*Lampropeltis*	*elapsoides*	FL	Miami-Dade	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH151582	*Lampropeltis*	*elapsoides*	FL	Palm Beach	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH141638	*Lampropeltis*	*elapsoides*	FL	Polk	*L. t. elapsoides*	X	X	*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
LSUMZ18342	*Lampropeltis*	*elapsoides*	FL	Santa Rosa	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
LSUMZ18350	*Lampropeltis*	*elapsoides*	FL	Santa Rosa	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
LSUMZ3244	*Lampropeltis*	*elapsoides*	FL	Santa Rosa	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
LSUMZ18905	*Lampropeltis*	*elapsoides*	FL	Santa Rosa	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
LSUMZ9474	*Lampropeltis*	*elapsoides*	FL	Sumter	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FLMNH140558	*Lampropeltis*	*elapsoides*	FL	Wakulla	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FTB1645	*Lampropeltis*	*elapsoides*	GA	Effingham	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FTB1311	*Lampropeltis*	*elapsoides*	GA	Lowndes	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
MHP9851	*Lampropeltis*	*elapsoides*	KY	Lyon	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
LSUMZ3361	*Lampropeltis*	*elapsoides*	LA	St. Tammany	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*

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LSUMZ2875	*Lampropeltis*	*elapsoides*	LA	St. Tammany	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
LSUMZ16038	*Lampropeltis*	*elapsoides*	LA	St. Tammany	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
LSUMZ18926	*Lampropeltis*	*elapsoides*	LA	Tangipahoa	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
LSUMZ8029	*Lampropeltis*	*elapsoides*	LA	West Feliciana	*L. t. elapsoides*	X	X	*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
LSUMZ2021	*Lampropeltis*	*elapsoides*	MS	Stone	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
LSUMZ2778	*Lampropeltis*	*elapsoides*	MS	Wilkinson	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FTB1641	*Lampropeltis*	*elapsoides*	NC	Brunswick	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FTB2109	*Lampropeltis*	*elapsoides*	NC	Carteret	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FTB1642	*Lampropeltis*	*elapsoides*	NC	Jones	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
NCSM74476	*Lampropeltis*	*elapsoides*	NC	Moore	*L. t. elapsoides*	X	X	*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FTB1754	*Lampropeltis*	*elapsoides*	NC	Richmond	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
NCSM74462	*Lampropeltis*	*elapsoides*	NC	Scotland	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
MHP9868	*Lampropeltis*	*elapsoides*	SC	Aiken	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
NCSM74542	*Lampropeltis*	*elapsoides*	SC	Chesterfield	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FTB1755	*Lampropeltis*	*elapsoides*	SC	Dorchester	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
NCSM74541	*Lampropeltis*	*elapsoides*	SC	Dorchester	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
FTB1644	*Lampropeltis*	*elapsoides*	SC	Jasper	*L. t. elapsoides*	X		*L. elapsoides*	*L. elapsoides*	*L. elapsoides*
DBS2052	*Lampropeltis*	*triangulum*	AR	Montgomery	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
DBS2053	*Lampropeltis*	*triangulum*	AR	Montgomery	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
DBS531	*Lampropeltis*	*triangulum*	AR	Polk	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
DBS622	*Lampropeltis*	*triangulum*	AR	Polk	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
FTB1632	*Lampropeltis*	*triangulum*	AR	Pulaski	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
FTB1527	*Lampropeltis*	*triangulum*	AR	Saline	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
FTB1817	*Lampropeltis*	*triangulum*	AZ	Cochise	*L. t. celaenops*	X	X	Western	Eastern/Western	*L. gentilis*
FTB1966	*Lampropeltis*	*triangulum*	AZ	Coconino	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB1967	*Lampropeltis*	*triangulum*	AZ	Coconino	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB1968	*Lampropeltis*	*triangulum*	AZ	Coconino	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB1969	*Lampropeltis*	*triangulum*	AZ	Coconino	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB1970	*Lampropeltis*	*triangulum*	AZ	Yavapai	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB1971	*Lampropeltis*	*triangulum*	AZ	Yavapai	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB1972	*Lampropeltis*	*triangulum*	AZ	Yavapai	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*

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FTB2224	*Lampropeltis*	*triangulum*	AZ	Yavapai	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB2225	*Lampropeltis*	*triangulum*	AZ	Yavapai	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB2226	*Lampropeltis*	*triangulum*	AZ	Yavapai	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
UCM61875	*Lampropeltis*	*triangulum*	CO	Mesa	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
MHP9699	*Lampropeltis*	*triangulum*	KS	Anderson	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
MHP7991	*Lampropeltis*	*triangulum*	KS	Leavenworth	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
MHP8310	*Lampropeltis*	*triangulum*	KS	Linn	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
MHP8504	*Lampropeltis*	*triangulum*	KS	Logan	*L. t. gentilis*	X		Western	Eastern/Western	*L. gentilis*
MHP8275	*Lampropeltis*	*triangulum*	KS	Ness	*L. t. gentilis*	X		Western	Eastern/Western	*L. gentilis*
MHP8496	*Lampropeltis*	*triangulum*	KS	Ness	*L. t. gentilis*	X		Western	Eastern/Western	*L. gentilis*
MHP7910	*Lampropeltis*	*triangulum*	KS	Rush	*L. t. gentilis*	X		Western	Eastern/Western	*L. gentilis*
MHP8549	*Lampropeltis*	*triangulum*	KS	Russell	*L. t. gentilis*	X		Western	Eastern/Western	*L. gentilis*
SRSU6564	*Lampropeltis*	*triangulum*	KS	Russell	*L. t. gentilis*	X		Western	Eastern/Western	*L. gentilis*
MHP8838	*Lampropeltis*	*triangulum*	KS	Washington	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
LSUMZ19609	*Lampropeltis*	*triangulum*	LA	Ascension	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
LSUMZ15926	*Lampropeltis*	*triangulum*	LA	Ascension	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
FTB1516	*Lampropeltis*	*triangulum*	LA	Calcasieu	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
LSUMZ2916	*Lampropeltis*	*triangulum*	LA	Iberville	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
LSUMZ2744	*Lampropeltis*	*triangulum*	LA	Iberville	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
LSUMZ14742	*Lampropeltis*	*triangulum*	LA	Jefferson	*L. t. amaura*	X	X	Western	Eastern/Western	*L. gentilis*
LSUMZ1509	*Lampropeltis*	*triangulum*	LA	Jefferson	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
FLMNH142915	*Lampropeltis*	*triangulum*	LA	Natchitoches	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
LSUMZ19572	*Lampropeltis*	*triangulum*	LA	St. James	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
LSUMZ187656	*Lampropeltis*	*triangulum*	LA	Washington	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
DBS559	*Lampropeltis*	*triangulum*	MT	Carbon	*L. t. multistriata*	X		Western	Eastern/Western	*L. gentilis*
DBS562	*Lampropeltis*	*triangulum*	MT	Carbon	*L. t. multistriata*	X	X	Western	Eastern/Western	*L. gentilis*
LJV10740	*Lampropeltis*	*triangulum*	MT	Carbon	*L. t. multistriata*	X		Western	Eastern/Western	*L. gentilis*
LJV10742	*Lampropeltis*	*triangulum*	MT	Carbon	*L. t. multistriata*	X		Western	Eastern/Western	*L. gentilis*
LJV10749	*Lampropeltis*	*triangulum*	MT	Carbon	*L. t. multistriata*	X		Western	Eastern/Western	*L. gentilis*
LJV10753	*Lampropeltis*	*triangulum*	MT	Carbon	*L. t. multistriata*	X		Western	Eastern/Western	*L. gentilis*
LJV10782	*Lampropeltis*	*triangulum*	MT	Yellowstone	*L. t. multistriata*	X		Western	Eastern/Western	*L. gentilis*

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SRSU6575	*Lampropeltis*	*triangulum*	NE	Cherry	*L. t. multistriata*	X		Western	Eastern/Western	*L. gentilis*
FTB1806	*Lampropeltis*	*triangulum*	NM	Torrance	*L. t. celaenops*	X		Western	Eastern/Western	*L. gentilis*
FTB1807	*Lampropeltis*	*triangulum*	NM	Torrance	*L. t. celaenops*	X		Western	Eastern/Western	*L. gentilis*
MVZ249826	*Lampropeltis*	*triangulum*	NV	Elko	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
DBS1304	*Lampropeltis*	*triangulum*	OK	Atoka	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
DBS1349	*Lampropeltis*	*triangulum*	OK	Atoka	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
DBS1387	*Lampropeltis*	*triangulum*	OK	Atoka	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
DBS1406	*Lampropeltis*	*triangulum*	OK	Atoka	*L. t. amaura*	X	X	Western	Eastern/Western	*L. gentilis*
LJV10693	*Lampropeltis*	*triangulum*	OK	Atoka	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
LJV10694	*Lampropeltis*	*triangulum*	OK	Atoka	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
DBS1805	*Lampropeltis*	*triangulum*	OK	Cherokee	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
TJC29	*Lampropeltis*	*triangulum*	OK	Cherokee	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
DBS1535	*Lampropeltis*	*triangulum*	OK	LeFlore	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
DBS532	*Lampropeltis*	*triangulum*	OK	LeFlore	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
LSUMZ8760	*Lampropeltis*	*triangulum*	OK	McCurtain	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
DBS76	*Lampropeltis*	*triangulum*	OK	Muskogee	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
LJV9335	*Lampropeltis*	*triangulum*	OK	Muskogee	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
DBS1754	*Lampropeltis*	*triangulum*	AR	Polk	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
DBS1452	*Lampropeltis*	*triangulum*	OK	Pushmataha	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
YPM13949	*Lampropeltis*	*triangulum*	OK	Pushmataha	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
LSUMZ105	*Lampropeltis*	*triangulum*	OK	Rogers	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
FTB1643	*Lampropeltis*	*triangulum*	OK	Sequoyah	*L. t. syspila*	X		Western	Eastern/Western	*L. gentilis*
MHP8727	*Lampropeltis*	*triangulum*	SD	Custer	*L. t. multistriata*	X		Western	Eastern/Western	*L. gentilis*
MHP8916	*Lampropeltis*	*triangulum*	SD	Custer	*L. t. multistriata*	X		Western	Eastern/Western	*L. gentilis*
MHP8917	*Lampropeltis*	*triangulum*	SD	Custer	*L. t. multistriata*	X		Western	Eastern/Western	*L. gentilis*
MHP9106	*Lampropeltis*	*triangulum*	SD	Custer	*L. t. multistriata*	X		Western	Eastern/Western	*L. gentilis*
FTB1805	*Lampropeltis*	*triangulum*	TX	Brazos	*L. t. amaura*	X	X	Western	Eastern/Western	*L. gentilis*
FTB1798	*Lampropeltis*	*triangulum*	TX	Burleson	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
FTB1532	*Lampropeltis*	*triangulum*	TX	Coleman	*L. t. annulata*	X		Western	Eastern/Western	*L. gentilis*
FTB1534	*Lampropeltis*	*triangulum*	TX	Coleman	*L. t. annulata*	X		Western	Eastern/Western	*L. gentilis*
FTB1797	*Lampropeltis*	*triangulum*	TX	Grimes	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*

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SRSU6519	*Lampropeltis*	*triangulum*	TX	Jeff Davis	*L. t. celaenops*	X		Western	Eastern/Western	*L. gentilis*
CLC698	*Lampropeltis*	*triangulum*	TX	McMullen	*L. t. annulata*	X		Western	Eastern/Western	*L. gentilis*
FTB1796	*Lampropeltis*	*triangulum*	TX	San Jacinto	*L. t. amaura*	X		Western	Eastern/Western	*L. gentilis*
FTB1531	*Lampropeltis*	*triangulum*	TX	Val Verde	*L. t. annulata*	X	X	Western	Eastern/Western	*L. gentilis*
FTB1790	*Lampropeltis*	*triangulum*	UT	Carbon	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB1787	*Lampropeltis*	*triangulum*	UT	Duschesne	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB1786	*Lampropeltis*	*triangulum*	UT	Emery	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB1792	*Lampropeltis*	*triangulum*	UT	Salt	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB1788	*Lampropeltis*	*triangulum*	UT	Toole	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB1789	*Lampropeltis*	*triangulum*	UT	Uinta	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB1791	*Lampropeltis*	*triangulum*	UT	Utah	*L. t. taylori*	X		Western	Eastern/Western	*L. gentilis*
FTB1985	*Lampropeltis*	*triangulum*	Costa Rica	Cartago	*L. t. gaigeae*	X	X	SA	SA	*L. micropholis*
FHGO2427	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
FHGO2663	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
FHGO2885	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
FHGO2906	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
FHGO2918	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
FHGO3021	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
FHGO3026	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
FHGO3090	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
FHGO4848	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
FHGO5766	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
FHGO5966	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
FHGO6217	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
FHGO7784	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X		SA	SA	*L. micropholis*
QCAZR5576	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
QCAZR6321	*Lampropeltis*	*triangulum*	Ecuador	Pichincha	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
USNM578513	*Lampropeltis*	*triangulum*	Panama	Cocle	*L. t. micropholis*	X	X	SA	SA	*L. micropholis*
FTB1568	*Lampropeltis*	*triangulum*	Mexico	Colima	*L. t. arcifera*	X	X	Colima	MX	*L. polyzona*
FTB1818	*Lampropeltis*	*triangulum*	Mexico	Colima	*L. t. arcifera*	X	X	Colima	MX	*L. polyzona*
JAC27958	*Lampropeltis*	*triangulum*	Mexico	Colima	*L. t. sinaloae*	X	X	Colima	MX	*L. polyzona*

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JAC30084	*Lampropeltis*	*triangulum*	Mexico	Colima	*L. t. arcifera*	X	X	Colima	MX	*L. polyzona*
JAC30103	*Lampropeltis*	*triangulum*	Mexico	Colima	*L. t. arcifera*	X	X	Colima	MX	*L. polyzona*
JAC30303	*Lampropeltis*	*triangulum*	Mexico	Colima	*L. t. arcifera*	X	X	Colima	MX	*L. polyzona*
JAC30515	*Lampropeltis*	*triangulum*	Mexico	Colima	*L. t. arcifera*	X	X	Colima	MX	*L. polyzona*
JAC30516	*Lampropeltis*	*triangulum*	Mexico	Colima	*L. t. arcifera*	X	X	Colima	MX	*L. polyzona*
JAC30658	*Lampropeltis*	*triangulum*	Mexico	Colima	*L. t. arcifera*	X	X	Colima	MX	*L. polyzona*
JAC30659	*Lampropeltis*	*triangulum*	Mexico	Colima	*L. t. arcifera*	X	X	Colima	MX	*L. polyzona*
PPC20	*Lampropeltis*	*triangulum*	Mexico	Jalisco	*L. t. arcifera*	X	X	Colima	MX	*L. polyzona*
L177306	*Lampropeltis*	*triangulum*	Mexico	Michoacan	*L. t. arcifera*	X	X	Colima	MX	*L. polyzona*
AMNH21975	*Lampropeltis*	*triangulum*	Mexico	Guerrero	*L. t. conanti*	X	X	Guerrero	MX	*L. polyzona*
AMNH22074	*Lampropeltis*	*triangulum*	Mexico	Guerrero	*L. t. conanti*	X	X	Guerrero	MX	*L. polyzona*
AMNH22361	*Lampropeltis*	*triangulum*	Mexico	Oaxaca	*L. t. conanti*	X	X	Oaxaca	MX	*L. polyzona*
AMNH22785	*Lampropeltis*	*triangulum*	Mexico	Oaxaca	*L. t. conanti*	X	X	Oaxaca	MX	*L. polyzona*
AMNH22786	*Lampropeltis*	*triangulum*	Mexico	Oaxaca	*L. t. conanti*	X	X	Oaxaca	MX	*L. polyzona*
FMQ4000	*Lampropeltis*	*triangulum*	Mexico	Hidalgo	*L. t. smithi*	X	X	Puebla	MX	*L. polyzona*
JLTV05	*Lampropeltis*	*triangulum*	Mexico	Hidalgo	*L. t. campbelli*	X	X	Puebla	MX	*L. polyzona*
AMNH22501	*Lampropeltis*	*triangulum*	Mexico	Puebla	*L. t. conanti*	X	X	Puebla	MX	*L. polyzona*
ISZ202	*Lampropeltis*	*triangulum*	Mexico	Puebla	*L. t. polyzona*	X		Puebla	MX	*L. polyzona*
ISZ203	*Lampropeltis*	*triangulum*	Mexico	Puebla	*L. t. polyzona*	X	X	Puebla	MX	*L. polyzona*
UOGV481	*Lampropeltis*	*triangulum*	Mexico	Puebla	*L. t. polyzona*	X		Puebla	MX	*L. polyzona*
JAC29865	*Lampropeltis*	*triangulum*	Mexico	Veracruz	*L. t. polyzona*	X	X	Puebla	MX	*L. polyzona*
LtLC 2	*Lampropeltis*	*triangulum*	Mexico	Jalisco	*L. t. arcifera*	X		Sonora	MX	*L. polyzona*
LtLC 3	*Lampropeltis*	*triangulum*	Mexico	Jalisco	*L. t. arcifera*	X		Sonora	MX	*L. polyzona*
PPC21	*Lampropeltis*	*triangulum*	Mexico	Jalisco	*L. t. nelsoni*	X	X	Sonora	MX	*L. polyzona*
HIR242	*Lampropeltis*	*triangulum*	Mexico	Michoacan	*L. t. nelsoni*	X	X	Sonora	MX	*L. polyzona*
JAC30542	*Lampropeltis*	*triangulum*	Mexico	Sinaloa	*L. t. sinaloae*	X	X	Sonora	MX	*L. polyzona*
JAC30582	*Lampropeltis*	*triangulum*	Mexico	Sinaloa	*L. t. sinaloae*	X	X	Sonora	MX	*L. polyzona*
JAC30602	*Lampropeltis*	*triangulum*	Mexico	Sinaloa	*L. t. sinaloae*	X	X	Sonora	MX	*L. polyzona*
JAC30571	*Lampropeltis*	*triangulum*	Mexico	Sonora	*L. t. sinaloae*	X	X	Sonora	MX	*L. polyzona*
DGM2241	*Lampropeltis*	*triangulum*	Mexico	Sonora	*L. t. sinaloae*	X	X	Sonora	MX	*L. polyzona*
FTB1964	*Lampropeltis*	*triangulum*	Mexico	Sonora	*L. t. sinaloae*	X	X	Sonora	MX	*L. polyzona*

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FTB1965	*Lampropeltis*	*triangulum*	Mexico	Sonora	*L. t. sinaloae*	X	X	Sonora	MX	*L. polyzona*
YPM13038	*Lampropeltis*	*triangulum*	CT	Fairfield	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
YPM13969	*Lampropeltis*	*triangulum*	CT	Fairfield	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1579	*Lampropeltis*	*triangulum*	IA	Madison	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
INHS19582	*Lampropeltis*	*triangulum*	IL	Iroquois	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1512	*Lampropeltis*	*triangulum*	IL	Jersey	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1513	*Lampropeltis*	*triangulum*	IL	Jersey	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
INHS19804	*Lampropeltis*	*triangulum*	IL	Jersey	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
INHS19805	*Lampropeltis*	*triangulum*	IL	Jersey	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
INHS18921	*Lampropeltis*	*triangulum*	IL	Kankakee	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
INHS19454	*Lampropeltis*	*triangulum*	IL	Monroe	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
INHS18261	*Lampropeltis*	*triangulum*	IL	Washington	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1793	*Lampropeltis*	*triangulum*	IN	Franklin	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FMNH267551	*Lampropeltis*	*triangulum*	IN	Lake	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FMNH259747	*Lampropeltis*	*triangulum*	IN	Lake	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FMNH268700	*Lampropeltis*	*triangulum*	IN	LaPorte	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
MHP7215	*Lampropeltis*	*triangulum*	KY	Hickman	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
MHP9886	*Lampropeltis*	*triangulum*	KY	Hickman	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
MHP9887	*Lampropeltis*	*triangulum*	KY	Menifee	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1587	*Lampropeltis*	*triangulum*	KY	Owsley	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
LSUMZ19650	*Lampropeltis*	*triangulum*	LA	La Salle	*L. t. amaura*	X	X	Eastern	Eastern/Western	*L. triangulum*
LSUMZ2291	*Lampropeltis*	*triangulum*	LA	La Salle	*L. t. amaura*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1829	*Lampropeltis*	*triangulum*	MA	Hampshire	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1634	*Lampropeltis*	*triangulum*	MD	Baltimore	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1635	*Lampropeltis*	*triangulum*	MD	Baltimore	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1637	*Lampropeltis*	*triangulum*	MD	Baltimore	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1640	*Lampropeltis*	*triangulum*	MD	Baltimore	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FMNH266865	*Lampropeltis*	*triangulum*	MN	Winona	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FMNH266870	*Lampropeltis*	*triangulum*	MN	Winona	*L. t. triangulum*	X	X	Eastern	Eastern/Western	*L. triangulum*
FTB2229	*Lampropeltis*	*triangulum*	MO	Iron	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
FTB2230	*Lampropeltis*	*triangulum*	MO	Iron	*L. t. syspila*	X	X	Eastern	Eastern/Western	*L. triangulum*

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FTB1839	*Lampropeltis*	*triangulum*	MO	Jackson	*L. t. syspila*	X	X	Eastern	Eastern/Western	*L. triangulum*
FTB1983	*Lampropeltis*	*triangulum*	MO	Ripley	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
FTB2231	*Lampropeltis*	*triangulum*	MO	Shannon	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
FTB2232	*Lampropeltis*	*triangulum*	MO	Shannon	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
FTB2233	*Lampropeltis*	*triangulum*	MO	Shannon	*L. t. syspila*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1636	*Lampropeltis*	*triangulum*	NC	Graham	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1804	*Lampropeltis*	*triangulum*	NJ	Burlington	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1638	*Lampropeltis*	*triangulum*	NJ	Hunterdon	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB952	*Lampropeltis*	*triangulum*	NJ	Ocean	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1000	*Lampropeltis*	*triangulum*	NY	Albany	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB551	*Lampropeltis*	*triangulum*	NY	Delaware	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB949	*Lampropeltis*	*triangulum*	NY	Orange	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB2410	*Lampropeltis*	*triangulum*	NY	Richmond	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB442	*Lampropeltis*	*triangulum*	NY	Suffolk	*L. t. triangulum*	X	X	Eastern	Eastern/Western	*L. triangulum*
AMNH18137	*Lampropeltis*	*triangulum*	NY	Suffolk	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1840	*Lampropeltis*	*triangulum*	NY	Sullivan	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB553	*Lampropeltis*	*triangulum*	NY	Sullivan	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
LSUMZ5589	*Lampropeltis*	*triangulum*	NY	Tompkins	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
LSUMZ5590	*Lampropeltis*	*triangulum*	NY	Tompkins	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB2299	*Lampropeltis*	*triangulum*	OH	Noble	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB2298	*Lampropeltis*	*triangulum*	OH	Tusc	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB2297	*Lampropeltis*	*triangulum*	OH	Vinton	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1171	*Lampropeltis*	*triangulum*	ON	Frontenac	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1973	*Lampropeltis*	*triangulum*	PA	Lackawanna	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1633	*Lampropeltis*	*triangulum*	PA		*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
MHP9876	*Lampropeltis*	*triangulum*	TN	Obion	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1639	*Lampropeltis*	*triangulum*	VA	Shenandoah	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB563	*Lampropeltis*	*triangulum*	VA	Shenandoah	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB647	*Lampropeltis*	*triangulum*	WI	Crawford	*L. t. triangulum*	X	X	Eastern	Eastern/Western	*L. triangulum*
FTB648	*Lampropeltis*	*triangulum*	WI	Crawford	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB1984	*Lampropeltis*	*triangulum*	WI	Iowa	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*

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LSUMZ1705	*Lampropeltis*	*triangulum*	WI	Walworth	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
FTB728	*Lampropeltis*	*triangulum*	WV	Tucker	*L. t. triangulum*	X		Eastern	Eastern/Western	*L. triangulum*
RB6	*Lampropeltis*	*alterna*	Mexico	Durango		X	X	Western	*L. alterna*	*L. alterna*
FTB2385	*Lampropeltis*	*alterna*	Mexico	Nuevo Leon		X	X	Western	*L. alterna*	*L. alterna*
RB5	*Lampropeltis*	*alterna*	Mexico	Nuevo Leon		X	X	Western	*L. alterna*	*L. alterna*
SRSU6522	*Lampropeltis*	*alterna*	TX	Crockett		X	X	Western	*L. alterna*	*L. alterna*
SRSU6521	*Lampropeltis*	*alterna*	TX	Crockett		X		Western	*L. alterna*	*L. alterna*
SRSU6516	*Lampropeltis*	*alterna*	TX	Jeff Davis		X		Western	*L. alterna*	*L. alterna*
SRSU6517	*Lampropeltis*	*alterna*	TX	Jeff Davis		X	X	Western	*L. alterna*	*L. alterna*
LSUMZ15977	*Lampropeltis*	*alterna*	TX	Presido		X	X	Western	*L. alterna*	*L. alterna*
FTB1811	*Lampropeltis*	*alterna*	TX	Sanderson		X		Western	*L. alterna*	*L. alterna*
FTB1184	*Lampropeltis*	*alterna*	TX	Val Verde		X		Western	*L. alterna*	*L. alterna*
LSUMZ15978	*Lampropeltis*	*alterna*	TX	Val Verde		X	X	Western	*L. alterna*	*L. alterna*
FTB1809	*Lampropeltis*	*alterna*	TX	Val Verde		X		Western	*L. alterna*	*L. alterna*
FTB1810	*Lampropeltis*	*alterna*	TX	Val Verde		X		Western	*L. alterna*	*L. alterna*
FTB1460	*Lampropeltis*	*californiae*	AZ	Pima		X	X			
FTB1530	*Lampropeltis*	*californiae*	CA	Kern		X	X			
MVZ175969	*Lampropeltis*	*calligaster*	NC	Wake		X	X			
DBS1073	*Lampropeltis*	*calligaster*	OK	Latimer		X	X			
LSUMZ40124	*Lampropeltis*	*extenuata*	FL	Citrus		X	X			
UF150109	*Lampropeltis*	*extenuata*	FL	Hernando		X	X			
FTB1626	*Lampropeltis*	*getula*	FL	Glades		X	X			
FTB966	*Lampropeltis*	*getula*	NJ	Cumberland		X	X			
DBS1570	*Lampropeltis*	*holbrooki*	OK	LeFlore		X	X			
TNHC61324	*Lampropeltis*	*holbrooki*	TX	Aransas		X	X			
FTB2221	*Lampropeltis*	*knoblochi*	AZ	Cochise		X	X			
FTB2075	*Lampropeltis*	*knoblochi*	Mexico	Sonora		X	X			
FTB1557	*Lampropeltis*	*mexicana*	Mexico	Aguascalientes		X				
RB3	*Lampropeltis*	*mexicana*	Mexico	Aguascalientes		X	X			
UANL5940	*Lampropeltis*	*mexicana*	Mexico	Durango		X	X			
FTB1573	*Lampropeltis*	*mexicana*	Mexico	Durango		X				

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FTB2252	*Lampropeltis*	*mexicana*	Mexico	Durango		X	X			
FTB2403	*Lampropeltis*	*mexicana*	Mexico	Nayarit		X				
RB4	*Lampropeltis*	*mexicana*	Mexico	Nuevo Leon		X	X	Western		
UANL Uncat. 6	*Lampropeltis*	*mexicana*	Mexico	San Luis Potosi		X				
UANL Uncat. 5	*Lampropeltis*	*mexicana*	Mexico	San Luis Potosi		X	X			
UANL Uncat. 3	*Lampropeltis*	*mexicana*	Mexico	San Luis Potosi		X	X			
UANL Uncat. 4	*Lampropeltis*	*mexicana*	Mexico	San Luis Potosi		X				
FTB1505	*Lampropeltis*	*nigra*	KY	Powell		X	X			
FTB1775	*Lampropeltis*	*nigra*	MS	Pontotoc		X	X			
FTB2080	*Lampropeltis*	*pyromelana*	AZ	Gila		X	X			
FTB2070	*Lampropeltis*	*pyromelana*	NV	White Pine		X	X			
FTB1558	*Lampropeltis*	*ruthveni*	Mexico	Michoacan		X	X			
RB2	*Lampropeltis*	*ruthveni*	Mexico	Michoacan		X	X			
RB1	*Lampropeltis*	*ruthveni*	Mexico	Queretaro		X	X			
FTB1554	*Lampropeltis*	*splendida*	NM	Valencia		X	X			
FTB1529	*Lampropeltis*	*splendida*	TX	Jeff Davis		X	X			
FTB2253	*Lampropeltis*	*webbi*	Mexico	Durango		X	X			
UANL5684	*Lampropeltis*	*webbi*	Mexico	Sinaloa		X	X			
FTB1168	*Lampropeltis*	*zonata*	CA	Napa		X	X			
FTB1211	*Lampropeltis*	*zonata*	CA	Santa Cruz		X	X			
FTB1854	*Arizona*	*elegans*	AZ	Graham		X	X			
FTB1937	*Arizona*	*elegans*	NM	Hidalgo		X	X			
DBS45	*Cemophora*	*coccinea*	OK	Muskogee		X	X			
FTB606	*Cemophora*	*coccinea*	SC	Union		X	X			

Appendix 2. Twelve loci used for *Lampropeltis* and PCR/sequencing protocols. The overall PCR conditions for all loci were the same with the exception of the annealing temperature (shown below) and used GoTaq Green MasterMix (Promega Corp.) according to the manufacturer's specifications, with a 90-s extension time. The polymerase chain reaction (PCR) products were cleaned using1 uL of ExoSap-IT (USB Corp.) per 10 uL of PCR product. The sequencing reaction consisted of 2 uL Beckman-Coulter DTCS, 1 uL primer (10 uM), 2 uL template and 5 uL deionized water. All loci are nuclear with the exception of cytochrome b (mitochondrial); GAD2 intron 15 is from the Z-chromosome. If the primer was developed for this project, the sequence is included. Internal primers used specifically for sequencing reactions are indicated with (*). For samples that were old/degraded, the internal primer was combined with a flanking primer to sequence the locus in two parts, using the internal primer's annealing temperature for the PCR.

Locus	**Protein Coding**	**Length**	**Primer Name/Sequence**	**Primer Source**	**Annealing Temperature ^{o}C**
Cytochrome b	Yes	1117bp	H14910	Burbrink et al. 2000	49^{o}
			THRSN2	Burbrink et al. 2000	49^{o}
			MxTriangF: 5'-CGA TTC TTT GCC YTA CAC TT-3'*	developed for this project	48^{o}
			MxTriangR: 5'-GAC TGA TAT GGR TGG AAT GGA-3'*	developed for this project	48^{o}
			Triangulum1F: 5'-ACA GAA YTA ACY AAC TGA CT-3'*	developed for this project	43.9^{o}
			Triangulum2R: 5'-ATT TTR TCR ATA TCH GAG TTT GT-3'*	developed for this project	43.3^{o}
NT3	Yes	481bp	NT3-F3	Noonan and Chippindale 2006	51^{o}
			NT3-R4	Noonan and Chippindale 2006	51^{o}
PRLR	Yes	552bp	PRLR-F1	Townsend et al. 2008	48^{o}
			PRLR-R3	Townsend et al. 2008	48^{o}
GAD2 intron 15	No	541bp	EST GAD2 15F: 5'-CAC ACA AAT GTY TGC TTC TGG-3'	developed for this project	48.3^{o}
			EST GAD2 16R: 5'-ATG CGG AAR AAA TTG ACC TTG TC-3'	developed for this project	48.3^{o}
			GAD15_16 intF: 5'-ACC TCA CAA TGA AGA TTT GTG-3'*	developed for this project	46^{o}
			GAD15_16 intR: 5'-GTG TAG ATG CTA CTG AAG CAA AGT C-3'*	developed for this project	48.6^{o}
NAV intron 5	No	561bp	NAV5F	Geffeney et al. 2005	55^{o}
			NAV6R	Geffeney et al. 2005	55^{o}
SPTBN intron 1	No	839bp	SPTBN1F APR-2010: 5'-TTG GTC GAT GCC AGT TGT A-3'	developed for this project	48.5^{o}
			SPTBN1R APR-2010: 5'-CAG GGT TTG TAA CCT KTC CA-3'	developed for this project	48.5^{o}
			SPTBN1 interF: 5'-TTT CCT TTC CAT TCC TTC TTT C-3'*	developed for this project	46^{o}
			SPTBN1 interR: 5'-GGC TGT CTG TTT GCA TCT TG-3'*	developed for this project	49^{o}
Vimentin intron 5	No	584bp	Vim Exon 5F	Zehner and Paterson 1983	55^{o}
			Vim Exon 6R	Zehner and Paterson 1983	55^{o}
CL4	No	373bp	CL4 F: 5'-CGC CTA AAA CTA ACA GTA GG-3'	developed for this project	45.5^{o}
			CL4 R: 5'-GTT CAG AGA GAT CTG ATT GC-3'	developed for this project	45.5^{o}
LAT Clone	No	705bp	CL LAT F: 5'-CCA GTG TGC TGG AAT TCA G-3'	developed for this project	45.5^{o}
			CL LAT R: 5'-TAT CTG CAG CAT TCA GGA-3'	developed for this project	45.5^{o}
2CL3	No	429bp	2CL3 F: 5'-TGC TGA ACT AGC AGT CAT-3'	developed for this project	45.5^{o}
			2CL3 R: 5'-GCT TTC CCA AGA GGA ATG AAA T-3'	developed for this project	45.5^{o}
2CL4	No	376bp	2CL4 F: 5'-ACT GGC AGG ATC CAG AA-3'	developed for this project	47^{o}
			2CL4 R: 5'-AAT CCA GCA GCC TTT GAC-3'	developed for this project	47^{o}
2CL8	No	466bp	2CL8F: 5'-CCC TCA ATC TAG CCC AGT-3'	developed for this project	48^{o}
			2CL8R: 5'-GAT TAG CAG GAA ACT CT-3'	developed for this project	48^{o}

Appendix 3. Taxonomic revision of *Lampropeltis triangulum.* Our results indicate seven species of milksnake that were formerly classified as subspecies of *L. triangulum*. Below, we list the seven species and details regarding their taxonomy and approximate or likely ranges based on our sampling. A map showing proposed ranges is also included in Figure 2.

Lampropeltis triangulum (Lacépède 1788)

The oldest subspecies within the proposed range of the Eastern lineage is the nominate subspecies, *L. t. triangulum,* with the holotype restricted to the vicinity of New York City (Schmidt 1953). We suggest that the Eastern lineage is designated as *L. triangulum*. The range of this species includes the entire range for *L. t. triangulum*. *Lampropeltis triangulum* would also synonimize the subspecies *L. t. syspila* and includes *L. t. syspila* and suspected "intergrades" that occur in Alabama, Indiana, Iowa, Illinois, Kentucky, Missouri, Mississippi, Tennessee, and possibly Arkansas north of the Arkansas River. Some milksnakes that have fallen under the subspecies *L. t. amaura* in northeastern Louisiana (specifically in La Salle Parish), would also be recognized as *L. triangulum*.

Lampropeltis gentilis (Baird and Girard 1853)

The oldest subspecies within the proposed range of the Western lineage is *L. t. gentilis*, originally described as a distinct species, with the lectotype from Wheeler County, Texas (Blanchard 1921). We suggest that the Western lineage is designated as *L. gentilis*. The range of *L. gentilis* includes that of the subspecies *L. t. gentilis*, and includes the ranges of the following subspecies, which it synonimizes: *L. t. amaura*, *L. t. celaenops*, *L. t. multistriata*, *L. t. taylori*. In addition, *L. gentilis* includes *L. t. annulata* in Texas and *L. t. syspila* from Nebraska, Kansas, and Oklahoma.

Lampropeltis elapsoides (Holbrook 1838)

Lampropeltis elapsoides was originally described as a species, with the holotype unknown, from South Carolina and Georgia (Williams 1988). We suggest that *L. elapsoides* continue to be designated as a species, following recent authors (Pyron and Burbrink 2009c, d). The range of *L. elapsoides* remains the same as that of the subspecies, being found across the southeastern US as far north as Virginia and Kentucky east of the Mississippi River and in eastern Louisiana. Suspected “intergrades” with *L. triangulum* from eastern Virginia to southern New Jersey are likely *L. triangulum* and not hybrids based on our migration analyses.

Lampropeltis annulata Kennicot 1861

The oldest known subspecies within the proposed range of the Tamaulipas lineage is *L. t. annulata*. *Lampropeltis annulata* was originally described as a distinct species, with the holotype from Matamoros, Tamaulipas, Mexico. We suggest that the Tamaulipas lineage is designated as *L. annulata*. *Lampropeltis annulata* is found in the Mexican states of Nuevo Leon, Queretaro and Tamaulipas. It is likely that members of this species are also found in Coahuila, eastern San Luis Potosi, and Hidalgo and thus synonimizes the subspecies *L. t. dixoni*.

L. polyzona Cope 1861

The oldest known subspecies within the proposed range of the Mexico lineage is *L. t. polyzona*, originally described as a species with the holotype from Jalapa, Veracruz, Mexico. We suggest the Mexico lineage is designated *L. polyzona*. *Lampropeltis polyzona* is found in the Mexican states of Colima, Guerrero, Hidalgo, Jalisco, Puebla, Michoacán, Oaxaca, Sinaloa, Sonora, and Veracruz. This synonimizes the subspecies *L. t. arcifera*, *L. t. conanti*, *L. t. campbelli*, *L. t. campbelli*, and *L. t. sinaloae*. It is likely that this species is also found in Guanajuato, Morelos, and Nayarit, and western San Luis Potosi which synonimizes *L. t. nelsoni*.

Lampropeltis abnorma (Bocourt 1866)

The oldest known subspecies within the proposed range of the Central America lineage is *L. t. abnorma*, originally described as a species with the holotype from Alta Verapaz, Guatemala. We suggest the Central America lineage is designated *L. abnorma*. *Lampropeltis abnorma* is found in southern Veracruz and southeastern Guerrero ranging south through Nicaragua, Honduras, and western Costa Rica. This species is possibly in southern Oaxaca, and likely Campeche, Chiapas, Quintana Roo, Tabasco, and Yucatan as well as Belize and El Salvador. The proposed range synonimizes *L. t. blanchardi, L. t. hondurensis, L. t. oligozona* and *L. t. stuarti* with *L. abnorma*.

Lampropeltis micropholis Cope 1861

The oldest known subspecies within the proposed range of the South America lineage is *L. t. micropholis*, originally described as a species with the holotype from Panama. We suggest the South America lineage is designated *L. micropholis*. *Lampropletis micropholis* ranges from eastern Costa Rica, throughout Panama, and south to Ecuador. It is likely found in Columbia and possibly Venezuela. This proposed range synonimizes *L. t. gaigeae* and *L. t. andesiana* with *L. micropholis*.

Appendix 4. Individuals sequenced for demographics. Identification numbers correspond to those of Appendix 1.

ID #	**Genus**	**Species**	**State/Country**	**County/State/Locality**
AMNH21940	*Lampropeltis*	*abnorma*	Mexico	Guerrero
AMNH22617	*Lampropeltis*	*abnorma*	Mexico	Veracruz
FTB1986	*Lampropeltis*	*abnorma*	Costa Rica	Alajuela
FTB2317	*Lampropeltis*	*abnorma*	Nicaragua	Central Nicaragua
FTB2320	*Lampropeltis*	*abnorma*	Nicaragua	Central Nicaragua
FTB2321	*Lampropeltis*	*abnorma*	Honduras	NW Honduras
FTB2322	*Lampropeltis*	*abnorma*	Honduras	Southern Honduras
FTB2323	*Lampropeltis*	*abnorma*	Honduras	Southern Honduras
FTB2386	*Lampropeltis*	*abnorma*	Honduras	Choluteca
FTB2387	*Lampropeltis*	*abnorma*	Honduras	Choluteca
FTB2411	*Lampropeltis*	*abnorma*	Costa Rica	Guanacaste
FTB0797	*Lampropeltis*	*elapsoides*	FL	Gulf
FTB1311	*Lampropeltis*	*elapsoides*	GA	Lowndes
FTB1589	*Lampropeltis*	*elapsoides*	FL	Palm Beach
FTB1591	*Lampropeltis*	*elapsoides*	FL	Leon
FTB1593	*Lampropeltis*	*elapsoides*	FL	Calhoun
FTB1597	*Lampropeltis*	*elapsoides*	FL	Polk
FTB1598	*Lampropeltis*	*elapsoides*	FL	MiamiDade
FTB1606	*Lampropeltis*	*elapsoides*	FL	Manatee
FTB1607	*Lampropeltis*	*elapsoides*	FL	Manatee
FTB1615	*Lampropeltis*	*elapsoides*	FL	Hendry
FTB1641	*Lampropeltis*	*elapsoides*	NC	Brunswick
FTB1642	*Lampropeltis*	*elapsoides*	NC	Jones
FTB1644	*Lampropeltis*	*elapsoides*	SC	Jasper
FTB1645	*Lampropeltis*	*elapsoides*	GA	Effingham

ID #	Genus	Species	State/Country	County/State/Locality
FTB1676	*Lampropeltis*	*elapsoides*	LA	Tangipahoa
FTB1679	*Lampropeltis*	*elapsoides*	LA	St. Tammany
FTB1691	*Lampropeltis*	*elapsoides*	MS	Wilkinson
FTB1693	*Lampropeltis*	*elapsoides*	MS	Stone
FTB1699	*Lampropeltis*	*elapsoides*	LA	St. Tammany
FTB1701	*Lampropeltis*	*elapsoides*	FL	Santa Rosa
FTB1702	*Lampropeltis*	*elapsoides*	LA	West Feliciana
FTB1703	*Lampropeltis*	*elapsoides*	LA	St. Tammany
FTB1755	*Lampropeltis*	*elapsoides*	SC	Dorchester
FTB1756	*Lampropeltis*	*elapsoides*	AL	Cleburne
FTB1794	*Lampropeltis*	*elapsoides*	AL	Bibb
FTB1795	*Lampropeltis*	*elapsoides*	AL	Bibb
FTB1974	*Lampropeltis*	*elapsoides*	SC	Chesterfield
FTB1976	*Lampropeltis*	*elapsoides*	NC	Scotland
FTB1977	*Lampropeltis*	*elapsoides*	SC	Dorchester
FTB1978	*Lampropeltis*	*elapsoides*	NC	Moore
FTB2109	*Lampropeltis*	*elapsoides*	NC	Carteret
MHP9851	*Lampropeltis*	*elapsoides*	KY	Lyon
CLC698	*Lampropeltis*	*gentilis*	TX	McMullen
DBS0562	*Lampropeltis*	*gentilis*	MT	Carbon
DBS1406	*Lampropeltis*	*gentilis*	OK	Atoka
DBS1452	*Lampropeltis*	*gentilis*	OK	Pushmataha
DBS1535	*Lampropeltis*	*gentilis*	OK	LeFlore
DBS2052	*Lampropeltis*	*gentilis*	AR	Montgomery
FTB1531	*Lampropeltis*	*gentilis*	TX	ValVerde
FTB1534	*Lampropeltis*	*gentilis*	TX	Coleman
FTB1605	*Lampropeltis*	*gentilis*	LA	Natchitoches
FTB1653	*Lampropeltis*	*gentilis*	NV	Elko

ID #	Genus	Species	State/Country	County/State/Locality
FTB1690	*Lampropeltis*	*gentilis*	LA	Iberville
FTB1700	*Lampropeltis*	*gentilis*	LA	Jefferson
FTB1786	*Lampropeltis*	*gentilis*	UT	Emery
FTB1789	*Lampropeltis*	*gentilis*	UT	Uinta
FTB1797	*Lampropeltis*	*gentilis*	TX	Grimes
FTB1798	*Lampropeltis*	*gentilis*	TX	Burleson
FTB1805	*Lampropeltis*	*gentilis*	TX	Brazos
FTB1806	*Lampropeltis*	*gentilis*	NM	Torrance
FTB1817	*Lampropeltis*	*gentilis*	AZ	Cochise
FTB1967	*Lampropeltis*	*gentilis*	AZ	Coconino
FTB1971	*Lampropeltis*	*gentilis*	AZ	Yavapai
FTB2224	*Lampropeltis*	*gentilis*	AZ	Yavapai
LJV10749	*Lampropeltis*	*gentilis*	MT	Carbon
LJV10753	*Lampropeltis*	*gentilis*	MT	Carbon
LJV10782	*Lampropeltis*	*gentilis*	MT	Yellowstone
MHP7910	*Lampropeltis*	*gentilis*	KS	Rush
MHP8504	*Lampropeltis*	*gentilis*	KS	Logan
MHP8549	*Lampropeltis*	*gentilis*	KS	Russell
MHP8727	*Lampropeltis*	*gentilis*	SD	Custer
SRSU6519	*Lampropeltis*	*gentilis*	TX	Jeff Davis
TJC29	*Lampropeltis*	*gentilis*	OK	Cherokee
FHGO2427	*Lampropeltis*	*micropholis*	Ecuador	Pichincha
FHGO2663	*Lampropeltis*	*micropholis*	Ecuador	Pichincha
FHGO2885	*Lampropeltis*	*micropholis*	Ecuador	Pichincha
FHGO2906	*Lampropeltis*	*micropholis*	Ecuador	Pichincha
FHGO2918	*Lampropeltis*	*micropholis*	Ecuador	Pichincha
FHGO3021	*Lampropeltis*	*micropholis*	Ecuador	Pichincha
FHGO3026	*Lampropeltis*	*micropholis*	Ecuador	Pichincha

ID #	Genus	Species	State/Country	County/State/Locality
FHGO3090	*Lampropeltis*	*micropholis*	Ecuador	Pichincha
FHGO4848	*Lampropeltis*	*micropholis*	Ecuador	Pichincha
FHGO5766	*Lampropeltis*	*micropholis*	Ecuador	Pichincha
FHGO5966	*Lampropeltis*	*micropholis*	Ecuador	Pichincha
FHGO6217	*Lampropeltis*	*micropholis*	Ecuador	Pichincha
FTB1985	*Lampropeltis*	*micropholis*	Costa Rica	Cartago
QCAZR5576	*Lampropeltis*	*micropholis*	Ecuador	Pichincha
QCAZR6321	*Lampropeltis*	*micropholis*	Ecuador	Pichincha
USNM578513	*Lampropeltis*	*micropholis*	Panama	Coclé
AMNH21975	*Lampropeltis*	*polyzona*	Mexico	Guerrero
AMNH22074	*Lampropeltis*	*polyzona*	Mexico	Guerrero
AMNH22361	*Lampropeltis*	*polyzona*	Mexico	Oaxaca
AMNH22501	*Lampropeltis*	*polyzona*	Mexico	Puebla
AMNH22785	*Lampropeltis*	*polyzona*	Mexico	Oaxaca
AMNH22786	*Lampropeltis*	*polyzona*	Mexico	Oaxaca
DGM2241	*Lampropeltis*	*polyzona*	Mexico	Sonora
FMQ4000	*Lampropeltis*	*polyzona*	Mexico	Hidalgo
FTB1568	*Lampropeltis*	*polyzona*	Mexico	Colima
FTB1818	*Lampropeltis*	*polyzona*	Mexico	Colima
FTB1821	*Lampropeltis*	*polyzona*	Mexico	Puebla
FTB1964	*Lampropeltis*	*polyzona*	Mexico	Sonora
FTB1965	*Lampropeltis*	*polyzona*	Mexico	Sonora
HIR242	*Lampropeltis*	*polyzona*	Mexico	Michoacan
JAC27958	*Lampropeltis*	*polyzona*	Mexico	Colima
JAC29865	*Lampropeltis*	*polyzona*	Mexico	Veracruz
JAC30084	*Lampropeltis*	*polyzona*	Mexico	Colima
JAC30103	*Lampropeltis*	*polyzona*	Mexico	Colima
JAC30303	*Lampropeltis*	*polyzona*	Mexico	Colima

ID #	Genus	Species	State/Country	County/State/Locality
JAC30515	*Lampropeltis*	*polyzona*	Mexico	Colima
JAC30516	*Lampropeltis*	*polyzona*	Mexico	Colima
JAC30542	*Lampropeltis*	*polyzona*	Mexico	Sinaloa
JAC30571	*Lampropeltis*	*polyzona*	Mexico	Sonora
JAC30582	*Lampropeltis*	*polyzona*	Mexico	Sinaloa
JAC30602	*Lampropeltis*	*polyzona*	Mexico	Sinaloa
JAC30658	*Lampropeltis*	*polyzona*	Mexico	Colima
JAC30659	*Lampropeltis*	*polyzona*	Mexico	Colima
JLTV05	*Lampropeltis*	*polyzona*	Mexico	Hidalgo
L177306	*Lampropeltis*	*polyzona*	Mexico	Michoacan
PPC20	*Lampropeltis*	*polyzona*	Mexico	Jalisco
PPC21	*Lampropeltis*	*polyzona*	Mexico	Jalisco
FTB0442	*Lampropeltis*	*triangulum*	NY	Suffolk
FTB0563	*Lampropeltis*	*triangulum*	VA	Shennandoaha
FTB0647	*Lampropeltis*	*triangulum*	WI	Crawford
FTB0728	*Lampropeltis*	*triangulum*	WV	Tucker
FTB0949	*Lampropeltis*	*triangulum*	NY	Orange
FTB1512	*Lampropeltis*	*triangulum*	IL	Jersey
FTB1521	*Lampropeltis*	*triangulum*	IL	Washington
FTB1524	*Lampropeltis*	*triangulum*	IL	Kankakee
FTB1525	*Lampropeltis*	*triangulum*	IL	Iroquois
FTB1580	*Lampropeltis*	*triangulum*	MN	Winona
FTB1581	*Lampropeltis*	*triangulum*	MN	Winona
FTB1582	*Lampropeltis*	*triangulum*	IN	Lake
FTB1584	*Lampropeltis*	*triangulum*	IN	Lake
FTB1635	*Lampropeltis*	*triangulum*	MD	Baltimore
FTB1638	*Lampropeltis*	*triangulum*	NJ	Hunterdon
FTB1640	*Lampropeltis*	*triangulum*	MD	Baltimore

ID #	**Genus**	**Species**	**State/Country**	**County/State/Locality**
FTB1678	*Lampropeltis*	*triangulum*	LA	La Salle
FTB1680	*Lampropeltis*	*triangulum*	LA	La Salle
FTB1687	*Lampropeltis*	*triangulum*	WI	Walworth
FTB1697	*Lampropeltis*	*triangulum*	NY	Tompkins
FTB1829	*Lampropeltis*	*triangulum*	MA	Hampshire
FTB1839	*Lampropeltis*	*triangulum*	MO	Jackson
FTB1840	*Lampropeltis*	*triangulum*	NY	Sullivan
FTB1973	*Lampropeltis*	*triangulum*	PA	Lackawanna
FTB1984	*Lampropeltis*	*triangulum*	WI	Iowa
FTB2229	*Lampropeltis*	*triangulum*	MO	Iron
FTB2230	*Lampropeltis*	*triangulum*	MO	Iron
FTB2231	*Lampropeltis*	*triangulum*	MO	Shannon
FTB2232	*Lampropeltis*	*triangulum*	MO	Shannon
FTB2298	*Lampropeltis*	*triangulum*	OH	Tusc
MHP9876	*Lampropeltis*	*triangulum*	TN	Obion
MHP9886	*Lampropeltis*	*triangulum*	KY	Hickman
MHP9887	*Lampropeltis*	*triangulum*	KY	Menifee
YPM13969	*Lampropeltis*	*triangulum*	CT	Fairfield

Appendix 5. Total number of individuals sequenced (and used in EBSP analyses) for each locus and the number of individuals used only in DNAsp analyses.

Species	**Locus**	**Total # of Individuals Sequenced**	**# of Individuals for DNAsp Analyses**
L. abnorma	2CL3	11	9
L. abnorma	2CL4	11	10
L. abnorma	2CL8	11	8
L. abnorma	CL4	11	11
L. abnorma	CYTB	11	11
L. abnorma	GAD2	11	11
L. abnorma	LATCL	11	10
L. abnorma	NT3	11	10
L. abnorma	PRLR	10	9
L. abnorma	SPTBN1	11	7
L. abnorma	VIM56	11	10
L. elapsoides	2CL8	20	15
L. elapsoides	CL4	31	31
L. elapsoides	CYTB	32	32
L. gentilis	2CL8	19	6
L. gentilis	CL4	29	24
L. gentilis	CYTB	31	31
L. micropholis	2CL8	16	12
L. micropholis	CYTB	12	11
L. micropholis	GAD2	14	13
L. micropholis	NT3	16	16
L. micropholis	PRLR	12	12
L. micropholis	SPTBN1	16	16
L. polyzona	2CL3	30	27
L. polyzona	2CL8	31	24
L. polyzona	CL4	25	23

Species	**Locus**	**Total # of Individuals Sequenced**	**# of Individuals for DNAsp Analyses**
L. polyzona	CYTB	31	23
L. polyzona	GAD2	24	22
L. polyzona	LATCL	23	22
L. polyzona	NAV56	29	17
L. polyzona	NT3	26	22
L. polyzona	PRLR	30	22
L. polyzona	SPTBN1	31	25
L. polyzona	VIM56	30	23
L. triangulum	2CL8	20	20
L. triangulum	CL4	33	29
L. triangulum	CYTB	34	34

Appendix 6. Specimens photographed for geometric morphometric analyses. Snout-vent length was used to determine whether a specimen was a juvenile (see Chapter 3 for details). Museum abbreviations are as follows: American Museum of Natural History (AMNH), Smithsonian National Museum of Natural History (UNSM), Burbrink collection (FTB).

ID #	**Species**	**Sex**	**SVL (cm)**	**Country**	**State**	**County**	**Juvenile**
AMNH 12676	*L. abnorma*	Female	145	Nicaragua	Unknown	Unknown	No
AMNH 12677	*L. abnorma*	Male	34	Nicaragua	Unknown	Unknown	Yes
AMNH 12678	*L. abnorma*	Female	35	Nicaragua	Unknown	Unknown	Yes
AMNH 32572	*L. abnorma*	Female	135	Honduras	Unknown	Unknown	No
AMNH 36465	*L. abnorma*	Female	46	Central Am.	Unknown	Unknown	Yes
AMNH 70179	*L. abnorma*	Female	104	Honduras	Unknown	Unknown	No
AMNH 70198	*L. abnorma*	Female	28	Honduras	Morazan	Unknown	Yes
AMNH 75430	*L. abnorma*	Male	35	Nicaragua	Unknown	Unknown	Yes
AMNH 76132	*L. abnorma*	Male	35	Honduras	Unknown	Unknown	Yes
AMNH 77061	*L. abnorma*	Male	61.8	Mexico	Tabasco	Unknown	No
AMNH 158782	*L. abnorma*	Female	80	Mexico	Chiapas	Unknown	No
UNSM 25132	*L. abnorma*	Female	111.9	Guatemala	Peten	Unknown	No
UNSM 85121	*L. abnorma*	Female	99.7	Honduras	Unknown	Unknown	No
UNSM 121451	*L. abnorma*	Male	98	Mexico	Chiapas	Unknown	No
UNSM 508417	*L. abnorma*	Male	91.6	Honduras	Copan	Unknown	No
UNSM 570430	*L. abnorma*	Male	84.5	Honduras	Copan	Unknown	No
UNSM 570544	*L. abnorma*	Female	125.3	Honduras	Unknown	Unknown	No
AMNH 8455	*L. elapsoides*	Female	42	USA	Florida	Unknown	No
AMNH 9629	*L. elapsoides*	Female	16	USA	Kentucky	Unknown	Yes
AMNH 18090	*L. elapsoides*	Male	21.5	USA	Florida	Martin	Yes
AMNH 22433	*L. elapsoides*	Female	35	USA	Florida	Duval	No
AMNH 24346	*L. elapsoides*	Male	17	USA	Alabama	Unknown	Yes
AMNH 46746	*L. elapsoides*	Female	25	USA	Mississippi	Harrison	Yes
AMNH 62693	*L. elapsoides*	Female	32	USA	Florida	Dade	No
AMNH 63470	*L. elapsoides*	Male	34	USA	Florida	Miami-Dade	No
AMNH 63471	*L. elapsoides*	Female	43	USA	Florida	Miami-Dade	No

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH 63875	*L. elapsoides*	Male	27	USA	Louisiana	Tammany	Yes
AMNH 63961	*L. elapsoides*	Female	37	USA	Georgia	Wilcox	No
AMNH 74738	*L. elapsoides*	Male	33	USA	Florida	Marion	No
AMNH 84324	*L. elapsoides*	Female	25	USA	Virginia	Lancaster	Yes
AMNH 93067	*L. elapsoides*	Male	30	USA	South Carolina	Jasper	Yes
AMNH 97526	*L. elapsoides*	Female	40	USA	South Carolina	Jasper	No
AMNH 97527	*L. elapsoides*	Male	26.5	USA	South Carolina	Jasper	Yes
AMNH 97528	*L. elapsoides*	Female	29.5	USA	South Carolina	Jasper	Yes
AMNH 99066	*L. elapsoides*	Female	36	USA	Georgia	Emanuel	No
AMNH 107674	*L. elapsoides*	Male	35	USA	Florida	Putnam	No
AMNH 117699	*L. elapsoides*	Male	33	USA	South Carolina	Jasper	No
AMNH 121628	*L. elapsoides*	Male	42	USA	Florida	Volusia	No
AMNH 129521	*L. elapsoides*	Male	56	USA	North Carolina	Hyde	No
AMNH 158777	*L. elapsoides*	Male	19	USA	Louisiana	Assumption	Yes
FTB1755	*L. elapsoides*	Female	34.5	USA	South Carolina	Unknown	No
FTB1756	*L. elapsoides*	Female	38	USA	Alabama	Unknown	No
UNSM2305	*L. elapsoides*	Female	34.2	USA	Florida	Valuscia	No
UNSM2384	*L. elapsoides*	Male	36.7	USA	South Carolina	Charleston	No
UNSM 9689	*L. elapsoides*	Female	36.7	USA	Charleston	South Carolina	No
UNSM 28251	*L. elapsoides*	Female	34.3	USA	Florida	Dade	No
UNSM 36566	*L. elapsoides*	Female	38.4	USA	Florida	Dade	No
UNSM 85324	*L. elapsoides*	Female	47.4	USA	Florida	Monroe	No
UNSM 204238	*L. elapsoides*	Male	31.5	USA	Florida	Monroe	No
USNM 210070	*L. elapsoides*	Male	29.5	USA	Florida	Brevard	No
AMNH 3726	*L. gentilis*	Female	17	USA	Kansas	Unknown	No
AMNH 7705	*L. gentilis*	Male	55	USA	Oklahoma	Creek	No
AMNH 8788	*L. gentilis*	Male	28	USA	Nebraska	Garden	No
AMNH 76165	*L. gentilis*	Male	40	USA	Texas	Jefferson	No
AMNH 85392	*L. gentilis*	Female	67	USA	Texas	Brazos	No

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH 86932	*L. gentilis*	Male	33	USA	Texas	Calhoun	No
AMNH 86933	*L. gentilis*	Male	46	USA	Texas	Calhoun	No
AMNH 95951	*L. gentilis*	Male	48	USA	Arkansas	Polk	No
AMNH 107041	*L. gentilis*	Female	22	USA	Louisiana	St Charles	No
AMNH 107363	*L. gentilis*	Male	51	USA	Texas	Val Verde	No
AMNH 108159	*L. gentilis*	Male	20.5	USA	Colorado	Cheyenne	No
AMNH 126479	*L. gentilis*	Female	66	USA	Texas	Bexar	No
AMNH 158765	*L. gentilis*	Unknown	18.5	USA	Louisiana	Assumption	No
AMNH 158766	*L. gentilis*	Female	39	USA	Louisiana	St. Charles	No
AMNH 158767	*L. gentilis*	Male	31	USA	Louisiana	Terrebonne	No
AMNH 158768	*L. gentilis*	Female	49	USA	Louisiana	Terrebonne	No
AMNH 158769	*L. gentilis*	Unknown	16	USA	Louisiana	Terrebonne	No
AMNH 158770	*L. gentilis*	Female	31	USA	Texas	Brazos	No
AMNH 158776	*L. gentilis*	Female	22.5	USA	Louisiana	Tangipahoa	No
AMNH 158781	*L. gentilis*	Female	38	USA	Kansas	Unknown	No
USNM 1841	*L. gentilis*	Female	53.2	USA	Texas	Cameron	No
USNM 7116	*L. gentilis*	Male	51.9	USA	Texas	Bexar	No
USNM 17031	*L. gentilis*	Female	59.4	USA	Texas	Cameron	No
USNM 17032	*L. gentilis*	Female	60.7	USA	Texas	Cameron	No
USNM 88769	*L. gentilis*	Male	59.7	USA	Kansas	Cowley	No
USNM 197622	*L. gentilis*	Male	72.3	USA	Kansas	Doniphan	No
USNM 307595	*L. gentilis*	Female	55.7	USA	Kansas	Wabunsee	No
USNM 321516	*L. gentilis*	Male	70.1	USA	Texas	Webb	No
USNM 330227	*L. gentilis*	Female	56.7	USA	Kansas	Doniphan	No
USNM 335578	*L. gentilis*	Male	46.1	USA	Utah	Unknown	No
USNM 561129	*L. gentilis*	Male	46.5	USA	Texas	Webb	No
AMNH 13429	*L. micropholis*	Male	97	Ecuador	Unknown	Unknown	No
AMNH 13431	*L. micropholis*	Female	27	Ecuador	Unknown	Unknown	Yes
AMNH 17263	*L. micropholis*	Female	140	Costa Rica	Unknown	Unknown	No

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH 17490	*L. micropholis*	Male	28	Colombia	Unknown	Unknown	Yes
AMNH 17491	*L. micropholis*	Female	31	Colombia	Unknown	Unknown	Yes
AMNH 19993	*L. micropholis*	Female	26.5	Colombia	Magdalena	Unknown	Yes
AMNH 49225	*L. micropholis*	Female	29	Rep Panama	Chiriqui	Unknown	Yes
AMNH 73328	*L. micropholis*	Male	38	Panama	Chiriqui	Unknown	Yes
AMNH 75632	*L. micropholis*	Female	103	Panama	Unknown	Unknown	No
AMNH 108480	*L. micropholis*	Female	130.5	Colombia	Unknown	Unknown	No
AMNH 109744	*L. micropholis*	Male	122.9	Colombia	Unknown	Unknown	No
AMNH 113633	*L. micropholis*	Female	107.9	Columbia	Unknown	Unknown	No
AMNH 129759	*L. micropholis*	Male	91	Panama	Panama	Unknown	No
AMNH 159495	*L. micropholis*	Female	106.4	Columbia	Unknown	Unknown	No
UNSM 211026	*L. micropholis*	Female	79.9	Ecuador	Unknown	Unknown	No
AMNH 3525	*L. polyzona*	Female	70	Mexico	Sinaloa	Unknown	No
AMNH 3526	*L. polyzona*	Female	72	Mexico	Sinaloa	Unknown	No
AMNH 3527	*L. polyzona*	Unknown	45	Mexico	Sinaloa	Unknown	Yes
AMNH 4279	*L. polyzona*	Female	48.8	Mexico	Veracruz	Unknown	No
AMNH 4280	*L. polyzona*	Female	65.3	Mexico	Veracruz	Unknown	No
AMNH 15252	*L. polyzona*	Male	50	Mexico	Puebla	Unknown	Yes
AMNH 19646	*L. polyzona*	Male	31	Mexico	Veracruz	Unknown	Yes
AMNH 19647	*L. polyzona*	Female	76	Mexico	Jalisco	Unknown	No
AMNH 19649	*L. polyzona*	Female	25.5	Mexico	Jalisco	Unknown	Yes
AMNH 19650	*L. polyzona*	Female	19	Mexico	Jalisco	Unknown	Yes
AMNH 19702	*L. polyzona*	Female	24	Mexico	Jalisco	Unknown	Yes
AMNH 63713	*L. polyzona*	Female	70	Mexico	Sonora	Unknown	No
AMNH 63714	*L. polyzona*	Male	65	Mexico	Sonora	Unknown	No
AMNH 64269	*L. polyzona*	Male	31	Mexico	Veracruz	Unknown	Yes
AMNH 64270	*L. polyzona*	Male	34.5	Mexico	Veracruz	Unknown	Yes
AMNH 64271	*L. polyzona*	Male	39	Mexico	Veracruz	Unknown	Yes
AMNH 64272	*L. polyzona*	Male	44	Mexico	Veracruz	Unknown	Yes

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH 65739	*L. polyzona*	Female	40	Mexico	Oaxaca	Unknown	Yes
AMNH 68013	*L. polyzona*	Female	45	Mexico	Oaxaca	Unknown	Yes
AMNH 68884	*L. polyzona*	Female	28	Mexico	Oaxaca	Unknown	Yes
AMNH 71364	*L. polyzona*	Female	71	Mexico	Jalisco	Unknown	No
AMNH 72488	*L. polyzona*	Male	22.5	Mexico	Guerrero	Unknown	Yes
AMNH 76424	*L. polyzona*	Male	23	Mexico	Puebla	Unknown	Yes
AMNH 76425	*L. polyzona*	Male	26	Mexico	Puebla	Unknown	Yes
AMNH 76426	*L. polyzona*	Male	24	Mexico	Puebla	Unknown	Yes
AMNH 76427	*L. polyzona*	Male	25	Mexico	Puebla	Unknown	Yes
AMNH 78674	*L. polyzona*	Female	32	Mexico	Nayarit	Unknown	Yes
AMNH 78761	*L. polyzona*	Female	38	Mexico	Nayarit	Unknown	Yes
AMNH 85753	*L. polyzona*	Female	85	Mexico	Colima	Unknown	No
AMNH 90705	*L. polyzona*	Female	68	Mexico	Sinaloa	Unknown	No
AMNH 90706	*L. polyzona*	Male	75	Mexico	Sinaloa	Unknown	No
AMNH 90707	*L. polyzona*	Female	60	Mexico	Sinaloa	Unknown	No
AMNH 90708	*L. polyzona*	Female	65	Mexico	Sinaloa	Unknown	No
AMNH 90710	*L. polyzona*	Male	31	Mexico	Sinaloa	Unknown	Yes
AMNH 93422	*L. polyzona*	Female	75.2	Mexico	Veracruz	Unknown	No
AMNH 100386	*L. polyzona*	Female	39	Mexico	Sinaloa	Unknown	Yes
AMNH 102958	*L. polyzona*	Female	45	Mexico	Oaxaca	Unknown	Yes
AMNH 106572	*L. polyzona*	Female	85	Mexico	Hidalgo	Unknown	No
AMNH 107626	*L. polyzona*	Male	83	Mexico	Sinaloa	Unknown	No
AMNH 109032	*L. polyzona*	Female	78	Mexico	Sinaloa	Unknown	No
AMNH 117991	*L. polyzona*	Male	49	Mexico	Jalisco	Unknown	Yes
AMNH 134684	*L. polyzona*	Female	80	Mexico	Sinaloa	Unknown	No
AMNH 138602	*L. polyzona*	Male	67.5	Mexico	Jalisco	Unknown	No
AMNH 158723	*L. polyzona*	Male	74.5	Mexico	Oaxaca	Unknown	No
AMNH 158779	*L. polyzona*	Male	74	Mexico	Nayarit	Unknown	No
AMNH 158780	*L. polyzona*	Male	52	Mexico	Nayarit	Unknown	Yes

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
UNSM 12121	*L. polyzona*	Male	53.2	Mexico	Veracruz	Unknown	No
UNSM 25008	*L. polyzona*	Female	83.4	Mexico	Veracruz	Unknown	No
UNSM 25009	*L. polyzona*	Female	59.6	Mexico	Veracruz	Unknown	No
UNSM 25192	*L. polyzona*	Male	73.1	Mexico	Veracruz	Unknown	No
UNSM 110823	*L. polyzona*	Male	88.8	Mexico	Veracruz	Unknown	No
UNSM 212218	*L. polyzona*	Male	112.9	Mexico	Jalisco	Unknown	No
AMNH2314	*L. triangulum*	Male	76.5	USA	New York	Queens	No
AMNH2382	*L. triangulum*	Male	23	USA	Pennsylvania	Unknown	Yes
AMNH3168	*L. triangulum*	Male	55	USA	North Carolina	Unknown	No
AMNH3530	*L. triangulum*	Female	68	USA	New Jersey	Essex	No
AMNH3681	*L. triangulum*	Male	58	USA	Missouri	Unknown	No
AMNH3682	*L. triangulum*	Female	27	USA	Missouri	Unknown	Yes
AMNH3721	*L. triangulum*	Female	29.5	USA	New York	Unknown	Yes
AMNH3725	*L. triangulum*	Male	84.5	USA	New York	Unknown	No
AMNH3729	*L. triangulum*	Male	79.5	USA	New York	Unknown	No
AMNH3730	*L. triangulum*	Female	57.5	USA	New York	Unknown	No
AMNH3731	*L. triangulum*	Female	69.5	USA	New York	Unknown	No
AMNH3732	*L. triangulum*	Female	46.5	USA	New York	Unknown	No
AMNH3733	*L. triangulum*	Male	74	USA	New York	Unknown	No
AMNH3735	*L. triangulum*	Male	57	USA	New Jersey	Essex	No
AMNH3737	*L. triangulum*	Female	81.5	USA	New Jersey	Essex	No
AMNH3740	*L. triangulum*	Female	63	USA	New Jersey	Hudson	No
AMNH7540	*L. triangulum*	Male	31.5	USA	New Jersey	Unknown	Yes
AMNH8258	*L. triangulum*	Female	52.5	USA	North Carolina	Transylvania	No
AMNH8420	*L. triangulum*	Male	56	USA	North Carolina	Avery	No
AMNH17758	*L. triangulum*	Female	50	USA	Pennsylvania	Pike	No
AMNH19441	*L. triangulum*	Male	20.5	USA	Connecticut	Fairfield	Yes
AMNH23084	*L. triangulum*	Male	66	USA	Indiana	Marshall	No
AMNH28660	*L. triangulum*	Male	45	USA	New York	Suffolk	No

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH 28937	*L. triangulum*	Female	23.5	USA	North Carolina	Haywood	Yes
AMNH 29957	*L. triangulum*	Male	81	USA	New York	Nassau	No
AMNH 31847	*L. triangulum*	Male	24	USA	Connecticut	Unknown	Yes
AMNH 36522	*L. triangulum*	Male	75.5	USA	Connecticut	Unknown	No
AMNH 36540	*L. triangulum*	Male	40.5	USA	North Carolina	Unknown	No
AMNH 43933	*L. triangulum*	Female	30	USA	New Jersey	Union	Yes
AMNH 43940	*L. triangulum*	Female	45	USA	New Jersey	Somerset	No
AMNH 46394	*L. triangulum*	Male	63	USA	New York	Rockland	No
AMNH 58631	*L. triangulum*	Female	23.5	USA	New York	Suffolk	Yes
AMNH 60038	*L. triangulum*	Male	49	USA	Maine	Unknown	No
AMNH 60419	*L. triangulum*	Female	62	USA	New Hampshire	Unknown	No
AMNH 62086	*L. triangulum*	Male	30.5	USA	Vermont	Rutland	Yes
AMNH 62675	*L. triangulum*	Male	94.5	USA	New York	Suffolk	No
AMNH 63879	*L. triangulum*	Female	23.5	USA	New Jersey	Ocean	Yes
AMNH 63880	*L. triangulum*	Male	63	USA	New Jersey	Burlington	No
AMNH 63881	*L. triangulum*	Male	80	USA	New Jersey	Burlington	No
AMNH 63882	*L. triangulum*	Male	72	USA	New Jersey	Burlington	No
AMNH 63884	*L. triangulum*	Male	54	USA	New Jersey	Burlington	No
AMNH 64042	*L. triangulum*	Male	18	USA	Connecticut	Unknown	Yes
AMNH 64088	*L. triangulum*	Male	44	USA	New Jersey	Warren	No
AMNH 64099	*L. triangulum*	Male	38	USA	Wisconsin	Dodge	No
AMNH 64778	*L. triangulum*	Male	101.5	USA	New York	Orange	No
AMNH 64842	*L. triangulum*	Male	58	USA	Maine	Oxford	No
AMNH 66357	*L. triangulum*	Male	100	Canada	Ontario	Unknown	No
AMNH 66359	*L. triangulum*	Female	68.5	USA	Pennsylvania	Lehigh	No
AMNH 66554	*L. triangulum*	Female	71	USA	New Jersey	Bergen	No
AMNH 67139	*L. triangulum*	Male	18.8	USA	Delaware	Newcastle	Yes
AMNH 67298	*L. triangulum*	Male	92	USA	Vermont	Rutland	No
AMNH 67649	*L. triangulum*	Female	18	USA	New Jersey	Burlington	Yes

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH 67650	*L. triangulum*	Female	17	USA	New Jersey	Ocean	Yes
AMNH 68995	*L. triangulum*	Male	19.5	USA	Maryland	Cecil	Yes
AMNH 72432	*L. triangulum*	Male	94	USA	New Jersey	Hunterdon	No
AMNH 74874	*L. triangulum*	Female	31.5	USA	Virginia	Giles	Yes
AMNH 77545	*L. triangulum*	Male	20.5	USA	Maryland	Washington	Yes
AMNH 84242	*L. triangulum*	Male	105.5	USA	New Jersey	Monmouth	No
AMNH 84307	*L. triangulum*	Female	76	USA	New Hampshire	Grafton	No
AMNH 84308	*L. triangulum*	Male	84	USA	New Hampshire	Grafton	No
AMNH 85380	*L. triangulum*	Female	71.5	USA	North Carolina	Yancey	No
AMNH 86797	*L. triangulum*	Female	32	USA	Virginia	Lancaster	Yes
AMNH 88059	*L. triangulum*	Female	82	USA	New Jersey	Hudson	No
AMNH 88239	*L. triangulum*	Female	70	USA	New Jersey	Hudson	No
AMNH 88421	*L. triangulum*	Female	78	USA	New York	Suffolk	No
AMNH 90477	*L. triangulum*	Male	40.5	USA	New Jersey	Middlesex	No
AMNH 90478	*L. triangulum*	Female	16	USA	New Jersey	Monmouth	Yes
AMNH 90626	*L. triangulum*	Female	84.5	USA	New York	Suffolk	No
AMNH 91944	*L. triangulum*	Female	71	USA	New Jersey	Middlesex	No
AMNH 92761	*L. triangulum*	Male	42	USA	New Jersey	Burlington	No
AMNH 92762	*L. triangulum*	Female	16.5	USA	New Jersey	Burlington	Yes
AMNH 92961	*L. triangulum*	Male	70	USA	New York	Dutchess	No
AMNH 93033	*L. triangulum*	Male	59	USA	New Jersey	Passaic	No
AMNH 93036	*L. triangulum*	Male	17.5	USA	New Jersey	Passaic	Yes
AMNH 93038	*L. triangulum*	Unknown	17	USA	New Jersey	Passaic	Yes
AMNH 93040	*L. triangulum*	Female	38.5	USA	New Jersey	Passaic	No
AMNH 93041	*L. triangulum*	Male	54.5	USA	New Jersey	Bergen	No
AMNH 93042	*L. triangulum*	Female	49.5	USA	New Jersey	Bergen	No
AMNH 93043	*L. triangulum*	Female	26	USA	New Jersey	Hudson	Yes
AMNH 93045	*L. triangulum*	Male	71	USA	New York	Sullivan	No
AMNH 93049	*L. triangulum*	Female	55	USA	New Jersey	Middlesex	No

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH93050	*L. triangulum*	Male	81.5	USA	New Jersey	Middlesex	No
AMNH93053	*L. triangulum*	Male	26	USA	New Jersey	Ocean	Yes
AMNH93663	*L. triangulum*	Female	58	USA	New Jersey	Middlesex	No
AMNH93665	*L. triangulum*	Female	78.5	USA	New York	Ulster	No
AMNH93666	*L. triangulum*	Female	23.5	USA	New York	Ulster	Yes
AMNH94899	*L. triangulum*	Male	63	USA	New Jersey	Middlesex	No
AMNH95507	*L. triangulum*	Male	83.5	USA	New York	Orange	No
AMNH96908	*L. triangulum*	Male	85.5	USA	West Virginia	Raleigh	No
AMNH97228	*L. triangulum*	Male	18.5	USA	New York	Tompkins	Yes
AMNH97288	*L. triangulum*	Male	56.5	USA	New York	Sullivan	No
AMNH97563	*L. triangulum*	Male	72	USA	New Jersey	Bergen	No
AMNH97568	*L. triangulum*	Male	18	USA	New Jersey	Passaic	Yes
AMNH97569	*L. triangulum*	Male	83	USA	New Jersey	Middlesex	No
AMNH97570	*L. triangulum*	Male	82	USA	New Jersey	Middlesex	No
AMNH97571	*L. triangulum*	Male	78.5	USA	New York	Putnam	No
AMNH97808	*L. triangulum*	Female	63.5	USA	Vermont	Windham	No
AMNH97816	*L. triangulum*	Male	32	USA	New York	Erie	Yes
AMNH101060	*L. triangulum*	Female	73	USA	New Jersey	Ocean	No
AMNH101061	*L. triangulum*	Male	64	USA	New Jersey	Ocean	No
AMNH101063	*L. triangulum*	Male	63	USA	New Jersey	Ocean	No
AMNH101268	*L. triangulum*	Female	94	USA	New Jersey	Bergen	No
AMNH101269	*L. triangulum*	Male	90	USA	New Jersey	Bergen	No
AMNH101270	*L. triangulum*	Male	41	USA	New Jersey	Bergen	No
AMNH102630	*L. triangulum*	Female	76	USA	New York	Rockland	No
AMNH102631	*L. triangulum*	Female	59	USA	New York	Rockland	No
AMNH102632	*L. triangulum*	Male	90	USA	New York	Rockland	No
AMNH103198	*L. triangulum*	Male	118	USA	New York	Richmond	No
AMNH103233	*L. triangulum*	Male	18.7	USA	New Jersey	Bergen	Yes
AMNH103234	*L. triangulum*	Male	17.8	USA	New Jersey	Bergen	Yes

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH 103235	*L. triangulum*	Male	18	USA	New Jersey	Bergen	Yes
AMNH 103236	*L. triangulum*	Male	17.3	USA	New Jersey	Bergen	Yes
AMNH 103237	*L. triangulum*	Female	18.3	USA	New Jersey	Bergen	Yes
AMNH 103238	*L. triangulum*	Female	18.4	USA	New Jersey	Bergen	Yes
AMNH 104640	*L. triangulum*	Male	70.5	USA	New Jersey	Morris	No
AMNH 104650	*L. triangulum*	Male	81	USA	New Jersey	Middlesex	No
AMNH 104651	*L. triangulum*	Male	52	USA	New Jersey	Middlesex	No
AMNH 104652	*L. triangulum*	Female	41	USA	New Jersey	Middlesex	No
AMNH 104653	*L. triangulum*	Female	70	USA	New Jersey	Middlesex	No
AMNH 104654	*L. triangulum*	Female	74	USA	New Jersey	Middlesex	No
AMNH 104807	*L. triangulum*	Female	93	USA	New Jersey	Middlesex	No
AMNH 104808	*L. triangulum*	Female	19.5	USA	New Jersey	Middlesex	Yes
AMNH 104809	*L. triangulum*	Female	20	USA	New Jersey	Middlesex	Yes
AMNH 104810	*L. triangulum*	Female	21	USA	New Jersey	Middlesex	Yes
AMNH 104811	*L. triangulum*	Female	19.5	USA	New Jersey	Middlesex	Yes
AMNH 104812	*L. triangulum*	Male	19.5	USA	New Jersey	Middlesex	Yes
AMNH 104813	*L. triangulum*	Female	19.5	USA	New Jersey	Middlesex	Yes
AMNH 104814	*L. triangulum*	Male	20	USA	New Jersey	Middlesex	Yes
AMNH 104815	*L. triangulum*	Male	20	USA	New Jersey	Middlesex	Yes
AMNH 104816	*L. triangulum*	Female	19	USA	New Jersey	Middlesex	Yes
AMNH 104817	*L. triangulum*	Male	18.5	USA	New Jersey	Middlesex	Yes
AMNH 104818	*L. triangulum*	Male	20.5	USA	New Jersey	Middlesex	Yes
AMNH 104820	*L. triangulum*	Female	19.5	USA	New Jersey	Middlesex	Yes
AMNH 104821	*L. triangulum*	Unknown	20	USA	New Jersey	Middlesex	Yes
AMNH 104822	*L. triangulum*	Male	19	USA	New Jersey	Middlesex	Yes
AMNH 105895	*L. triangulum*	Male	22	USA	Connecticut	Fairfield	Yes
AMNH 106280	*L. triangulum*	Male	68	USA	New Jersey	Ocean	No
AMNH 107624	*L. triangulum*	Male	62	USA	New Jersey	Bergen	No
AMNH 107625	*L. triangulum*	Male	67	USA	New Jersey	Middlesex	No

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH107677	*L. triangulum*	Male	68.5	USA	New Jersey	Morris	No
AMNH107679	*L. triangulum*	Male	69	USA	Maine	Hancock	No
AMNH108341	*L. triangulum*	Male	39.5	USA	New Jersey	Middlesex	No
AMNH109507	*L. triangulum*	Male	59	USA	New Jersey	Middlesex	No
AMNH109508	*L. triangulum*	Male	67	USA	New York	Orange	No
AMNH109510	*L. triangulum*	Male	92	USA	New York	Bronx	No
AMNH109511	*L. triangulum*	Female	93	USA	New York	Rockland	No
AMNH109512	*L. triangulum*	Male	69	USA	New Jersey	Somerset	No
AMNH111626	*L. triangulum*	Male	91	USA	New York	Orange	No
AMNH113045	*L. triangulum*	Male	93	USA	New Jersey	Bergen	No
AMNH113046	*L. triangulum*	Male	73	USA	New Jersey	Monmouth	No
AMNH113563	*L. triangulum*	Male	54.5	USA	Pennsylvania	Bucks	No
AMNH113618	*L. triangulum*	Male	50	USA	New Jersey	Bergen	No
AMNH118889	*L. triangulum*	Female	26	USA	New York	Rockland	Yes
AMNH119316	*L. triangulum*	Male	55	USA	Connecticut	Fairfield	No
AMNH119317	*L. triangulum*	Male	19	USA	Connecticut	Fairfield	Yes
AMNH119318	*L. triangulum*	Male	92	USA	Connecticut	New London	No
AMNH119651	*L. triangulum*	Female	40	USA	Connecticut	Fairfield	No
AMNH119652	*L. triangulum*	Male	70	USA	Connecticut	Hartford	No
AMNH119653	*L. triangulum*	Male	86	USA	Connecticut	Litchfield	No
AMNH120495	*L. triangulum*	Male	55	USA	Connecticut	Hartford	No
AMNH121576	*L. triangulum*	Male	20	USA	Ohio	Wood	Yes
AMNH121579	*L. triangulum*	Female	86	USA	Ohio	Highland	No
AMNH121580	*L. triangulum*	Male	97	USA	Ohio	Highland	No
AMNH121581	*L. triangulum*	Male	82	USA	Ohio	Ross	No
AMNH121582	*L. triangulum*	Male	78	USA	Ohio	Ross	No
AMNH121583	*L. triangulum*	Female	18	USA	Ohio	Lawrence	Yes
AMNH121584	*L. triangulum*	Male	20.5	USA	Ohio	Fulton	Yes
AMNH121586	*L. triangulum*	Male	20	USA	Ohio	Putnam	Yes

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH121587	*L. triangulum*	Male	19	USA	Ohio	Ottawa	Yes
AMNH121589	*L. triangulum*	Male	29	USA	Ohio	Hancock	Yes
AMNH121591	*L. triangulum*	Male	45	USA	Ohio	Richland	No
AMNH121592	*L. triangulum*	Female	43	USA	Ohio	Richland	No
AMNH121593	*L. triangulum*	Female	23	USA	Ohio	Scioto	Yes
AMNH121595	*L. triangulum*	Male	80	USA	Ohio	Champaign	No
AMNH121598	*L. triangulum*	Male	58.5	USA	Ohio	Pike	No
AMNH121600	*L. triangulum*	Male	34	USA	Ohio	Hocking	Yes
AMNH121601	*L. triangulum*	Female	41	USA	Ohio	Hocking	No
AMNH121602	*L. triangulum*	Male	66	USA	Ohio	Hocking	No
AMNH121603	*L. triangulum*	Male	80	USA	Ohio	Hocking	No
AMNH121604	*L. triangulum*	Unknown	90	USA	Ohio	Hocking	No
AMNH121606	*L. triangulum*	Female	60	USA	Ohio	Ashtabula	No
AMNH121607	*L. triangulum*	Female	90	USA	Ohio	Muskingum	No
AMNH121608	*L. triangulum*	Male	33	USA	Ohio	Geauga	Yes
AMNH121609	*L. triangulum*	Female	56	USA	Ohio	Geauga	No
AMNH121610	*L. triangulum*	Female	78	USA	Ohio	Geauga	No
AMNH121611	*L. triangulum*	Male	42	USA	Ohio	Trumbull	No
AMNH121612	*L. triangulum*	Male	103	USA	Ohio	Trumbull	No
AMNH121614	*L. triangulum*	Male	41.5	USA	Ohio	Lucas	No
AMNH121615	*L. triangulum*	Male	59	USA	Ohio	Lucas	No
AMNH121616	*L. triangulum*	Male	91	USA	Ohio	Lucas	No
AMNH121621	*L. triangulum*	Female	65	USA	Ohio	Lucas	No
AMNH121625	*L. triangulum*	Male	19	USA	Indiana	Noble	Yes
AMNH121626	*L. triangulum*	Unknown	80	USA	Indiana	Brown	No
AMNH121904	*L. triangulum*	Male	87	USA	Ohio	Crawford	No
AMNH121906	*L. triangulum*	Male	110	USA	Ohio	Wood	No
AMNH121907	*L. triangulum*	Female	49	USA	Ohio	Ashtabula	No
AMNH123209	*L. triangulum*	Male	97.5	USA	Connecticut	Fairfield	No

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH123210	*L. triangulum*	Male	19	USA	Connecticut	Hartford	Yes
AMNH124942	*L. triangulum*	Female	36	USA	New Hampshire	Grafton	No
AMNH125057	*L. triangulum*	Male	73.5	USA	Connecticut	Litchfield	No
AMNH125058	*L. triangulum*	Female	58	USA	Connecticut	Windham	No
AMNH125520	*L. triangulum*	Male	87	USA	New York	Jefferson	No
AMNH127397	*L. triangulum*	Unknown	22.5	USA	Connecticut	Fairfield	Yes
AMNH127400	*L. triangulum*	Male	67	USA	Connecticut	Litchfield	No
AMNH127401	*L. triangulum*	Female	49.5	USA	Connecticut	Litchfield	No
AMNH127402	*L. triangulum*	Male	85	USA	Connecticut	Litchfield	No
AMNH127403	*L. triangulum*	Female	27	USA	Connecticut	Middlesex	Yes
AMNH127404	*L. triangulum*	Male	21	USA	Connecticut	Middlesex	Yes
AMNH127405	*L. triangulum*	Male	60.5	USA	Connecticut	New Haven	No
AMNH128051	*L. triangulum*	Male	68	USA	Connecticut	Hartford	No
AMNH128052	*L. triangulum*	Male	21	USA	Connecticut	Hartford	Yes
AMNH128053	*L. triangulum*	Male	93	USA	Connecticut	Hartford	No
AMNH128054	*L. triangulum*	Female	68	USA	Connecticut	Hartford	No
AMNH128055	*L. triangulum*	Male	70	USA	Connecticut	Litchfield	No
AMNH128056	*L. triangulum*	Male	69	USA	Connecticut	New London	No
AMNH128057	*L. triangulum*	Male	35	USA	Connecticut	Tolland	No
AMNH128058	*L. triangulum*	Male	21	USA	Connecticut	Tolland	Yes
AMNH128140	*L. triangulum*	Male	69	USA	Massachusetts	Berkshire	No
AMNH128141	*L. triangulum*	Female	17.5	USA	Massachusetts	Berkshire	Yes
AMNH128625	*L. triangulum*	Male	28	USA	New Jersey	Ocean	Yes
AMNH128626	*L. triangulum*	Female	80	USA	New Jersey	Passaic	No
AMNH130067	*L. triangulum*	Male	66	USA	Connecticut	Fairfield	No
AMNH130068	*L. triangulum*	Male	65	USA	Connecticut	Litchfield	No
AMNH130069	*L. triangulum*	Female	73.5	USA	Connecticut	Middlesex	No
AMNH130070	*L. triangulum*	Male	98	USA	Connecticut	New Haven	No
AMNH130071	*L. triangulum*	Female	65	USA	Connecticut	New Haven	No

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH130130	*L. triangulum*	Male	81.5	USA	Massachusetts	Berkshire	No
AMNH130131	*L. triangulum*	Female	80	USA	Massachusetts	Berkshire	No
AMNH130132	*L. triangulum*	Female	65.5	USA	Massachusetts	Berkshire	No
AMNH130133	*L. triangulum*	Female	63.5	USA	Massachusetts	Berkshire	No
AMNH130153	*L. triangulum*	Male	88	USA	Vermont	Chittenden	No
AMNH130194	*L. triangulum*	Male	104.5	USA	New York	Westchester	No
AMNH130231	*L. triangulum*	Male	58	USA	West Virginia	Berkeley	No
AMNH130614	*L. triangulum*	Female	72	USA	Connecticut	Fairfield	No
AMNH130615	*L. triangulum*	Male	85	USA	Connecticut	Hartford	No
AMNH130616	*L. triangulum*	Female	75	USA	Connecticut	Hartford	No
AMNH130617	*L. triangulum*	Female	86	USA	Connecticut	Hartford	No
AMNH130618	*L. triangulum*	Female	81	USA	Connecticut	Hartford	No
AMNH130621	*L. triangulum*	Female	75	USA	Connecticut	Middlesex	No
AMNH130626	*L. triangulum*	Male	65	USA	Connecticut	Windham	No
AMNH130684	*L. triangulum*	Female	85.5	USA	Rhode Island	Newport	No
AMNH130685	*L. triangulum*	Female	48	USA	Rhode Island	Washington	No
AMNH130720	*L. triangulum*	Female	20	USA	Massachusetts	Berkshire	Yes
AMNH130721	*L. triangulum*	Male	41	USA	Massachusetts	Berkshire	No
AMNH130722	*L. triangulum*	Female	78	USA	Massachusetts	Berkshire	No
AMNH130723	*L. triangulum*	Female	51	USA	Massachusetts	Berkshire	No
AMNH130724	*L. triangulum*	Female	27.5	USA	Massachusetts	Berkshire	Yes
AMNH130725	*L. triangulum*	Male	81	USA	Massachusetts	Berkshire	No
AMNH130726	*L. triangulum*	Female	32	USA	Massachusetts	Berkshire	Yes
AMNH130728	*L. triangulum*	Female	92	USA	Massachusetts	Berkshire	No
AMNH130789	*L. triangulum*	Female	67	USA	New York	Columbia	No
AMNH130790	*L. triangulum*	Female	30.5	USA	New York	Columbia	Yes
AMNH130792	*L. triangulum*	Male	99	USA	New York	Westchester	No
AMNH130898	*L. triangulum*	Female	100.5	USA	Rhode Island	Bristol	No
AMNH130899	*L. triangulum*	Male	81.5	USA	Rhode Island	Newport	No

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH130900	*L. triangulum*	Female	72.5	USA	Rhode Island	Providence	No
AMNH130901	*L. triangulum*	Female	87.5	USA	Rhode Island	Providence	No
AMNH133026	*L. triangulum*	Female	66	USA	New York	Orange	No
AMNH133470	*L. triangulum*	Female	31.5	USA	Connecticut	Hartford	Yes
AMNH133512	*L. triangulum*	Female	76.5	USA	Rhode Island	Newport	No
AMNH133587	*L. triangulum*	Male	79	USA	Massachusetts	Berkshire	No
AMNH133588	*L. triangulum*	Male	40	USA	Massachusetts	Berkshire	No
AMNH133589	*L. triangulum*	Female	64.5	USA	Massachusetts	Berkshire	No
AMNH134280	*L. triangulum*	Female	29	USA	Connecticut	New London	Yes
AMNH134366	*L. triangulum*	Male	19.5	USA	Massachusetts	Berkshire	Yes
AMNH134367	*L. triangulum*	Female	20.5	USA	Massachusetts	Berkshire	Yes
AMNH134488	*L. triangulum*	Male	90.5	USA	New York	Columbia	No
AMNH134489	*L. triangulum*	Male	70	USA	New York	Dutchess	No
AMNH134491	*L. triangulum*	Female	70.5	USA	New York	Dutchess	No
AMNH134741	*L. triangulum*	Male	79.5	USA	Rhode Island	Newport	No
AMNH134742	*L. triangulum*	Female	94.5	USA	Rhode Island	Providence	No
AMNH134833	*L. triangulum*	Female	51	USA	Rhode Island	Kent	No
AMNH135242	*L. triangulum*	Unknown	19.5	USA	Connecticut	Unknown	Yes
AMNH135243	*L. triangulum*	Male	76	USA	Connecticut	Fairfield	No
AMNH135244	*L. triangulum*	Female	50	USA	Connecticut	Fairfield	No
AMNH137033	*L. triangulum*	Female	21	USA	Rhode Island	Newport	Yes
AMNH137069	*L. triangulum*	Male	73	USA	Rhode Island	Providence	No
AMNH137717	*L. triangulum*	Male	19	USA	Connecticut	Litchfield	Yes
AMNH137780	*L. triangulum*	Male	87	USA	Massachusetts	Barnstable	No
AMNH137791	*L. triangulum*	Male	24.5	USA	Rhode Island	Bristol	Yes
AMNH137801	*L. triangulum*	Male	92	USA	Rhode Island	Newport	No
AMNH137850	*L. triangulum*	Female	102.5	USA	Rhode Island	Washington	No
AMNH137877	*L. triangulum*	Male	42	USA	Rhode Island	Kent	No
AMNH137883	*L. triangulum*	Male	71.5	USA	Rhode Island	Providence	No

ID #	**Species**	**Sex**	**SVL (cm)**	**Country**	**State**	**County**	**Juvenile**
AMNH138645	*L. triangulum*	Male	92.5	USA	New York	Ulster	No
AMNH138955	*L. triangulum*	Female	75.5	USA	Rhode Island	Washington	No
AMNH138990	*L. triangulum*	Male	65	USA	Rhode Island	Kent	No
AMNH138997	*L. triangulum*	Male	22	USA	Massachusetts	Nantucket	Yes
AMNH139075	*L. triangulum*	Male	89	USA	Connecticut	Litchfield	No
AMNH139102	*L. triangulum*	Female	41	USA	Connecticut	Tolland	No
AMNH139382	*L. triangulum*	Female	65	USA	New Jersey	Morris	No
AMNH139385	*L. triangulum*	Female	39	USA	New Jersey	Unknown	No
AMNH139386	*L. triangulum*	Female	17	USA	New Jersey	Ocean	Yes
AMNH139387	*L. triangulum*	Male	73	USA	Virginia	Unknown	No
AMNH139388	*L. triangulum*	Male	69	USA	Virginia	Unknown	No
AMNH139389	*L. triangulum*	Male	56	USA	Virginia	Unknown	No
AMNH139390	*L. triangulum*	Male	68	USA	Virginia	Alleghany	No
AMNH140042	*L. triangulum*	Female	86	USA	New York	Suffolk	No
AMNH140050	*L. triangulum*	Female	49	USA	Connecticut	Litchfield	No
AMNH141754	*L. triangulum*	Female	24.5	USA	Rhode Island	Providence	Yes
AMNH141757	*L. triangulum*	Male	83	USA	Rhode Island	Providence	No
AMNH141758	*L. triangulum*	Female	72	USA	Rhode Island	Providence	No
AMNH141759	*L. triangulum*	Male	83	USA	Rhode Island	Washington	No
AMNH142250	*L. triangulum*	Male	23	USA	New York	Suffolk	Yes
AMNH142254	*L. triangulum*	Male	89	USA	Connecticut	Litchfield	No
AMNH142255	*L. triangulum*	Male	29.5	USA	Connecticut	Windham	Yes
AMNH146538	*L. triangulum*	Male	81	USA	New York	Dutchess	No
AMNH146539	*L. triangulum*	Male	69	USA	New York	Dutchess	No
AMNH146540	*L. triangulum*	Male	44	USA	New York	Dutchess	No
AMNH146542	*L. triangulum*	Female	68	USA	New York	Putnam	No
AMNH147179	*L. triangulum*	Male	79.5	USA	New York	Dutchess	No
AMNH147180	*L. triangulum*	Male	78	USA	New York	Orange	No
AMNH147181	*L. triangulum*	Female	20	USA	New York	Orange	Yes

ID #	Species	Sex	SVL (cm)	Country	State	County	Juvenile
AMNH 147182	*L. triangulum*	Male	58	USA	New York	Orange	No
AMNH 147186	*L. triangulum*	Female	36.5	USA	Connecticut	Fairfield	No
AMNH 147188	*L. triangulum*	Male	74.5	USA	Connecticut	New London	No
AMNH 147777	*L. triangulum*	Female	82.5	USA	Connecticut	New London	No
AMNH 148681	*L. triangulum*	Female	35.5	USA	New York	Orange	No
AMNH 148682	*L. triangulum*	Male	19.5	USA	New York	Orange	Yes
AMNH 148683	*L. triangulum*	Female	72	USA	New York	Orange	No
AMNH 148685	*L. triangulum*	Male	46.5	USA	New York	Dutchess	No
AMNH 151596	*L. triangulum*	Female	19.5	USA	Connecticut	Middlesex	Yes
AMNH 151597	*L. triangulum*	Male	66	USA	Pennsylvania	McKean	No
AMNH 154321	*L. triangulum*	Female	22.5	USA	New York	Putnam	Yes
AMNH 154341	*L. triangulum*	Male	41	USA	New Jersey	Sussex	No
AMNH 154346	*L. triangulum*	Female	17	USA	Connecticut	New Haven	Yes
AMNH 154347	*L. triangulum*	Female	18	USA	Connecticut	New Haven	Yes
AMNH 154404	*L. triangulum*	Male	44	USA	Connecticut	Windham	No
AMNH 154405	*L. triangulum*	Male	38	USA	Connecticut	Windham	No
AMNH 154419	*L. triangulum*	Male	90	USA	New York	Orange	No
AMNH 154420	*L. triangulum*	Male	76	USA	New York	Orange	No
AMNH 154421	*L. triangulum*	Male	54	USA	New York	Orange	No
AMNH 154658	*L. triangulum*	Female	79	USA	Connecticut	Windham	No
AMNH 155590	*L. triangulum*	Female	95	USA	Connecticut	Litchfield	No
AMNH 155594	*L. triangulum*	Male	21	USA	New York	Orange	Yes
AMNH 155784	*L. triangulum*	Male	62	USA	Connecticut	Litchfield	No
AMNH 159930	*L. triangulum*	Male	17.5	USA	New York	Orange	Yes
AMNH 159931	*L. triangulum*	Female	19	USA	New York	Orange	Yes

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