

SYSTEMATICS AND HISTORICAL BIOGEOGRAPHY OF THE
LAMPROPELTININE SNAKES

by

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Abstract

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Comparative studies in biology require phylogenetic hypotheses to make inferences about the processes which have shaped the evolutionary history of organisms. Thus, a complete phylogenetic estimate of a diverse group offers an excellent opportunity for examining the factors which have promoted the diversification of ecomorphological assemblages. Here, I detail such a study of the New World snake tribe Lampropeltini. The lampropeltinines comprise 31 currently recognized species, occurring from Canada to northern South America, inhabiting most major North American biomes, and exhibiting an unusual temperate peak in species richness. The Lampropeltini also exhibit an array of ecomorphological diversity, with adult sizes differing by an order of magnitude, specialization for both endothermic and ectothermic diets, and the evolution of putative Batesian mimicry of venomous coral and rattle snakes in several species. A new phylogeny inferred using multiple nuclear and mitochondrial genes allows for the stabilization of the taxonomy of the Lampropeltini. Subsequent analyses reveal that the extratropical increase in species richness is attributable to a combination of historical biogeographic factors related to the Tropical Conservatism Hypothesis (Wiens & Donoghue 2004), which are proposed as a general explanation for the in-situ

evolution of biodiversity, dubbed the ‘Biogeographical Conservatism Hypothesis.’

The ecomorphological diversification of the Lampropeltini occurred primarily along an axis of adult body size, with which is observed the correlated evolution of diet and color pattern. This pattern of correlated evolution of putatively unrelated characters suggests that body size may be a primary determinant of morphological diversification when multiple traits are linked to variables such as body size.

Finally, examining the factors which drove lineage formation at the species level through a phylogeographic assessment of the transcontinentally distributed Common Kingsnake (*Lampropeltis getula*) reveals patterns of allopatric speciation due to both phylogenetic niche conservatism and niche divergence. This suggests that niche preferences are labile on short evolutionary timescales, and that speciation can occur simultaneously in both geographic and ecological dimensions. This phylogeographic estimate also allows for a systematic assessment of the taxonomy of the Common Kingsnake group, which is revised to include five species corresponding to the major phylogeographic lineages.

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INTRODUCTION

Systematics and Historical Biogeography of the Lampropeltine Snakes

The discipline of systematics has undergone rapid methodological development in recent years, resulting in a surge in studies using molecular phylogenetic techniques to infer the evolutionary relationships of organisms (Hillis et al. 1996; Page & Holmes 1998; Felsenstein 2004). The revolution in molecular phylogenetic methods in the latter portion of the twentieth century has opened new frontiers in biology, beyond any single application for systematics, phylogenetics, or taxonomy. The ability to consider multiple organisms not just in an evolutionary context, but in an explicitly phylogenetic context is the central principle of the comparative method in biology (Harvey & Pagel 1991). This has been facilitated to an unprecedented degree by the ability to rapidly generate robust phylogenetic estimates of any group one wishes to investigate, using large amounts of sequence data from independent loci (Brinkmann & Phillippe 2008; Edwards 2009).

However, as opportunities for generating new phylogenies for groups which have not yet been studied decrease, studies using molecular phylogenies must become more thorough investigations of organismal biology, rather than simply inferences of evolutionary relationships (e.g., Wiens 2008a). Thus, the question then becomes not what is the phylogeny of a particular group, but what we can learn about the ecology, behavior, physiology, evolutionary history, etc. of the group using the phylogeny? More importantly, what can we learn that we could not have inferred without a phylogeny? Molecular systematics is ultimately a tool to allow us to make fundamental discoveries about biological processes which were elusive without an estimate of evolutionary relationships. Thus, a phylogeny of a diverse group containing all known species, combined with detailed information for those species regarding ecomorphological diversity and variation, will allow for such an investigation.

This is illustrated here through several detailed sets of analyses concerning the systematics, historical biogeography, and ecomorphological diversification of the New World snake tribe Lampropeltini. The group comprises at least 31 well-defined species which range from Canada to northern South America (see Conant & Collins [1998] and Stebbins [2003]). Species from the tribe are common in many areas, and may comprise up to 37% of snake taxa in local assemblages in areas such as west Texas (Werler & Dixon 2000). Species inhabit most terrestrial biomes in North and South America, from mesic eastern forests, central plains, and western deserts, to tropical rainforests (Williams 1978; Schulz 1996; Conant & Collins 1998; Stebbins 2003). The lampropeltinines also exhibit an unusual temperate peak in species richness, with most species occurring in subtropical regions of North America (Conant & Collins 1998; Stebbins 2003).

Additionally, the group exhibits enormous variation in ecology and morphology, with adult body sizes varying by an order of magnitude (<40cm to >200cm; Conant & Collins 1998). Dietary preferences range from endotherms to snake, lizard, or squamate egg specialists, to broad generalists (Conant & Collins 1998; Rodriguez-Robles et al. 1999; Rodriguez-Robles & Greene 1999; Rodriguez-Robles & de Jesus-Escobar 1999). Several lampropeltinine species also exhibit some of the classic cases of Batesian mimicry of venomous coral and rattle snakes (Garstka 1982; Savage & Slowinskii 1992; Conant & Collins 1998). I have gathered extensive molecular, ecological, and morphological data to test several suites of hypotheses related to the phylogenetic relationships, the evolution of biodiversity, the ecomorphological diversification, and the causes of species-level lineage formation within the Lampropeltini. I also present a taxonomic revision of the group as a whole, and the species *Lampropeltis getula*.

CHAPTER I: SYSTEMATICS

Neogene diversification and taxonomic stability in the snake tribe Lampropeltini

(Serpentes: Colubridae)

The impact of climate change during the Pleistocene on organisms living in temperate areas of the world has been documented for more than fifty years (Rand 1948). A major impact of Pleistocene glacial cycles on temperate vertebrates is hypothesized to be the isolation of species into allopatric populations (Avice & Walker 1998). Refugial isolation and subsequent lineage formation during the Pleistocene has been invoked as a primary mechanism resulting in modern species diversity (Johnson & Cicero 2004). Under the Pleistocene speciation model, these glacial cycles acted as a ‘species pump,’ producing the majority of extant organisms inhabiting temperate areas. These arguments, usually focused on dating the origin of sister species using a fixed rate of divergence in birds, have suggested that the majority of speciation events occurred in the Pleistocene (Johnson & Cicero 2004; Weir & Schluter 2007). In a more limited study, Avice et al. (1998) suggested that the Pleistocene was also important for speciation in reptiles, amphibians, and fish as well.

In contrast to the Pleistocene diversification model, it has been shown that many speciation events in birds occurred much earlier than the Pleistocene (Zink & Slowinski 1995; Klicka & Zink 1997), potentially calling into question the effects of glacial cycles on the formation of species. Unfortunately, most of these studies have invoked a molecular clock using a fixed rate of mtDNA substitution over time, and thus did not consider the impact of molecular rate variation for assessing error in divergence dates. The effect of Pleistocene climatic cycles on speciation has not been investigated in other large groups of vertebrates, particularly those that may be more sensitive to the effects of glacial cycles, such as ectotherms like reptiles. We investigate these patterns in a major New World (NW) group of colubroid snakes, the tribe Lampropeltini, inferring a robust

phylogeny for the group and estimating the timing of speciation and diversification. We use multiple nuclear and mitochondrial genes and ‘relaxed phylogenetics’ methods (Drummond et al. 2006) to estimate phylogeny and dates of origin while accounting for the possibility of rate heterogeneity across branches.

The colubroid snakes are a diverse (>2500 species), globally distributed group (Lawson et al. 2005) which date to the early Cenozoic (Burbrink & Pyron 2008). Of the several NW representatives of the group (Natricinae, Crotalinae, Elapinae, Colubrinae and Xenodontinae), the colubrine tribe Lampropeltini is one of the most conspicuous and well-studied (Williams 1978; Rodriguez-Robles & De Jesus-Escobar 1999). The lampropeltinines (rat, corn, and fox [*Pantherophis*, *Bogertophis*, and *Pseudelaphe*], king and milk [*Lampropeltis*], short-tailed [*Stilosoma*], bull, gopher, and pine [*Pituophis*], glossy [*Arizona*], scarlet [*Cemophora*] and long-nosed [*Rhinocheilus*] snakes) are common constrictors, distributed from Canada to Ecuador (Williams 1978; Conant & Collins 1998; Stebbins 2003). Several recent studies have found that the Lampropeltini form a monophyletic clade endemic to the NW, thus rendering the cosmopolitan genus *Elaphe* paraphyletic (Rodriguez-Robles & de Jesus-Escobar 1999; Utiger et al. 2002; Burbrink & Lawson 2007).

Based primarily on trees inferred using mitochondrial evidence, the taxonomy of the group is in a state of flux and the monophyly of several genera (i.e., *Pantherophis*, *Pituophis*, and *Lampropeltis*) has been disputed, including the erection of a new genus (*Mintonius*) for the fox snakes (*Pantherophis vulpinus*; Bryson et al. 2007; Burbrink & Lawson 2007; Collins & Taggart 2008). Additionally, while many phylogeographic studies have used mtDNA to investigate biogeographic structure (Burbrink 2001, 2002;

Mulcahy 2008; Rodriguez-Robles & de Jesus-Escobar 2000), higher-level phylogenies based solely or primarily on mitochondrial data have not been well-supported (Rodrigues-Robles & de Jesus-Escobar 1999; Burbrink & Lawson 2007). Thus, multiple independent loci are desirable to infer phylogenies and estimate tree-based quantities such as divergence times (i.e., Wiens 2008a). Here, we present a phylogeny based on three nuclear genes (3368bp), two of which are newly presented in this study, and six mitochondrial genes (4926bp). We included representatives from all 31 of the traditionally described species of lampropeltinine. We use this phylogeny to address hypotheses regarding the timing of origin and diversification of the lampropeltinines, as well as generate a revised taxonomy of the group.

Methods and Materials

Specimen Acquisition and DNA Sequencing

We collected tissue samples and DNA extracts for the 31 commonly recognized species of lampropeltinines (Conant & Collins 1998; Rodriguez-Robles & De Jesus-Escobar 1999; Stebbins 2003) and two outgroups (*Coronella austriaca* and *Rhinechis scalaris*; Burbrink & Lawson 2007). Additional sequence data was obtained only from existing datasets for the additional outgroup taxa *Gonyosoma oxycephalum* and *Elaphe carinata* (Utiger et al. 2002; Burbrink & Lawson, 2007). In addition to the new markers sequenced specifically for this study (SPTBN1; Vimentin Introns 4 and 5), we combined the nuclear and mitochondrial datasets of Rodriguez-Robles & De Jesus-Escobar (1999, 2000), Utiger et al. (2002) and Burbrink & Lawson (2007), which yielded six mitochondrial (mtDNA) loci (*cyt-b*, COI, ND1, ND2, ND4 and 12S) and one single copy

(scnDNA) nuclear locus (*c-mos*). Genomic DNA was extracted using Qiagen DNEasy kits (Qiagen Corp.). Taxa which were missing any of the above genes were sequenced using the primers listed in the given references.

In addition, we sequenced three single copy, non protein coding nuclear fragments: SPTBN1 (Matthee et al. 2001) and Vimentin Introns 4 and 5 (Zehner & Paterson 1983). We developed intron-spanning primer sets for SPTBN1 (Matthee et al. 2001) and Vimentin Introns 4 and 5 (Zehner & Paterson 1983) by comparing homologous regions from GenBank, and by sequencing taxa using the degenerate primers used by the original authors. These fragments were chosen based on two criteria: i) existing as single copy in other tetrapods, with a ii) reasonable amount of reported inter-specific divergence in other groups, suggesting that evolutionary rates would be high enough to yield phylogenetic signal at recent time scales. The following primer sets were used for amplification and sequencing: SPTBN1: SPTBN1SeqF 5'-ATA CAG GCT GAG CGA GTG AGA-3', SPTBN1-SeqR 5'-AGC TGA CAT AGC TCT TGG TAA CA-3'; Vimentin Intron 4: VimExon4SeqF 5'-AAG CCC AAA TCC AGG ATC A-3', VimIntron5R 5'-AGC ATA AGG GAG GAC ATA AAA-3'; Vimentin Intron 5: VimExon5F 5'-AAC AAT GAT GCC CTG CGC CA-3', VimExon6R 5'-CAA TAT CAA GAG CCA TCT TTA CAT T-3'.

Gene fragments were amplified by polymerase chain reactions (PCR) using GoTaq Green Master Mix (Promega Corp.) according to the manufacturer's protocol. The existing genes (12S, *c-mos*, *cyt-b*, COI, ND1, ND2, and ND4) were amplified and sequenced at the annealing temperatures given in the original references. The annealing temperature used for amplifying and sequencing with the SPTBN1 primers was 51

degrees; for Vimentin Intron 4, 54 degrees; and for Vimentin Intron 5, 49 degrees, with an extension time of 90 seconds. Reaction products were cleaned using EXO-SapIT (USB Corp., 1 μ l per 10 μ l product) and sequenced using Dye Terminator Cycle Sequencing reaction chemistry (Beckman-Coulter Corp.) according to the manufacturer's protocol at the annealing temperatures noted above. Fragments were analyzed on a Beckman-Coulter CEQ-8000 automated sequencer. Protein coding segments (*c-mos*, *cyt-b*, COI, ND1, ND2, and ND4) were edited and aligned by eye in the program Sequencher (Genecodes Corp.) after confirming an absence of stop codons suggesting a pseudogene. Intron fragments and 12S were aligned using the MUSCLE algorithm (Edgar 2004) implemented in the program Geneious (Biomatters Ltd.) using the default parameters.

Phylogenetic Inference

To test hypotheses regarding the timing of diversification within the Lamproletini, we inferred phylogenies using both Maximum Likelihood (ML) and Bayesian inference (BI) methods. For BI and ML analyses, we conducted mixed-, partitioned-model analyses on the concatenated data, partitioning by gene and by codon position (where applicable). The best fit evolutionary model for each gene was chosen by AIC in the program MrModeltest2 (Nylander 2004). These models were as follows: HKY (*c-mos*), HKY+ Γ (SPTBN1), HKY+ Γ +I (COI), GTR+ Γ (Vimentin Introns 4 and 5), and GTR+ Γ +I (12S, *cyt-b*, ND1, ND2, ND4). The appropriate model was then applied to each partition in the concatenated dataset. Both BI tree inference and divergence time estimation were performed using the program BEASTv1.4.8 (Drummond et al. 2006; Drummond & Rambaut, 2007). The analysis was run for fifteen million generations, with

the first 2.5 million discarded as burnin. Stationarity was assumed when the effective sample size (ESS) reached >200 for all parameters (as per Drummond et al. 2006), which occurred prior to 2.5 million generations. The analyses were repeated multiple times to ensure that the chains were independently converging and not merely sampling local optima. The ML analyses were performed using the program RAxMLv7.0.3 (Stamatakis 2006) under the same partitioning scheme, though RAxML only allows the use of the GTR model, which was enforced with the addition of Γ -distributed rate heterogeneity (GTRGAMMA). Support was assessed by performing one thousand non-parametric bootstrap (BS) replicates of the topology. To examine the potential impact of nuclear/mitochondrial gene tree incongruence (e.g., Sota & Vogler 2001), we also repeated these analyses on the individual mtDNA and scnDNA datasets separately.

Divergence Time Estimation

To test temporal and evolutionary hypotheses regarding species richness, we estimated divergence times using the relaxed clock method of Drummond et al. (2006), implemented in the program BEASTv1.4.8 (Drummond & Rambaut, 2007). The data were partitioned by gene and given the same models used in the tree inference. The conditions of the analyses are described identical to those of the phylogenetic inference. Rates and times were estimated under the uncorrelated lognormal tree prior, with a birth-death prior on speciation and Jeffrey's priors on the substitution model parameters. Five fossil constraints taken from the paleoherpetological literature which putatively represent extinct progenitors of modern taxa were placed on the tree. Using a lognormal distribution, we enforced fossil constraints in the most conservative manner possible,

interpreting the age of the fossil as the mean of a time horizon during which an internal bifurcation took place, with the 95% prior distribution representing soft bounds on the divergence time of that node (*sensu* Yang & Rannala 2006). Fossil information was taken from Holman (2000) as follows:

- (a) The root of the tree (*Gonyosoma oxycephalum*) was given a prior credible interval (PCI) of 17.8-57.6Ma, with a mean of 32Ma, based on the oldest known colubrine fossil *Texasophis galbreathi* from the Orellan of the Oligocene.
- (b) The MRCA of the Lampropeltini was given a mean date of 20.6Ma (PCI=11.4-37.1Ma) based on the oldest known putative lampropeltine, *Pseudocemophora cf. antiqua* from the late Arikareean of the Miocene.
- (c) The divergence between the rat snakes (*Pantherophis*) and the pine snakes (*Pituophis*) was given a mean date of 15.5Ma (PCI=9.5-25.3Ma) based on the oldest known rat snake, *Elaphe (Pantherophis) kansensis* from the early Barstovian of the Miocene.
- (d) The divergence between the genera *Lampropeltis* and *Cemophora* was given a mean date of 13.75Ma (PCI=8.4-24.4Ma) based on the oldest known kingsnake, *Lampropeltis similis*, from the medial Barstovian of the Miocene.
- (e) The divergence between the sister taxa *Lampropeltis getula* and *Stilosoma extenuatum* was given a mean date of 6.8Ma (PCI=4.75-9.94Ma) based on the oldest known fossils of *L. getula* and *Stilosoma vetustum* from the middle Hemphillian of the Miocene.

Results

Phylogenetic Analysis

The combination of sequences added in this study and the existing Genbank data resulted in a concatenated alignment with a length of 8294bp (3368 scnDNA, 4926 mtDNA) of data per species. The new sequences have been deposited on Genbank (accession numbers available as an online supplement to Pyron & Burbrink 2009a) and TreeBase (S2278, M4334). After minor trimming for quality, the lengths of the new nuclear intron alignments were as follows: SPTBN1, 1106bp; Vimentin Intron 4, 1064bp; and Vimentin Intron 5, 631bp. With some exceptions, the results of our phylogenetic analysis are broadly similar to those of Burbrink & Lawson (2007). Preliminary analyses supported recognition of two taxa of previously indeterminate status, the scarlet kingsnake (*Lampropeltis elapsoides*; Collins 1991) and the Baja California gopher snake (*Pituophis vertebralis*; Rodriguez-Robles & de Jesus-Escobar 2000; Stebbins 2003), which were included in the analyses as distinct terminals (Fig. 1). The Lampropeltini are found to be monophyletic, with the OW *Coronella austriaca* the sister to the lampropeltinines (Fig. 1). Most internal relationships are highly supported by both Bayesian posterior probability (>0.95) and bootstrap proportion (BS >0.70).

Though not fully resolved, the results from the nuclear genes alone are broadly concordant with the combined analyses (Fig. 1) and the mtDNA-only analysis. All the terminal species are differentiable based on the scnDNA analyses. While the mtDNA-only analysis yields weak support for paraphyly of the rat, corn, and fox snakes (*Pantherophis*, including *Mintonius*) with respect to the pine, bull, and gopher snakes (*Pituophis*), which is the result presented in Burbrink & Lawson (2007), both the combined mtDNA+scnDNA and separate scnDNA analyses provide strong support for

the reciprocal monophyly of those genera (Fig. 1). The scnDNA analyses yield well-supported sister relationships between *Pi. melanoleucus* and *Pi. ruthveni*, and between *Pi. catenifer* and *Pi. vertebralis*, a different result from the combined analyses.

Several phylogeographic lineages which had been elevated to species status using mtDNA alone are found here to be divergent based solely on nuclear evidence (i.e., *Pantherophis obsoletus* complex, *Pa. guttatus* complex, *Pituophis melanoleucus* complex; Burbrink et al. 2000; Burbrink 2002; Rodriguez-Robles & de Jesus-Escobar 2000). The scnDNA and mtDNA are also in concordance for other major features, such as the placement of *Senticolis* and corroboration of Bryson et al. (2007) in finding that *Stilosoma extenuatum* is sister to *Lampropeltis getula*. The scnDNA suggest a slightly different arrangement of the genus *Lampropeltis* (including a sister relationship between *L. calligaster* and *L. triangulum*, and between *L. alterna* and *L. mexicana*), though this was not well supported. In light of the potential polyphyly suggested by Bryson et al. (2007), further research may be necessary to fully resolve relationships in this genus.

Divergence Time Estimation

The Lampropeltini diverged from OW relatives (*Coronella*) approximately 24.4 (19.0-30.5) Ma, and share a NW ancestor approximately 22.9 (17.9-28.8) Ma (Fig. 2). These results are broadly consistent with those of Burbrink & Lawson (2007), supporting the hypothesis of a trans-Beringian dispersal into the NW during the late Oligocene or early Miocene, as this was the only viable route during that time period. The lampropeltinines appear to have diversified primarily during the Miocene, with divergences between extant species occurring primarily during the late Miocene and

Pliocene (Fig. 2). Unlike patterns reported for groups such as birds (Johnson & Cicero 2004), the majority of terminal speciation events in the Lampropeltini predate the Pleistocene. Additionally, the diversification of all genera occurred in the mid or early-Miocene (Fig. 2).

Discussion

Historical Origins and Diversification

Diversification of the modern genera and most species in the Lampropeltini occurred during the mid-Miocene and early Pliocene (Fig. 2). All diversification events at the generic level occurred in the early or mid- Miocene, and the majority of speciation events predate the Pleistocene (Fig. 2). Of the 31 currently described extant species, 9 originated during the Miocene, with error estimates from the 95% Highest Posterior Density (HPD) rejecting a younger Pliocene origin (Fig. 2). Thirteen extant species originated during the Pliocene, and an additional seven occur near the Miocene/Pliocene boundary, which included the Pliocene in the 95% HPD (Fig. 2). Two terminal species pairs, *Pantherophis emoryi/slowinskii* and *P. alleghaniensis/spiloides* cannot reject an early Pleistocene origin in the lower tail of the 95% HPD. Only *P.*

alleghaniensis/spiloides are estimated to have diverged after the Pliocene/Pleistocene boundary (1.7Ma; Fig. 2).

These dates are consistent with the fossil record, which indicates that many extant species were present by the late Miocene (reviewed in Holman 2000). As the majority of the lampropeltinine species occur in temperate NA (~25 species; Conant & Collins 1998; Stebbins 2003), this indicates that diversification in this group was not heavily influenced

by Pleistocene glacial cycles. Diversification in the group was coincident with the mid-Miocene climatic optimum (~15Ma), and continued during the formation of the Northern Hemisphere ice sheets (Zachos et al. 2001). Along with some divergence dating studies on birds, our results provides an additional piece of evidence suggesting that not all major radiations of vertebrate taxa are attributable to recent (i.e., Pleistocene) climatic cycles, at least in NA (e.g., Zink & Slowinski 1995; Klicka & Zink 1997). In contrast to some studies on organisms such as birds (e.g., Johnson & Cicero 2004), the Pleistocene ‘species pump’ does not appear to be responsible for diversification in the Lampropeltini.

In terms of the degree and direction of diversification with respect to morphological and ecological specialization, the greatest amount of diversity appears early in the history of the Lampropeltini. For instance, the splits between the smaller kingsnakes and milksnakes and the larger rat and pine occurred during the Miocene (~19Ma), while the more recent phylogeographic divergences exhibit less apparent ecological and morphological differentiation. In contrast, however, some relatively young sister species (e.g. *Lampropeltis getula* and *Stilosoma extenuatum*, ~6Ma) exhibit a great degree of morphological specialization (Conant & Collins 1998), and other species such as the milksnake (*Lampropeltis triangulum*) exhibit large amounts of ecological and morphological diversity within a putative single species (Williams 1978). Thus, the underlying relationships between the temporal aspects of phylogenetic diversification, morphological variation, and ecological specialization require further study.

Improved Phylogenetic Resolution

The use of multiple nuclear and mitochondrial genes and total sampling of the recognized species diversity has greatly improved resolution of the phylogeny of the lampropeltinines over previous efforts (Rodriguez-Robles & de Jesus-Escobar 1999; Burbrink & Lawson 2007). We find strong support for the monophyly of the rat snakes (*Pantherophis*) and the pine snakes (*Pituophis*), as well as for the placement of the enigmatic monotypic genera *Arizona*, *Cemophora*, *Rhinocheilus*, *Senticolis*, *Pseudelaphe* and *Stilosoma*. In addition, we find that many phylogeographic lineages which were elevated to species based on mtDNA (*Pi. melanoleucus*, *Pi. catenifer*, *Pi. ruthveni*; *Pa. obsoletus*, *Pa. alleghaniensis*, *Pa. spiloides*, *Pa. bairdi*; *Pa. guttatus*, *Pa. emoryi*, *Pa. slowinskii*) are also well differentiated by nuclear evidence alone, though we did not examine population variation to ensure reciprocal monophyly. This suggests that mtDNA markers are likely sufficient for tracking phylogeographic structure and identifying species in many cases (Zink & Barrowclough 2008). Despite this, we still recommend that researchers attempt to corroborate mtDNA phylogeographic estimates with nuclear markers. The other caveat implied by these findings is that species diversity in the Lampropeltini, especially potentially cryptic phylogeographic lineages, may be even greater than the currently recognized species which we have sampled here. In particular, the milksnake (*Lampropeltis triangulum*) ranges from Canada to Ecuador and consists of more than 20 subspecies (Williams 1978), suggesting a strong potential for further undescribed specific diversity.

Notes on Taxonomy

Based on the results of this study (Fig. 1), some discussion of taxonomic nomenclature is necessary to accurately reflect historical evolutionary relationships within the Lampropeltini, and to promote greater understanding of current diversity. With regard to the clade consisting of the rat and pine snakes and relatives, the taxonomic conclusions of Burbrink & Lawson (2007) and Collins & Taggart (2008) are shown to be inaccurate. The monophyletic genus *Pantherophis* (Fitzinger 1843) is restored for the fox, rat and corn snakes (*Pa. alleghaniensis*, *Pa. bairdi*, *Pa. emoryi*, *Pa. guttatus*, *Pa. obsoletus*, *Pa. spiloides*, and *Pa. slowinskii*), while the monophyletic *Pituophis* (Holbrook 1842) is preserved for the pine snakes (*Pi. melanoleucus*, *Pi. catenifer*, *Pi. deppei*, *Pi. lineaticollis*, *Pi. ruthveni*, *Pi. vertebralis*). The genus *Mintonius* (Collins & Taggart 2008) is now considered a junior synonym of *Pantherophis*. We have placed the genus *Stilosoma* (Brown 1890) into synonymy with *Lampropeltis* (Fitzinger 1843), rendering the emendation of the sole species from the erstwhile genus *L. extenuata* (short-tailed kingsnake). We find that *L. elapsoides* is well differentiated from *L. triangulum* (Collins 1991), and that *Pi. vertebralis* is distinct from *Pi. catenifer* (Rodriguez-Robles & de Jesus-Escobar 2000; Stebbins 2003). The continued recognition of the genera, *Arizona*, *Bogertophis*, *Cemophora*, *Pseudelaphe*, *Rhinocheilus* and *Senticolis* is warranted since they do not render any of the other genera within the Lampropeltini paraphyletic.

CHAPTER II: DISTRIBUTION

Can the tropical conservatism hypothesis explain temperate species richness patterns? An inverse latitudinal biodiversity gradient in the New World snake tribe

Lampropeltini

A latitudinal gradient of species richness, wherein the tropics contain more taxa than temperate areas, is one of the oldest recognized patterns in ecology and evolutionary biology (Wallace 1878; Brown & Lomolino 1998; Willig et al. 2003; Hillebrand 2004; Mittelbach et al. 2007). Numerous hypotheses have been proposed to explain these observations (Pianka 1966; Rohde 1992; Willig et al. 2003; Wiens & Donoghue 2004; Mittelbach et al. 2007), all of which ultimately attempt to address the three factors directly affecting biodiversity: speciation, extinction and dispersal (Ricklefs 1987). Most of these explanations can be classified into three broad, though potentially overlapping categories of ecological, temporal, and evolutionary hypotheses (Mittelbach et al. 2007).

Ecological explanations suggest that the tropics evolved and maintain greater species diversity due to environmental effects, such as higher environmental productivity, increased available niche breadth, larger geographic areas, or some combination thereof allowing more species to co-exist locally (review in Willig et al. 2003). Temporal explanations assume that earlier origination and longer duration of clades in the tropics allowed for the development of higher biodiversity through the steady accumulation of species over time, such as through the time-for-speciation/center-of-origin effects (TFS/COO; Ricklefs & Schluter 1993; Brown & Lomolino 1998; Stephens & Wiens 2003a; Wiens & Donoghue 2004; Stevens 2006). Evolutionary hypotheses suggest that higher rates of tropical speciation or temperate extinction are responsible for the diversity gradient (see Rohde 1992; Cardillo et al. 2005; Ricklefs 2006; Weir & Schluter 2007). Additionally, some explanations indicate that the latitudinal species richness gradient is merely a spatial artifact of the overlap of species' ranges within a given area, called the Mid-Domain Effect (MDE; Colwell et al. 2004).

Few of these hypotheses offer explanations which are specific to a situation where species diversity increases towards the equator. For instance, ecological patterns such as Rapoport's Rule (decreasing latitudinal extent of ranges at lower latitudes; Stevens 1989) may exist independently of biodiversity gradients. Temporal explanations for higher tropical species richness, which invoke the TFS/COO effects, should also predict higher temperate species richness for groups which originated in temperate zones (Stephens & Wiens 2003a; Smith et al. 2005). Evolutionary rate variation could also exist in groups with higher temperate diversity if the rate differences were insufficient to overcome TFS/COO effects, or the differences in rate were complementary (i.e. increased extinction in the temperate areas combined with decreased speciation in the tropics). Finally, spatial patterns such as the MDE would also predict higher species richness for any area at the center of a bounded domain, not only the tropics.

If any of these explanations account for both high tropical diversity as well as inverse latitudinal gradients caused by higher temperate species richness, this would suggest that these hypotheses are not restricted to explaining higher diversity in the tropics only. We test these methods to explain inverse species gradients in the widely distributed New World (NW) snake tribe Lampropeltini, the rat, king, and milk snakes and relatives. The lampropeltinines comprise at least 31 species, ranging from Canada to northern South America (SA; Williams 1978; Conant & Collins 1998). A previous study indicated that the lampropeltinines dispersed into the NW in the late Oligocene or early Miocene and shared a common ancestor approximately 25Ma (Burbrink & Lawson 2007). The group exhibits far higher species richness in temperate areas of North America (NA; ~27 species) than in the neotropics (NT; ~4 species). This is unusual for

ectotherms such as reptiles and amphibians, which tend to have their greatest diversity in the tropics (Ricklefs et al. 2007; Wiens 2007a), although similar patterns have been observed in temperate clades of turtles and frogs (Stephens & Wiens 2003a,b; Smith et al. 2005). Determining why these snakes exhibit higher temperate species richness will help provide a general explanation for distributions of biodiversity on a global scale.

Using a robust phylogenetic estimate for the tribe containing all extant species (Pyron & Burbrink 2009a; Ch. 1), we assess several temporal, evolutionary, ecological, and spatial explanations for the observed inverse latitudinal species richness gradient. First, we infer the ancestral area of the group to test for the presence of the TFS/COO effect (Ricklefs & Schluter 1993; Stephens & Wiens 2003a). Second, we determine if phylogenetic niche conservatism of relevant ecological traits (e.g., Ricklefs & Latham 1992) may have limited dispersal between temperate and tropical areas (e.g., Wiens & Donoghue 2004; Smith et al. 2005). Third, we test for latitudinal variation in diversification rates to assess potential evolutionary explanations for the observed gradient (e.g., Rohde 1992; Weir & Schluter 2007). Fourth, we determine if Rapoport's rule (Stevens 1989) exists in this group independently of high temperate species richness, suggesting that its presence in groups with high tropical diversity is merely correlative. Finally, we test whether the observed latitudinal gradient in species richness is merely a spatial artifact due to the MDE (Colwell et al. 2004), centered on temperate latitudes.

A temperate origin of the group would suggest that historical biogeography and phylogenetic niche conservatism is the primary determinant of species richness, indicating that long term presence in temperate areas and limited tropical dispersal allowed more time for speciation to increase biodiversity (e.g., Stevens 2006; Wiens et al.

2006, 2009). Alternatively, a tropical origin of the extant lampropeltinines would suggest that latitudinal variation in diversification rates (e.g. Cardillo et al. 2005; Ricklefs 2006; Weir & Schluter 2007) and/or large scale biogeographic dispersal was responsible for the observed biodiversity gradients. Rapoport's rule could be rejected as a hypothesis for higher tropical species richness if it is found to be significant in the lampropeltinine snakes, indicating that the pattern can arise independently of species richness patterns (e.g., Rohde 1996; Gaston et al. 1998). Presence of the MDE would suggest that the pattern of higher temperate species richness fits an artefactual null model (Colwell et al. 2004), and is not indicative of an underlying biological process. Additionally, combinations of these effects are possible. Our data indicate that historical biogeographic processes, rather than spatial effects or ecological or evolutionary rate variation, are responsible for the distribution of species richness in the lampropeltinines.

Methods & Materials

Phylogenetic Hypothesis

Our phylogenetic estimate of the lampropeltinines is based on a dataset comprising 8294bp of nuclear and mtDNA from nine genes (Pyron & Burbrink 2009a; Ch. 1). This phylogeny contains representatives of all 31 currently recognized lampropeltinine species, and consists of 6 mtDNA fragments (12S, *cyt-b*, COI, ND1, ND2, ND4) and four nuclear fragments from three loci (*c-mos*, *SPTBN1*, *Vimentin* Intron 4, and *Vimentin* Intron 5). We use the Maximum Likelihood (ML) estimate of the topology and branch lengths, which is well supported by non-parametric bootstrap proportion and Bayesian posterior probabilities, as well as the Bayesian divergence time

estimates for the internal nodes (Pyron & Burbrink 2009a; Ch. 1). We used this phylogenetic estimate and divergence times for the following analyses.

Ancestral Area Reconstruction

The ancestral area of the lampropeltinines was estimated using the maximum likelihood dispersal-extinction-cladogenesis model implemented in the program LAGRANGE2.0.1 (Ree et al. 2005; Ree & Smith 2008). The terminal ingroup species were coded as occurring in one or a combination of four primary NW biogeographic areas based on occurrence records (see below): the neotropics (NT; south of the Tropic of Cancer at 23.4 degrees, excepting the Mexican plateau in central Mexico south to the Trans-Mexican Volcanic Belt; see Schultz 2005), eastern NA (E; east of the Mississippi River), central NA (C; between the Mississippi River and the Western Continental Divide, south to the Tropic of Cancer) and western NA (W; from the continental divide to the Pacific ocean, south to the Tropic of Cancer). Species occurring on the Mexican plateau, which are likely to cross the continental divide, were coded as both C and W.

A number of species occur within short distances south of the Tropic of Cancer, but are not considered to be neotropical, occurring either on the Mexican plateau or in the Sierra Madre Occidental/Oriental mountains. A second analysis was performed in which all such taxa were coded as also occurring in the neotropics, to assure that bias in biogeographic coding did not affect the ancestral state reconstructions. A fifth state, the Old World (OW) was coded for the outgroup taxa, and the ancestral state of the tree was constrained to be OW. The topology used was the dated chronogram (Fig. 3), with branch lengths equal to absolute time (Ree & Smith 2008). Dispersal probabilities between areas

were unconstrained to avoid excessive parameterization; the geographic formation of the NW remained relatively constant over the time period of interest and dispersal between the areas was not given any directional weighting. The model was also not constrained by time for the same reason. Rates of dispersal and local extinction were assumed to be constant (Ree & Smith 2008).

Phylogenetic Niche Conservatism

To test for the presence of phylogenetic niche conservatism and infer the possible effects on dispersal, we gathered 4564 presence records comprising all 31 ingroup species and the Old World sister taxon *Coronella austriaca* (data available as an online supplement to Pyron & Burbrink 2009b; Fig. 4). Occurrences were obtained from museum records, primarily from the HerpNet database. Points with GPS data were used as is, all other records were georeferenced to the common locality provided. We extracted climatic data for these points from the WORLDCLIM dataset projected at 30 second spatial resolution, which comprises 19 variables describing averages and seasonal variation in temperature and precipitation, (Hijmans et al. 2005). We then performed principal components analysis (PCA) to isolate the factors accounting for primary variance in ecological niche (e.g., Wiens et al. 2006).

To test for significant phylogenetic niche conservatism and infer the ancestral niche of the lampropeltinines, we reconstructed the scores for the significant PC axes on the phylogeny using Phylogenetic Generalized Least Squares (PGLS; Martins & Hansen 1997) in the program COMPAREv4.6b (Martins 2004), using the branch lengths estimated from the ML analysis (Pyron & Burbrink 2009a; Ch. 1). We truncated the

phylogeny to only include *Coronella austriaca* as an outgroup, which has been reliably inferred to be the immediate outgroup to the lampropeltinines (Utiger et al. 2002; Burbrink & Lawson 2007). We then plotted the ancestral reconstructions against the PC scores of the extant species to test for broad scale conservatism of ecological preferences through time.

To test for phylogenetic conservatism of specific ecological traits limiting dispersal between the temperate and tropical areas, we identified the variables which had the highest positive and negative loadings on the PC axis of greatest separation, and interpret them to be the primary range limiting variables of the temperate and tropical species (*sensu* Smith et al. 2005; Wiens et al. 2006). We log-transformed the means of each variable for each species and tested for significant niche conservatism in these variables using the λ statistic (Pagel 1999) in the program BayesTraits (Pagel & Meade 2006). A λ of 0 indicates no phylogenetic structure, while a λ of 1 indicates perfect correlation with the phylogeny (but see Revell et al. [2008] for caveats). The presence of niche conservatism was calculated using the standard likelihood ratio test comparing a model in which λ is set from 0 to 1, where λ is allowed to take its ML value (Pagel & Meade 2006). All analyses were performed in the program BayesTraits (Pagel & Meade 2006).

Latitudinal Variation in Diversification Rates

To test for latitudinal differences in diversification rate, *sensu* Weir & Schluter (2007), we performed linear regression analyses for the 10 sister species pairs inferred in the primary phylogenetic analysis. We regressed the age of the divergence of each

species pair against the mean latitudinal midpoint of that pair. If higher speciation rates are responsible for increased biodiversity, then species pairs should be youngest where species richness is highest (a positive relationship). The signature of higher extinction rates would be a negative relationship, with younger species pairs where diversity is lowest (Weir & Schluter 2007). A nonsignificant relationship between latitudinal midpoint and species pair age would suggest that little or no major geographic variation exists in net diversification rate.

Rapoport's Rule

We tested for the presence of the Rapoport effect, decreasing latitudinal extent of ranges with decreasing latitude (Stevens 1989), using both the raw and the phylogenetically corrected latitudinal midpoints and extents of each species. We characterized the latitudinal midpoint and extent of all 31 extant species using the georeferenced locality records. The occurrence records were used to calculate the minimum and maximum latitude of each species. The latitudinal range is the difference between the min and max latitude, while the latitudinal midpoint is the mean of the two. We performed linear regression analyses with the raw data. We also used phylogenetically generalized least squares (PGLS; Martins & Hansen 1997) and Felsenstein's phylogenetically independent contrasts (F/PIC; Felsenstein 1985) to control for phylogenetic non-independence of the location of the species in the program COMPARE v4.6b (Martins 2004).

Species Distribution Patterns

We tested for a fit to the null MDE model (Colwell et al. 2004) using the program Mid-Domain Null (McCain 2004). To account for the possibility of domain specification bias, we ran three different analyses, one with the domain equal to the latitudinal extent of the Lampropeltini (-3 to 51 degrees north), one in which the domain was the Northern Hemisphere (0 to 90 degrees north) and one in which the domain range was the entire globe (-90 to 90 degrees north). Since the domain must have one bound be zero, we transformed the midpoints of each species by addition so that for any analysis, the lowest latitudinal value was zero (i.e., the domain -90 to 90 became 0 to 180). We sampled both the empirical ranges and the empirical midpoints without replacement, and ran 50000 replicates of each analysis. We tested for a significant departure from the expected values of species richness from the null model (the midpoint between the upper and lower 95% Confidence Intervals) at each latitudinal bin using a χ^2 test with degrees of freedom equal to one minus the number of degrees of latitude in the domain.

Results

Phylogenetic Analysis

The primary results of the DNA sequencing, phylogenetic analysis and divergence time estimation are given in Chapter 1. We reconstructed ancestral areas (see below) on the dated chronogram from Pyron & Burbrink (2009a; see Figs. 1, 2).

Ancestral Area Reconstruction

The ancestral area of the extant lampropeltinines is inferred to be in temperate central North America (Fig. 3), between the Western Continental Divide and the

Mississippi River. The earliest putative lampropeltinine fossils are known from this area (Wyoming) dating to the early Miocene (Holman 2000). The results from LAGRANGE suggest that the extant lampropeltinines have persisted in central NA for >20Ma.

Excepting the unobservable dispersal events of the terminal species *Senticolis triaspis* and *Pseudelaphe flavirufa*, the first dispersal into the neotropics at an internal bifurcation occurred approximately 6.6Ma in the late Miocene (*Pituophis lineaticollis+deppei*; Fig. 3). Dispersal into eastern and western NA was also recent (<10Ma). Thus, the TFS/COO effect appears to explain the high temperate species richness of the lampropeltinines due to the longer time span during which biodiversity could accumulate in temperate NA.

Phylogenetic Niche Conservatism

The first six PC axes account for 97.24% of the total variance (Fig. 4; only PC 1 & 2 are shown). The PCA scores reveal that the temperate and tropical species are separated into two primary centroids, with the greatest amount of differentiation occurring along PC 1 (Fig. 4). The two variables with the highest loadings on PC1 are BIO7 (Temperature Annual Range; 0.84) and BIO13 (Precipitation of the Wettest Period; -0.87) and this indicates that the primary ecological separation between the temperate and tropical species occurs along a gradient of rainy-season precipitation and temperature seasonality. Temperate species occupy drier, more seasonal habitats, whereas tropical species inhabit wetter areas with less annual temperature variation (Fig. 4).

Our phylogenetic reconstructions also indicate that the ancestral lampropeltinines inhabited a temperate niche. The OW sister taxon to the Lampropeltini, *Coronella austriaca*, inhabits a temperate niche which occurs well within the range of the NW

temperate species, and the PC reconstructions at all of the internal nodes are temperate as well (Fig. 4). However, while BIO7 exhibits significant phylogenetic signal ($\lambda=1.07$, $LR=172.35$, $p<0.001$), BIO13 does not ($\lambda\sim 0$, $p>0.05$). We extended this analysis to the next two variables which loaded most heavily on PC1, BIO16 (Precipitation of Wettest Quarter) and BIO4 (Temperature Seasonality). The same pattern was observed, where BIO16 does not exhibit significant phylogenetic signal ($\lambda\sim 0$, $p>0.05$), while BIO4 does ($\lambda=1.08$, $LR=242.49$, $p<0.001$). This indicates that temperature seasonality is the primary phylogenetically conserved limiting environmental factor, while lampropeltine species are more tolerant of variation in precipitation. Since there have only been 4 tropical invasions, all of which are single dispersals of terminal species and not diversified clades of taxa (Fig. 3), this strongly suggests that an evolutionarily conserved preference for a temperate ancestral niche has limited dispersal into the tropics.

Latitudinal Variation in Diversification Rate

No significant relationship was found between the age of sister species divergence and mean latitudinal midpoint ($r^2=0.0007$, $p=0.94$; Fig. 5). This indicates that there is no geographically influenced bias in the timing of currently observable speciation events, suggesting that species turnover rates are similar at varying latitudes and net diversification rates do not vary latitudinally (Weir & Schluter 2007).

Rapoport's Rule

Linear regression analysis does not reveal a significant relationship between the raw values of the latitudinal extent of each species and their latitudinal midpoint

($b=0.081$, $r^2=0.007$, $p=0.67$). The relationship is also not significant when the phylogenetic non-independence of the species is taken into account using both PGLS ($b=0.12$, $r^2=0.006$, $p=0.69$) and FIC ($b=0.22$, $r^2=0.01$, $p=0.55$). However, these relationships appear to be heavily influenced by two outliers, *Lampropeltis triangulum* and *Senticolis triaspis*, the only two species whose ranges span both temperate North America and the neotropics. The relationship between the raw values becomes significant with the removal of *L. triangulum* ($b=0.37$, $r^2=0.14$, $p=0.05$; Fig. 6) and of both species ($b=0.50$, $r^2=0.25$, $p=0.01$). Though no justification for the exclusion of *S. triaspis* is evident, the validity of *L. triangulum* as a single taxon or even a monophyletic species complex is in dispute (Bryson et al., 2007). The species is extremely polymorphic (~25 subspecies; Williams, 1978), and preliminary phylogeographic analyses suggests that it consists of multiple lineages which appear to conform to the pattern otherwise observed (Ruane et al. unpubl. data). To test the effect of removing *L. triangulum* as a potential outlier on the phylogenetic comparative analyses, we substituted the mean of the latitudinal extents and midpoints of *L. getula* and *L. extenuata*, the closest relatives of *L. triangulum* (Fig. 3). This yields a significant relationship for both PGLS ($b=0.41$, $r^2=0.13$, $p=0.05$) and FIC ($b=0.71$, $r^2=0.20$, $p=0.01$). Thus, we tentatively conclude the presence of the Rapoport effect, which, despite ostensibly allowing higher species density at lower latitudes, seems to have no direct relationship to the distribution of species richness.

Species Distributions

Results from Mid-Domain Null for all three domains exhibited similar patterns; only the data from the empirical domain (-3 to 51 degrees North) are shown (Fig. 7). The observed number of species across the domain was significantly different than predicted by the null MDE model using the empirical ranges of the species sampled without replacement ($\chi^2=216.1$, $p<0.01$, $df=53$). Species richness was higher than predicted at latitudes above 23 degrees north, and lower than predicted below 23 degrees north (Fig. 7). Rejection of the MDE null model indicates that the observed species richness pattern is not explained by a random distribution of species through geographic space, centered on a temperate latitude.

Discussion

Though the vast majority of species richness gradients exhibit decreasing diversity with increasing latitude (Hillebrand 2004), this is not always the case. In addition to the Lampropeltini, organisms such as pond turtles (Stephens & Wiens 2003a,b), Holarctic tree frogs (Smith et al. 2005), penguins (Cody 1966), and ichneumonid parasites (Owen & Owen 1974) also exhibit extratropical peaks in diversity. It has been suggested that the observed commonality of latitudinal gradients in species richness is likely to have a common cause (Pianka 1966; Rohde 1992). As we have noted, many explanations for biodiversity gradients do not exclusively mandate high tropical species richness. Furthermore, not all of the proposed explanations are necessarily causative (Rohde 1992; Pianka 1966). As such, a generalized explanation for latitudinal biodiversity gradients does not necessarily hinge on gradients specific to higher tropical diversity. A common mechanism that describes species richness gradients, regardless of

whether the center of diversity is tropical or temperate, would provide a far more robust explanation for observed patterns of global species richness than those restricted to explaining high tropical diversity.

Historical biogeography, as exemplified by TFS/COO effect, best explains the pattern of high temperate species richness in the Lampropeltini. The latitudinal distribution of taxa indicates significantly higher species richness at temperate latitudes than would be expected under the null MDE model, indicating that the diversity in the temperate areas is due to non-random processes. No differences were detected in latitudinal diversification rate, suggesting that variation in extinction or speciation rates between temperate and tropical areas has not contributed to the observed patterns. The group originated in temperate NA in the Miocene, leaving >20Ma for speciation to increase biodiversity in the temperate areas and only dispersed to the tropics relatively recently. Therefore, there has not been enough time for diversity to accumulate from these young tropical lineages (<6Ma). This is similar to the more common reverse patterns observed in many groups that exhibit high tropical species richness, where early origin occurred in the tropics with only recent dispersal to temperate regions explains higher tropical diversity (Stevens 2006; Wiens et al. 2006, 2009).

The question then becomes: why was tropical dispersal so infrequent? The tropics exhibited a far greater latitudinal extent in the past, but began to recede no later than 30Ma (Behrensmeyer et al. 1992; Morley 2000). The lampropeltinines dispersed into temperate North America across Beringia (Burbrink & Lawson 2007) during or after the expansion of the temperate areas to their current extent, and presumably existed in close proximity to the tropics for most of their history. Yet, dispersal into the tropics occurred

only four times, and only in terminal taxa and not diverse tropical clades. The most likely explanation for a lack of tropical dispersals is phylogenetic niche conservatism (Wiens & Graham 2005), predicting a strong preference for a temperate ancestral niche. Several authors have cautioned against direct inference of evolutionary patterns or niche conservatism based solely on the presence of phylogenetic signal (Revell et al. 2008). However, the OW sister groups of the Lampropeltini all inhabit temperate areas in the Palearctic, a condition which is inferred to have existed for at least 30Ma (Burbrink & Lawson 2007).

The reconstruction of ancestral niche indicates that the Lampropeltini have existed in temperate environmental conditions since their origin (Figs. 3, 4). Thus, an evolutionary preference for temperate environments appears to have led to climatic constraints on dispersal into the neotropics, based primarily on temperature seasonality gradients. This also explains the large number of present day lampropeltinine species which abut but do not enter the neotropics in Mexico (Figs. 3, 6, 7). Some authors have also suggested the importance of interspecific competition in limiting species ranges (e.g. MacArthur 1972; Case & Taper 2000). Given both the strong indications of phylogenetic niche conservatism (Fig. 4) and the extensive penetration and coexistence of tropical lampropeltinine species into Central and South America (Figs. 3, 6, 7), this is may only be a secondary factor.

Remarkably, the primary explanations we find for the observed patterns of high temperate species richness in the lampropeltinines are the latitudinal inverse of the predictions made by the Tropical Conservatism Hypothesis (TCH; Wiens & Donoghue 2004) for groups exhibiting high tropical species richness. The TCH makes three

predictions. First, groups with high tropical diversity will have originated in the tropics and dispersed to temperate regions only recently, wherein the TFS/COO effect accounts for increased tropical diversity. Second, temperate dispersals will be limited due to phylogenetic conservatism of the tropical ancestral niche. Third, more clades will have arisen in the tropics due to their large former extent. Here, we find that inverse of the TCH predicts that groups with high temperate species richness originated in temperate areas and dispersed to the tropics only recently, that a preference for the ancestral temperate niche likely limited tropical dispersal, and that dispersal into and diversification in the NW was related to the expansion of the temperate areas to their current extent. Thus, it would appear that the underlying processes which the TCH proposes are the primary factors responsible for high tropical species richness are not intrinsic to the tropics. Rather than dub this the ‘Temperate Conservatism Hypothesis,’ we propose that these processes be generally referred to as the ‘Biogeographical Conservatism Hypothesis.’

Identical underlying patterns have now been shown to be responsible for several latitudinal biodiversity gradients regardless of their area of origin: the time-for-speciation effect acting in the geographic area of origin (e.g., Ricklefs & Schluter 1993), a phylogenetically conserved preference for an ancestral niche (e.g., Smith et al. 2005; Wiens et al. 2006), and the geographic availability of suitable habitat (e.g., Fine & Ree 2006). These processes can explain both high temperate and tropical diversity without reference to the MDE, as well as in the presence of potential red herrings such as Rapoport’s rule. We suggest that these factors may underlie similar gradients of both temperate and tropical latitudinal origin in numerous other taxa, and should form a

starting point for future investigations of latitudinal species richness gradients. It should be noted that there is still no firm explanation as to why instances of high tropical species richness vastly outnumber those of high temperate diversity. The TCH suggests that more groups likely originated in the tropics during the occupancy of their larger former extent (Wiens & Donoghue 2004). Alternatively, some research has indicated that at deeper phylogenetic time scales, there may indeed be higher rates of tropical diversification, leading to more clades of tropical origin (Wiens 2007a). Explanations for global biodiversity should take into account the possibility that both the BCH as well as evolutionary rate differences explain different aspects of species richness at different phylogenetic time scales (Mittelbach et al. 2007; Wiens 2007a).

CHAPTER III: DIVERSIFICATION

Body size as a primary determinant of ecomorphological diversification and the evolution of mimicry in the lampropeltine snakes (Serpentes: Colubridae)

The underlying causes of phylogenetic correlations between traits are a central question in systematics and evolutionary biology (Losos 1996). While the evolution of coadaptive suites of functionally related traits is well known (e.g., Huey & Pianka 1981; Brodie 1992; Cooper 2007), morphological integration of functionally unrelated traits may also occur through shared correlations with other characters of primary selective importance. For instance, studies have indicated that evolutionary correlations may exist between seemingly unrelated traits, such as vocalization and dietary preference in Galapagos finches, where both are tied to the underlying morphological variable of beak shape (Podos 2001). Perhaps most fundamentally, body size is seen to be related to numerous aspects of ecomorphological diversification and morphological adaptation in organisms such as the squamates *Anolis* and three-spine sticklebacks (Losos et al. 1994; Nagel & Schluter 1998). While numerous studies have investigated the evolutionary roots of body size diversification (e.g., Moen & Wiens 2009) and size-independent relationships among other traits (e.g., Bickel & Losos 2002; Bergmann et al. 2009), relatively few studies have directly investigated the effects of body size evolution on the diversification of traits in a quantitative context (see Losos 1994, 1996).

As body size is one of the most fundamentally important aspects of organismal morphology (e.g., LaBarbera 1989; Roy 2008), the evolution of size may play a larger role in the development of phenotypic diversity that is not directly related to body mass (e.g., color pattern, etc.) than is commonly discussed. For such traits, constraints on body size evolution may be ultimately responsible for both the potential diversity and observed differentiation of those traits. Thus, size may represent a single shared axis of diversification for traits which are correlated with gross morphology.

Such characters would thus share directional changes in and limitations on their diversification – not by being functionally related to each other, but rather by being correlated with and governed by body size. Therefore, potential adaptations and subsequent diversification in these traits may ultimately be controlled by body size evolution. Thus, evolutionary correlations between unrelated traits may be explained by shared relationships of those traits with body size (e.g., Bickel & Losos 2002). These patterns could be dismissed as simple body size scaling (e.g., LaBarbera 1989), suggesting that observed correlations between functionally unrelated characters do not represent meaningful evolutionary patterns. However, this may overlook the importance of body size in the evolutionary development of traits which are strongly related to size (e.g., Kozak et al. 2009; Goodman et al. 2009).

A thorough investigation of evolutionary relationships between multiple morphological and life history traits may be most easily accomplished by studying organisms with reduced morphologies such as snakes, in which potentially confounding morphological variables such as limb elements, dermal armor, and sexual ornamentations are absent (Vitt & Caldwell 2009). The extreme reduction in body form in snakes leaves length (typically snout-vent length, SVL) as the primary determinant of adult body size, which varies by nearly two orders of magnitude (~10 cm to ~10 m) in extant species, with a tendency towards moderate (~1.0m) sizes (Boback & Guyer 2003). One character most limited by body size is gape, the cross-sectional area of the open mouth (King 2002). This affects predatory efficiency and thus may define dietary preference and limit prey size (King 2002; Rodríguez-Robles et al. 1999b). Other characters putatively related to body size include anti-predator traits, particularly Batesian mimetic color patterns, as

mimics should match the model in color pattern as well as body size (review in Greene & McDiarmid 1981). Thus, for snakes, body size may be a determining factor with respect to both obviously size-linked characters, such as predatory specialization and diet preference, and characters less clearly related to size, such as color pattern.

A prime candidate group for such an analysis is the New World (NW) tribe Lampropeltini. The lampropeltinines comprise approximately 31 species, which shared a New World (NW) common ancestor in the mid-Miocene (~23Ma; Burbrink & Lawson 2007; Pyron & Burbrink 2009a; Ch. 1). They exhibit an extremely diverse array of ecomorphological forms and body size, and range from Canada to Central and South America (CA, SA; Conant & Collins 1998; Pyron & Burbrink 2009b; Stebbins 2003; Williams 1978; Ch. 2). Diet compositions range from entirely ectothermic prey (*L. extenuata*), to entirely endothermic ones (*Pituophis* and *Pantherophis* [part]; Table 1). Color patterns in the group include classic cases of Batesian mimicry of venomous rattle- and coral snakes, as well as drab cryptic patterns (Greene & McDiarmid 1981; Garstka 1982; Conant & Collins 1998; Stebbins 2003).

Excepting obviously coadaptive or functionally related suites of traits, there are three questions which can be asked about morphologically diverse groups such as the lampropeltinines with respect to the evolutionary history of trait diversification. First, do evolutionary relationships exist between seemingly unrelated traits, suggestive of an underlying axis of correlated diversification? Second, is this attributable to a key morphological variable such as body size (e.g., LaBarbera 1989; Brown et al. 1993; Martin & Palumbi 1993)? Finally, was trait diversification related to abiotic (e.g.,

climatic or environmental; Smith et al. 1995; Brakefield & French 1999), or biotic (e.g., interspecific interactions; Losos 1994; Robertson 1998; Moen & Wiens 2009) factors?

We tested for phylogenetic correlations in putatively unrelated suites of traits related to predatory specialization (e.g., gape size and diet preference) and adaptations for predator avoidance (e.g., Batesian mimetic color patterns) in the Lampropeltini. With respect to the first question, a lack of a significant relationship would indicate the absence of correlated diversification, suggestive of unconstrained diversification across multiple axes of character space. Regarding the second question, we test if any significant relationships can be explained by shared correlations with body size, indicating an underlying axis of diversification in those traits, governed by body size. For the third question, we tested whether current climatic or environmental factors exhibit any relationship with ecomorphological diversity in the lampropeltinines.

Since the geographic distribution of species richness and lineage formation in the group has been shown to be heavily influenced by climate (Pyron & Burbrink 2009b,c; Ch. 2, 4), we investigated the possible influence of these ecological factors on phenotypic diversity in the group. If individual traits are not correlated with each other, variation might instead be related to geographic (e.g. latitude) or climatic factors. However, if correlations between traits exist due to shared relationships with body size, then variation in overall diversity may be explained through a relationship between ecological factors and body size, a common pattern in many organisms including snakes (e.g., Smith et al. 1995; Ashton & Feldman 2003). A lack of ecological correlates of trait diversity may indicate that diversification was potentially driven by interspecific interactions, such as competition driving niche differentiation (e.g., Moen & Wiens 2009). Pyron & Burbrink

(2009a,b; Ch. 1, 2) presented a robust, multi-gene phylogenetic estimate of the Lampropeltini which sampled representatives of all currently described extant species, along with divergence time estimates and ancestral area reconstructions for the group which we use to test these hypotheses.

Methods & Materials

Phylogeny and Divergence Time Estimates

All subsequent analyses are based on the phylogenetic estimate and associated divergence times presented by Pyron & Burbrink (2009a; Ch. 1). The 31 commonly recognized extant species were included as terminal taxa. The analyses were based on a dataset consisting of six mitochondrial genes (12-S, COI, *cyt-b*, ND1, ND2, ND4) and four fragments of three nuclear genes (*c-mos*, Vimentin Introns 4 & 5, SPTBN1), totaling 8294bp of sequence data per species. Here, we use the topology and branch lengths from the Maximum Likelihood (ML) analysis performed in the program RaxMLv7.0.3 (Stamatakis, 2006), and the Bayesian divergence time estimates from the program BEASTv1.4.8 (Drummond & Rambaut 2007). In the phylogenetic analyses of Pyron & Burbrink (2009a; Ch. 1), the nominal subspecies *L. t. triangulum* was used as the terminal taxon to represent the complex. For these analyses, the morphological measurements, dietary compositions, and color pattern information are for *L. t. triangulum* only. A more detailed description of these methods is given by Pyron & Burbrink (2009a; Ch. 1).

Ancestral Areas and Ecological Data

We used the occurrence data, climatic data, and ML reconstructions of ancestral areas for internal nodes on the phylogeny presented by Pyron & Burbrink (2009b; Ch. 2). Ancestral area reconstructions were performed in the program LAGRANGE2.0.1 (Ree & Smith 2008). The presence dataset consists of 4564 occurrence records for all 31 species and the Old World (OW) outgroup taxon *Coronella austriaca*, which were used to calculate latitudinal midpoints, ranges, and extents for all species. For the presence localities, climatic data was extracted from the 19 BIOCLIM variables in the WorldClim dataset (Hijmans et al. 2005), representing averages, ranges, and extremes in yearly, quarterly, and monthly temperature and precipitation projected at 30s spatial resolution. Ecological niche estimates were calculated using Principal Components Analysis (PCA) to extract the primary axes of climatic variability from the 19 BIOCLIM variables. Here, we used the first PC axis, which accounts for the differences in niche between temperate and tropical lampropeltinine species (Pyron & Burbrink 2009b; Ch. 2).

Morphological and Color Pattern Data

To test hypotheses regarding morphological variation, we measured four primary variables related to adaptations for feeding morphology (body size [SVL], head width [HW], jaw length [JL] and head height [HH]) for 728 specimens, comprising 29 of the 31 extant species of lampropeltinine species and *C. austriaca*. For the species *Lampropeltis ruthveni* and *Pituophis vertebralis*, we were unable to directly measure any specimens for the cranial morphology characters, but SVL measurements for both species were obtained from published works (Blanchard [1921] and Stull [1940], respectively), for a total of 757 specimens (Table 1; data available as an online supplement to Pyron & Burbrink

2009d). We corrected for allometry by regressing HH/SVL against SVL, and excluding individuals whose ratio occurred prior to the asymptote (Burbrink, 2001). We extracted the primary axes of gross morphological variation PCA, from the measurements of SVL, HW, JL, and HH.

We calculated gape, the area of the open jaws with jaw length and head width as the major and minor axes, using the formula $(\pi * JL * HW) / 4$ after King (2002). For *L. ruthveni* and *P. vertebralis*, we calculated the predicted mean value of gape for both species based on mean SVL, using the exponential function describing the curve which relates gape to SVL in the allometrically corrected dataset. All morphological variables were log transformed (ln) prior to analyses. Batesian mimicry of both coral snakes (Greene & McDiarmid 1981) and rattlesnakes (Garstka 1982) is observed in the lampropeltinines (Table 1). For the discrete phylogenetic correlation analyses of the evolution of Batesian mimicry, we coded the presence of coral and rattle snake mimicry as binary character (0=non-mimic, 1=mimic) based on both the presence of the tricolored mimetic pattern class and published accounts or hypotheses of putative mimicry for the species (Table 1). As we are concerned with the potential expression of these patterns, polymorphic (i.e., species with potentially mimetic and non-mimetic individuals) were coded as mimics (1).

Dietary Composition

For all 31 species and *C. austriaca*, we recorded diet preference as a continuous character, average proportion of ectothermic prey ranging from 0 to 1, based on published reports (Table 1). For species which represent recently elevated

phylogeographic lineages (e.g. *Pa. guttatus*, *emoryi*, and *slowinskii*, and *Pa. obsoletus*, *alleghaniensis*, *spiloides*, and *bairdi*), diet compositions for the original species were generalized across the lineages. Two poorly known Mexican species, *L. ruthveni* and *L. mexicana*, yielded only scattered reports of dietary records of ectothermic prey; analyses were run with both species given a null value of 0.5, and 0.75, representing the known proportion of ectothermic prey items. Results were similar for both analyses, and the latter are reported. Diet proportion was arcsine transformed prior to analyses.

Phylogenetic Comparative Analyses

We first reconstructed ancestral states for SVL, diet preference, mimicry, and the PC axes explaining a significant portion of morphological variation to examine how the morphological and life history traits varied through time. We then tested for significant associations between traits related to morphology (SVL, gape), predatory specialization (diet preference), and predator avoidance (mimicry) to assess evolutionary relationships between traits and potential relationships with underlying explanatory variables such as body size. All analyses were performed using both non-phylogenetic methods (linear and logistic regression of raw data; TIPS) and phylogenetic comparative methods (PCM) to assess potential differences between atemporal and evolutionary relationships between traits.

PCM analyses were conducted using PGLS (Martins & Hansen 1997) and Felsenstein's Independent Contrasts (FIC; Felsenstein 1985) implemented in the program COMPAREv4.6b (PGLS-relationships & PGLS-ancestor models; Martins 2004). In almost all cases, results from the three methods were highly similar (see Ord & Martins

2006); thus, we report only the PGLS results, unless disagreement exists between the methods. Ancestral states with respect to mimicry were inferred using the DISCRETE model (Pagel 1994) implemented in Mesquite v.2.5 (Maddison & Maddison 2008). We used the ML topology and both the ML branch lengths and the chronogram branch lengths from Pyron & Burbrink (2009a; Ch. 1), truncated to include only *C. austriaca*. Both yielded similar results; as we are interested in trait evolution in an explicitly temporal context, we report the results using the chronogram branch lengths.

For all regression analyses which involved multiple comparisons, we assessed significance using a Bonferroni correction of $\alpha_{0.05/n}$. We first estimated the size-dependent component of gape by regressing gape against SVL. We then tested for a size-independent relationship between gape and diet and mimicry by taking the standard residuals of gape regressed against SVL, and regressing those against dietary preference (% ectothermy) and mimicry (logistic regression of presence or absence). Significance for the latter two analyses was assessed using a Bonferroni-corrected $\alpha_{0.05/2}=0.025$. We then tested whether significant phylogenetic relationships exist between the putatively unrelated traits of dietary preference and mimicry. Finally, we examined whether or not body size exhibited a significant relationship with dietary preference and the presence of mimicry, as predicted. Significance for the latter two analyses was again assessed using a Bonferroni-corrected level of $\alpha_{0.05/2}=0.025$ to account for the multiple comparisons.

To test for a significant relationship between geography and the development and diversification of the morphological variables, we regressed the ecomorphological traits against the latitudinal midpoint of each species, calculated from the occurrence records from Pyron & Burbrink (2009b; Ch. 2). For analysis of climatic factors, we used the first

PC axis derived from the 19 BIOCLIM variables, which encompasses the primary climatic factors shown to have influenced current and historical distributions in the lampropeltinines (Pyron & Burbrink, 2009b; Ch. 2). We then regressed the significant traits against each of the ecological and geographic variables. Analyses were performed using both non-phylogenetic (linear and logistic regression) and PCM analyses (PGLS and FIC), with Bonferroni corrections for multiple comparisons.

Results

Evolutionary History of Diversity

As per Pyron & Burbrink (2009a,b; Ch. 1, 2), the NW MRCA of the lampropeltinines originated in the early Miocene (~23 Ma), and occupied a temperate niche in Central NA. The PGLS reconstructions of gape, SVL, and diet composition indicate that this ancestral lineage resembled the OW outgroup species *C. austriaca*, with an SVL of approximately 72 cm, and endotherms comprising a slight majority of the diet (Fig. 8). The DISCRETE reconstructions of ancestral states with respect to the presence of mimicry using the Mk1 model selected by Mesquite, indicate that the early lampropeltinine lineages did not exhibit mimetic color patterns (Fig. 8). However, a portion of known diversity in the *L. triangulum* group was excluded, including potential mimics in CA and SA, due to a lack of information of the phylogeography of the species. Preliminary results have indicated that the species is not monophyletic (see Bryson et al. 2007; Pyron & Burbrink 2009a; Ch. 1); thus, we included only the nominate subspecies.

In the early Miocene (~18 Ma), the lampropeltinines diversified into three major clades (Fig. 8). Two clades (A and C; Fig. 8), the rat and pine snakes (*Bogertophis*,

Pituophis, *Pantherophis*, and *Pseudelaphe*), developed large adult body sizes (>100 cm); one specimen of the Bull snake (*Pi. catenifer*) was the largest snake ever observed in NA at 267 cm (Devitt et al. 2007). Both clades exhibit primarily endothermic diets. The third clade (B), comprised primarily of the king snakes (*Lampropeltis*) and relatives, evolved smaller adult body sizes and primarily ectothermic diets (Fig. 8). In the latter clade, the reconstructions of mimicry suggest that species may have exhibited putatively mimetic color patterns as early as 16 Ma, with the first unambiguous reconstruction of a mimetic color pattern occurring ~11.5 Ma (Fig. 8). The lineage leading to *Senticolis* appears to have undergone relatively little change (Fig. 8). The PCA scores revealed that >95% of all variation in gross morphology occurs along the first axis (PC1), along which all four variables (SVL, HW, JL, and HH) load strongly negatively (<-0.95). Plotted against time, the Miocene differentiation in gross morphology between clade B and clades A and C is clearly visible, while *Senticolis* exhibits little change from the centroid (Fig. 9).

Phylogenetic Correlation of Life History Traits

Analyses indicate that gape is strongly related to adult body size ($r=0.96$, $p=1.8 \times 10^{-19}$), suggesting that while snakes are gape limited predators (King 2002; Rodríguez-Robles et al. 1999b), this limitation may be ultimately due to the underlying geometric relationship between gape and body size. There is no significant relationship between the standard residuals of gape against SVL and either diet composition ($r=0.14$, $p=0.18$) or the presence of mimicry ($r=0.36$, $p=0.08$), indicating that there is no body size-independent relationship between gape, and either dietary preference or mimetic color pattern. Dietary composition (% ectothermy) shares a strong positive relationship

with the presence of mimicry ($r=0.56$, $p=0.009$), suggesting that mimics are more likely to consume ectothermic prey. A significant relationship between these putatively unrelated traits suggests that both may be dependent on an underlying variable, such as body size.

Adult body size (SVL) exhibits a strong negative relationship with dietary preference (% ectothermy; $r=-0.84$, $p=3.7 \times 10^{-10}$). Larger snakes show an increasing preference for endothermic prey, while smaller snakes exhibit primarily ectothermic diets. This is likely due to the increase in maximum possible prey volume for larger snakes, which are known to drop smaller prey items from their diets (King 2002; Rodríguez-Robles et al. 1999b). As with dietary preference, the presence of mimicry exhibits a significant negative relationship with SVL ($r=-0.44$, $p=0.015$). However, while the TIPS and PGLS results are significant at a Bonferroni-corrected level of $\alpha_{0.05/2}=0.025$, the FIC results are not ($r=-0.35$, $p=0.054$). This appears to be due to the presence of the outlier *Lampropeltis extenuata*, a very small non-mimic (Table 1). Removing the effect of *L. extenuata* by assigning it the same SVL value as its sister taxon *L. getula* (Fig. 8; Table 1) yields highly significant results for both PGLS ($r=-0.68$, $p=3.3 \times 10^{-5}$) and FIC ($r=0.59$, $p=0.0006$). This suggests that body size is related to mimicry within a certain range of SVL values; that it is possible to be both too large as well as too small to be an effective mimic, likely based on model size (e.g., Greene & McDiarmid 1981). These results suggest that adult body size is the primary determinant of the presence, and likely the effectiveness of the display of mimetic color patterns (see Greene & McDiarmid 1981), as well as dietary composition (King 2002; Rodríguez-Robles et al. 1999b).

Ecological Correlates of Diversity

Our analyses of the influence of climatic and geographic factors affecting evolution and diversity suggest that there is little impact of ecological variables on morphological diversification in the lampropeltinines. We found no significant relationship between the latitudinal midpoint of the species and SVL ($r=-0.07$, $p=0.99$), diet ($r=0.27$, $p=0.33$), or mimicry ($r=0.07$, $p=0.99$). The same pattern holds for the measurements of ecological niche, where the PC1 axis exhibits no significant relationship with SVL ($r=0.04$, $p=0.99$), dietary preference ($r=-0.17$, $p=0.33$), or mimicry ($r=0.11$, $p=0.62$). This indicates that the evolution of body size, diet preference, and the presence of mimetic color patterns are not strongly influenced by the primary ecological conditions experienced by the organisms, or by the geographic location of the species. Though we did not specifically test for the latter, the lack of climatic or geographic correlates of diversity suggests a role for interspecific interactions driving the evolution of these traits, a fundamental tenet of theories of competitive niche differentiation (see Losos, 2000).

Ecomorphological Diversification and Color Pattern Evolution

The primary axis of diversification in the lampropeltinines thus appears to be defined by adult body size. Plotted against diet preference, this reveals the underlying axis of diversification in the Lampropeltini (Fig. 10). The hypothesized presence of extrinsic biotic constraints on the evolution of mimicry (e.g. the presence of a model species, and body size concordance between the mimic and the model; Greene & McDiarmid 1981; Pfennig et al. 2001), along with the correlation between diet and body size, reveals the presence of a 'key zone' for mimicry (Fig. 10). Putative mimicry has

developed or persisted only in snakes within a certain size range (~40-70 cm) and exhibiting a primarily ectothermic diet. For comparison, adult sizes of the most common model species in NA are similar for both coral snakes; with *Micruroides euryxanthus* rarely larger than 50 cm, and *Micrurus fulvius* rarely larger than 80 cm; and rattlesnakes, with *Crotalus lepidus* rarely larger than 80 cm (Campbell & Lamar 2004).

Thus, *L. extenuata* may be too small to be an effective mimic of the larger coral snakes, while *L. t. triangulum* exists well outside the range of any venomous coral snakes and has a primarily endothermic diet. Information on this optimal range for mimicry may be used to elucidate the phylogenetic origin of these color patterns in the lampropeltinines. We determined whether or not the reconstructed body sizes and diet proportions occur in the key zone for mimicry, and hypothesize that tricolored patterns first evolved at the node subtending the genera *Cemophora* and *Lampropeltis*, despite the ambiguous reconstruction of mimicry at that node from the DISCRETE analysis. This would indicate that mimicry first evolved in the lampropeltinines during the mid Miocene, approximately 13.7 Ma (10.6-17.0 Ma).

Discussion

Ecomorphological Diversification

There are no large lampropeltinine snakes displaying the classic coral snake mimetic color pattern or an ectothermic diet. In contrast, there are no small lampropeltinines with primarily endothermic diets. Obvious biological explanations for these patterns are not easily rendered without considering how these characters are related phylogenetically and correlated with body size. The relationships among the traits

suggests a single underlying axis of radiation; that unconstrained diversification of these characters is not possible for these snakes. The major axis of diversification appears to be defined by adult body size (SVL), along which we observe the correlated diversification of gape, dietary composition, and the development of mimetic color patterns within a specific range of small body sizes and ectothermic diets (Fig. 10). We find a pattern of correlated trait evolution which is in turn explained by shared relationships of individual traits with body size. In most biological contexts, traits are size-corrected in order to produce size independent measurements for analysis. Here, size-dependent relationships between traits appear to be the primary determinant of ecomorphological diversification in the lampropeltinines.

Other researchers have found similar patterns (e.g. Bickel & Losos 2002; Pleguezuelos et al. 2007) of correlations between functionally related traits, but not between functionally unrelated traits when the effect of body size is removed. However, interpreting this as only revealing a null pattern attributable to body size scaling may overlook important aspects of evolutionary radiation related to the primary determinants of ecomorphological diversification (e.g., Schluter 2000; Stephens & Wiens 2009; Kozak et al. 2009). If traits such as predatory specialization, predator avoidance strategies, or morphological adaptations related to locomotor performance and/or habitat utilization are tied to single underlying variables such as body size (e.g., LaBarbera 1989), then diversification into available habitats and expansion into new niches will be dictated by any external constraints on body size (e.g., Glor et al. 2003), whether abiotic (e.g., Smith et al. 1995) or biotic (e.g., Moen & Wiens 2009). Thus, size-dependent interactions between traits may reveal as many important evolutionary patterns regarding adaptive

differentiation as size-independent relationships (e.g., Bickel & Losos 2002; Ord & Martins 2006; Goodman et al. 2009).

Diversification in adult body size is inferred to have proceeded from an intermediate body size, bi-directionally towards both extremes in three different clades (Figs. 8-10), a primary prediction for the effect of historical competition on body size evolution (e.g., Taper & Case 1985; Losos 1994; Moen & Wiens 2009). Thus, ecomorphological diversity in the lampropeltinines may ultimately stem from interspecific interactions, due to the dependence of traits such as diet and color pattern on body size. However, evolution in those traits may have been driven by interspecific interactions affecting body size evolution, rather than direct selection on the individual traits stemming from competition. While competitive differentiation along a body-size continuum is a well known and potentially widespread phenomenon (see Moen & Wiens 2009; Kozak et al. 2009), the effect that this pattern may have on overall ecomorphological differentiation in multiple unrelated traits may have been overlooked.

In the lampropeltinines, the lack of any ecological correlates of diversity for adult body size, gape, diet or color pattern suggests that adaptive differentiation was governed by interspecific interactions, such as competition. While the present-day climate is not an absolute facsimile of past environments, the significant effect that climate has been inferred to have had on species diversification in the lampropeltinines (see Pyron & Burbrink 2009b; Ch. 2) suggests that estimating past effects using current variables is not unreasonable. Thus, the lack of any significant relationship, particularly with geography (which most strongly affects species richness in the group), indicates that ecomorphological diversification may have been influenced by biotic factors, rather than

historical climatic or geographic effects. Potential factors include prey availability, the presence of model species for mimicry (e.g. Greene & McDiarmid 1981), and potentially, interspecific interactions such as competition (e.g. Losos et al. 1994; Moen & Wiens 2009). However, more explicit tests for phylogenetic evidence of competitive differentiation (e.g., Moen & Wiens 2009) will be required before any robust hypotheses of interspecific interactions driving the ecomorphological diversification of the lampropeltinines can be made.

Evolution of Color Pattern and Batesian Mimicry

The evolutionary constraints on the development of the tricolored pattern are apparently related to both body size and diet preference, as the ringed pattern is exhibited only by species in a narrow range of body sizes and diet compositions (Fig. 9). This ‘key zone’ for mimicry thus appears to represent the optimal range for the development of the tricolored ringed color pattern. Within a range of adult body size between ~40-70 cm SVL, species consuming primarily ectothermic prey, such as the scarlet snake (*Cemophora coccinea*), scarlet kingsnake (*L. elapsoides*), gray banded kingsnake (*L. alterna*), and mountain kingsnakes (*L. pyromelana* and *L. zonata*), all exhibit putatively mimetic color patterns, even if closely related species do not (Fig. 8). In contrast, larger species of lampropeltinine (e.g., *Pantherophis* and *Pituophis*) tend to exhibit a drab, likely cryptic or break-up color patterns (Schulz 1996; Conant & Collins 1998; Stebbins 2003). Very small species, such as *L. extenuata*, also exhibit less colorful saddled (cryptic) patterns (Conant & Collins 1998).

Body size evolution thus appears to affect phenotypic color pattern traits as well as more obviously size-dependent traits such as gape and diet preference. Most strikingly, however, is the existence of a key zone for the tricolored ring color pattern, suggesting that both increases and decreases in size can affect the evolutionary development of putative Batesian mimetic antipredator strategies. Additionally, ecomorph development in these snakes appears to stem more from size-specific factors, rather than ecological or habitat factors, as in the squamate genus *Anolis* (Losos 1994). Similar to *Anolis*, body size differentiation and by extension ecomorph adaptation, appears to have resulted from interspecific interactions. Thus, rather than predicting a continuum of ecomorphological forms, the body size dependent axis of diversification in the lampropeltinines appears to promote the development of distinct ecomorphs within certain ranges of body sizes.

Conclusions

In the lampropeltinines, presumably functionally unrelated traits related to predatory specialization and predatory avoidance exhibit evolutionary correlations, which are due to the shared dependence of those traits on body size. While the relationship between those traits disappears when body size is accounted for, dismissing the pattern as being due to simple scaling may overlook important aspects of ecomorphological diversification limited by variation in body size. Thus, the observed ecomorphological diversity of species assemblages with regard to numerous phenotypic, morphological, and life history traits may be determined by evolutionary constraints on body size. However, despite the wide range of ecological niches inhabited by the lampropeltinines, neither climatic nor geographic factors appear to influence body size or other traits, suggesting

that both might be potentially influenced by interspecific interactions such as competition. Thus, while the geographic distribution of lampropeltine species appears to have been driven by climatic factors, ecomorphological diversification in the group may have been driven by interspecific interactions such as competition. The evolution of mimetic color patterns appears to be constrained by both body size and dietary composition, appearing in smaller snakes consuming primarily ectothermic prey, providing a prime example of the limitations on diversity imposed by an underlying trait such as body size.

CHAPTER IV: SPECIATION (*LAMPROPELTIS GETULA*)

Lineage diversification in a widespread species: roles for niche divergence and conservatism in the Common Kingsnake, *Lampropeltis getula*

Speciation across geographic barriers can be influenced by both niche conservatism (e.g., Peterson et al. 1999; Kozak & Wiens 2006) and niche divergence in ecologically distinct habitats (e.g., Graham et al. 2004; Raxworthy et al. 2007; Rissler and Apodaca 2007). The idea that ancestral niches may be conserved across evolutionary time (phylogenetic niche conservatism; Ricklefs & Latham 1992; Wiens 2004) has recently gained a great deal of currency in the literature on speciation and the study of broad scale patterns of lineage formation (Peterson et al. 1999; Wiens & Graham 2005; Hawkins et al. 2006). Niche conservatism promotes allopatric divergence in fragmented habitats by limiting adaptation to new environments when populations maintain an ancestral niche. Alternatively, niche divergence may lead to lineage formation when populations adapt to new environments (Wiens 2004; Wiens & Graham 2005). The mechanism by which this happens is well-defined, in terms of the fragmentation of a continuous habitat which promotes geographic isolation, or dispersal into a new habitat, after which natural selection subsequently promotes ecological differentiation (Futuyma 1998; Coyne & Orr 2004). However, the relative importance of these processes for promoting lineage formation in single widespread species complexes has yet to be fully investigated. Whether both adaptive divergence and ancestral conservatism of ecological niche can influence lineage formation in widespread taxa crossing multiple distinct ecoregions and putative geographic barriers, is unknown.

At least three scenarios for allopatric speciation are possible when considering the roles of geographic barriers and ecological niche. The first is the presence of a geographic barrier dividing ecologically distinct populations, which indicates niche divergence across the barrier (e.g. Graham et al. 2004; Raxworthy et al. 2007). The second scenario

involves a barrier that divides ecologically similar populations, and indicates phylogenetic niche conservatism (e.g. Kozak & Wiens 2006). The final scenario is the presence of ecologically divergent populations on a continuous landscape without a physical barrier separating them, indicating population divergence promoted or at least reinforced by niche divergence (e.g. Gee 2004). While these patterns have been demonstrated on local geographic scales in various taxa, it is unknown how broadly distributed organisms, which inhabit a diversity of niches across their range, respond to physical barriers and environmental variation, and whether one or a combination of the above scenarios is the dominant mode of allopatric divergence within in wide-ranging taxa.

Ectotherms such as reptiles are well-suited for assessing the impact of environment and geography on lineage formation due to their low vagility and strong responses to environmental factors (e.g., Burbrink et al. 2008; Fontanella et al. 2008; Guiher & Burbrink 2008; Howes et al. 2006). The Common Kingsnake (*Lampropeltis getula*) is transcontinentally distributed in North America (NA; Conant & Collins 1998) and also appears ideal for investigating the roles of geographic barriers and ecological niche on the formation of lineages because of its ancient occupation of this region. Fossils have been found in the central part of the U.S. dating to the late-Miocene (Holman 2000) and by the Pliocene, the kingsnake had attained a distribution similar to the one inhabited today (Holman 2000; Parmley & Walker 2003).

To examine phylogeographic structure in the North American kingsnake, we assembled a range-wide molecular dataset of the mitochondrial gene cytochrome *b* (cyt-*b*). While many issues regarding the use of single mitochondrial gene estimates of

phylogeny have been raised (see Edwards & Beerli 2000), the use of mtDNA for estimating phylogeographic structure still has advantages (see Barrowclough & Zink 2009; Brito & Edwards 2008; Zink & Barrowclough 2008), particularly for tracking recent population divergence and associated ecological influences. While issues such as stochastic gene tree/species tree discordance may influence phylogenetic estimates, this does not directly affect the inference of local genetic structure using mitochondrial loci.

First, we use phylogenetic reconstructions and divergence time estimates to characterize the geographic population structure in the kingsnake relative to the presence of putative physical barriers in the range of the organism. Second, given the possible uncertainty in tree structure due to variance in the coalescent and the ancient age by which the current range had been attained (Pliocene), we test a scenario of structured divergence across North America derived from our maximum likelihood tree of the real data versus a null model of an unstructured widespread ancestor. Rejecting the null model will permit us to determine which clades can provide robust comparisons of ecological niche characteristics.

Similar to Kozak & Wiens (2006; 2007), we use statistical methods developed for assessing ecological niche models (ENMs) to infer processes of niche conservatism and niche divergence in the formation of lineages, where empirical results in the kingsnake may exhibit any or all of the following three patterns: i) niche conservatism at a physical barrier, ii) niche divergence at a physical barrier, and iii) niche divergence across biomes that lack physical barriers. Using predicted habitat suitability as a proxy for ecological niche, a lack of statistically significant differences between ENMs for lineages divided by geographic barriers would represent the signature of niche conservatism. Significant

differences in niche at a geographic barrier would represent niche evolution or divergence in allopatry. Significant niche differences in the absence of a physical barrier would indicate that lineage formation was promoted, or at least maintained, by niche divergence in ecologically heterogeneous environments.

Methods and Materials

Sequence Acquisition

We obtained 201 tissue samples of *Lampropeltis getula* taken throughout their known range and downloaded 60 gene sequences from a previous systematic study of *L. getula* (Krysko & Judd 2006) from Genbank (data available as a supplement to Pyron & Burbrink 2009c) for our phylogeographic analyses, a total of 261 samples (Fig. 11). For the tissue samples, we used standard methods of proteinase K digestion in lysis buffer followed by several rounds of phenol/CHCl₃ extraction (Sambrook & Russell 2001) or Qiagen DNEasy kits (tissue protocol) to obtain total genomic DNA from samples of shed skin, liver or muscle tissue or whole blood. The complete mitochondrial gene *cyt-b* was amplified using GoTaq Green Master Mix (Promega Corp.) according to the manufacturer's specifications, with a 90sec extension time. The PCR products were cleaned using 1µl of ExoSap-IT (USB Corp.) per 10µl of PCR product. The sequencing reaction consisted of 3µl Beckman-Coulter DTCS, 2µl primer (5µM), 3µl template and 2µl deionized water. Primers for the PCR and cycle sequencing reactions were as follows; *cyt-b* amplification: H14910 and THRSN2 (Burbrink et al., 2000), sequencing: LampSeq1F (5'-GTA ATT ACA AAC CTA CTA ACA GC-3') and LgetSeqRev2 (5'-TTT GTT CCT ART GGG TTR CTA GAG-3'). For some particularly old or degraded

templates, *cyt-b* was amplified and sequenced in two fragments, using H14910+LgetSeqRev2 and LampSeq1F+THRSN2. Nucleotide sequences were examined and aligned by eye using the program Sequencher 4.5 (Genecodes 2000). No sequences contained any gaps or any stop codons that would have suggested a pseudogene.

Phylogenetic Inference

To test for the presence of population divergence and assess the potential impact of geographic features on lineage formation, we inferred the phylogeographic structure of *L. getula* using Bayesian inference (BI) and Maximum Likelihood (ML) methods. For Bayesian phylogenetic inference we used the program MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001) to infer trees and nodal support. To assess model complexity, we tested a codon position partitioned General Time Reversible model with gamma distributed rate heterogeneity and a proportion of invariant sites (3GTR+ Γ +I) against a codon position partitioned HKY85+ Γ +I model using Posterior Bayes Factors (Kass & Raftery 1995), with PBF >10 considered strong support for the more parameterized model. Each analysis (two runs of four chains each) was run for 2×10^7 generations. Convergence was assessed using Gelman and Rubin's *r* statistic (Gelman et al. 1995). The lampropeltine species *Arizona elegans*, *Lampropeltis calligaster*, *Lampropeltis triangulum* and *Lampropeltis extenuata* were used as outgroups (accession numbers available as a supplement to Pyron & Burbrink 2009c). Maximum Likelihood (ML) analysis was performed using RAxMLv7.4.3 (Stamatakis 2006) with the same dataset used for the Bayesian analysis. A codon position partitioned GTRMIX model was used, and 1000 non-parametric bootstrap replicates (Felsenstein 1985) were performed to assess node

support. Bayesian posterior probabilities (Pp) greater than 95% are considered strong support for a clade, while bootstrap proportions (BS) greater than 0.7 are considered strong support (Hillis & Bull 1993; Felsenstein 2004).

Divergence Time Estimation

For divergence time estimation, we used a relaxed phylogenetics methods (Drummond et al. 2006) in the program BEAST v.1.4.8 (Drummond & Rambaut 2007). Divergence time estimation within species complexes is hampered by the necessity for a compromise between interspecific phylogenetic priors (tree priors) and intraspecific coalescent priors. It is inappropriate to combine inter- and intraspecific data in a single Bayesian divergence time analysis, as no prior can adequately account for both processes. We opt for the latter, combining our ingroup dataset with a single outgroup, the sister taxon *Lampropeltis extenuata* (Pyron & Burbrink 2009a) and using coalescent priors for inferring divergence times using the entire population level dataset. Although the inclusion of a non-conspecific outgroup taxon does not render all of the assumptions of the coalescent priors valid, this is a more conservative mode of analysis than attempting to date a large number of outgroups and a single representative from each lineage using only one mitochondrial gene.

We tested both increasing (logistic and exponential growth) and constant population size priors, which yielded similar results; we report the data from the constant population size prior. In addition, the inferred estimates are concordant with a multi-gene phylogenetic analysis of the tribe Lampropeltini (Pyron & Burbrink 2009a), suggesting that the estimated dates are robust to variation in priors. An uncorrelated lognormal tree

prior, and lognormal fossil priors were used for divergence time estimation under the relaxed-clock model in BEAST (Drummond et al. 2006) using the same model from the primary tree inference (3GTR+ Γ +I). The divergence between *Lampropeltis extenuata* and *Lampropeltis getula* was constrained to have occurred during the Hemphillian, based on the fossils *Stilosoma (Lampropeltis) vetustum* and *Lampropeltis getula* known from the middle Hemphillian, late Miocene (Holman 2000). The mean of the lognormal distribution was 6.875Ma (1.9278) with a standard deviation of 0.188, yielding a prior credible interval of 4.75-9.94Ma. No zero-offsets were used. The analysis was run for 10 million generations, the first 2.5 million of which were discarded as burnin. We assumed convergence when the effective sample size of the posterior probability distribution of all parameters was >200 (Drummond et al. 2006).

Historical Biogeography

We used coalescent simulations in Mesquite v.2.5 (Maddison & Maddison 2008) to determine if the perceived Structured Model from the ML tree fits the data better than a widespread Fragmented Ancestor, based on potential stochastic variance in the tree structure and the observed number of deep coalescences (Knowles & Maddison 2002; Fig. 12). The Fragmented Ancestor model posits that all population divergences were in effect concurrent and resulted from the fragmentation of the widely distributed range of a common ancestor. The presence of phylogeographic structure under this model would be due to differential extinction of ancestral haplotypes among areas (Knowles 2001a, b; Carstens et al. 2005). The Structured Model suggests that a wide-ranging common ancestor originating across the Central US (Pyron & Burbrink 2009b; Ch. 2) was first

fragmented into two ancestral populations at the Mississippi River and then each of those was subsequently fragmented during colonization towards the western and eastern US, respectively (Fig. 11).

For coalescent simulations, we first estimated N_e for *L. getula* in each of the five geographically distinct areas determined from phylogeographic analyses using values for Θ calculated in the program MIGRATE-N v.2.4 (Beerli 2008) under the following parameters: 15 short chains for 200 000 generations and four long chains for two million generations with four adaptive heating chains, sampled every 20 generations following a burn-in of 10 000 generations. Maximum likelihood estimates (MLE) were calculated three times to ensure convergence upon similar values for Θ . We converted Θ to N_e using the equation for maternally inherited mitochondrial DNA $\Theta = N_e\mu$, where $\mu = 3.0 \times 10^{-8}$ site/generation calculated in BEAST v.1.4.8 and a generation time of three years (Werler & Dixon 2000). We summed the estimates of N_e for all areas to calculate Total N_e and scaled the branch widths of our hypothesized population trees using the proportion of Total N_e that each area comprised. Internal branches on the Structured Model were scaled such that all branch widths summed to Total N_e at any single point in time (Carstens et al. 2004; Shepard & Burbrink 2008, 2009; Fig. 12).

The method of counting the number of deep coalescences assumes that deep coalescent events are due to incomplete lineage sorting and not migration among populations (Maddison 1997; Knowles & Maddison 2002). In cases where the number of deep coalescences may be inflated by recent migration, it is important to account for migration in simulations to build null models that better reflect history under a given scenario (Shepard & Burbrink 2009). Using the MLE of Total N_e , we simulated 500 gene

trees under a neutral coalescent process with migration on the Fragmented Ancestor model at a tree depth of 1 636 666 generations, which when based on a three year generation time is equivalent to 4.91Myr (the approximate age estimated for the first divergence within *L. getula* using the fossil calibrated relaxed phylogenetics method). To calculate the probability of migration per individual per generation for these simulations, we first multiplied values of M among adjacent populations (areas) calculated in MIGRATE-N v.2.4 (Beerli 2008) by the Θ of the receiving population to derive the number of immigrants per generation among pairs of adjacent populations. We divided these values by the estimated N_e of the source population to calculate the probability of emigration per individual per generation in the source population, and then calculated the mean of all population pairs to derive the average probability of migration per individual per generation.

We fit the simulated gene trees from the Fragmented Ancestor model into the Structured Model, calculated the number of deep gene coalescences (nDC), and built a distribution of nDC values. We then fit our reconstructed ML tree for *L. getula* to the Structured Model and calculated the nDC value. If this observed nDC falls below 95% of the distribution of nDC values calculated using the simulated gene trees (equivalent to one-tailed $P \leq 0.05$), then the Fragmented Ancestor model will be rejected in favor of the Structured Model. To calculate P values for the observed nDC values in these analyses, we fit the distribution of simulated nDC values to a normal distribution with the given mean and standard deviation (SD).

Niche Modeling

Although the predicted habitat suitability from the ENM results is not an absolute prediction of the true fundamental or realized niche of an organism, it should provide a reasonable proxy and allows for statistical hypothesis testing regarding expressed niche preferences, at least with regard to the major environmental conditions experienced by the organisms (Warren et al. 2008; review in Kozak et al. 2008). To assess the impact of ecological niche on the formation and maintenance of lineage separation, we modeled the predicted suitable habitat of the inferred lineages of *L. getula* using maximum entropy methods (Phillips et al. 2006; Elith et al. 2006) in the program Maxent v3.2.19. The nineteen BIOCLIM variables from the WorldClim dataset (Hijmans et al. 2005) were used at 30s spatial resolution (~1 km).

Many of the BIOCLIM variables are highly correlated, and the relative contribution of each variable to the model for each lineage is uncertain. Thus, we followed the protocol of Rissler & Apodaca (2007) and reduced the dataset to eleven biologically informative variables which are not significantly correlated across North America: BIO1-3, 7-9, and 15-19. In addition, we used the Level III Ecoregion designations for North America provided by the U.S. EPA and Commission for Environmental Cooperation. This layer, derived primarily from Omernik (1987), classifies North America into 182 distinct ecoregions based on biological and environmental ecosystem differentiation (CEC, 1997). The ecoregions were trimmed to the same extent as the BIOCLIM variables and projected at 30 second spatial resolution.

To train the model, 733 georeferenced presence localities were obtained, comprising the samples used in our phylogenetic analysis and additional georeferenced museum records (data available as a supplement to Pyron & Burbrink 2009c). The latter

were obtained either through the public web interface of museum collections, or through the HerpNet database (<http://herpnet.org>). Records with GPS coordinates were used as is; all other records were georeferenced to the reported locality using the description provided in the record. Occurrences were assigned to lineages based on the circumscribed area as inferred from the primary phylogeographic analysis, and niche models were constructed for each lineage.

We used auto features in Maxent (Phillips et al. 2006), set the regularization multiplier to the default (1.0), and allowed the algorithm to run to convergence (threshold of 0.00001). The resulting niche predictions were projected onto a map of the U.S. in DIVA-GIS, with the minimum training presence criterion used as the binary threshold for predicted suitability. We attempted to qualitatively assess the biological niche differentiation between the lineages by determining which variables in the model contributed the greatest proportion of entropy to the model from the table given in the Maxent output. Overlap in predicted suitable area was calculated between adjacent lineages by counting the number of 30 second pixels predicted as suitable for both lineages. This was converted to area by multiplying by 0.86 (30 seconds of arc equals 0.93km, thus a 30 second pixel equals 0.86km²).

Niche Differentiation

We assess differentiation in the predicted ecological niche models of the lineages using the newly developed niche equivalency methods of Warren et al. (2008). The program ENMTools uses two niche similarity metrics, Schoener's *D* (Schoener 1968) and the newly developed "Warren et al.'s" *I* (Warren et al. 2008). These statistics quantify

predicted niche similarity, and range from 0 (no overlap) to 1 (identical niche models). We used the test of niche equivalency in ENMTools, which evaluates equivalency between ecological niche models by comparing the observed values of D and I for the two models to a distribution of values of D and I based on randomized pseudoreplicates. This distribution is generated by randomly assigning occurrence points from both groups into one lineage or the other, simulating the potential overlap of a group of points occurring across a given geographical area (Warren et al. 2008). This allows for a one-tailed test of dissimilarity from random for two ecological niche models. As we are primarily reporting interactions between sister lineages, we did not employ the possible phylogenetic corrections for these analyses (Warren et al. 2008). We also did not employ the more stringent tests for niche equivalency given available background, as no biological justification for defining the available background is evident for a single widespread species complex. We calculated the observed D and I values and simulated distributions of D and I using 100 pseudoreplicates for all pairwise comparisons of the inferred lineages; only those with relevance to our hypotheses are reported. Due to computational constraints, the pseudoreplicates niche models were inferred using the 11 BIOCLIM variables and the Level III Ecoregions projected at 2.5 min spatial resolution.

Results

The *cyt-b* gene sequenced for *Lampropeltis getula* and all outgroup taxa measured 1117bp with no indels or stop codons in the reading frame for any sample. The sequences have been deposited on Genbank under the accession numbers FJ997648-FJ997848

(data available as a supplement to Pyron & Burbrink 2009c). For the BI phylogenetic analysis, we chose the 3GTR+ Γ +I model (PBF=32.76). Gelman and Rubin's r-statistic (Gelman & Rubin 1995) was less than 1.001 for all parameters after a burnin of 5×10^6 generations (split standard deviation among chains <0.01). Both analyses inferred five major lineages with generally strong support for both the lineages themselves and the relationships between them (Fig. 11, 13). The primary geographic lineages are as follows:

- 1) *Eastern*: A lineage comprising the kingsnakes of the eastern seaboard of the United States, from New Jersey to the Florida Keys and extending to the Apalachicola region in the Florida panhandle and southeast Alabama.
- 2) *Mississippi*: This lineage ranges through the greater Mississippi River drainage east of the Mississippi River, from southern Illinois east to Ohio and western West Virginia in the north, to the Tennessee and Alabama river drainages of Georgia and Alabama in the south.
- 3) *Central*: The Central lineage inhabits the Great Plains and Mississippi River valley west of the Mississippi River, from Iowa and Nebraska in the north to west-central Texas and the western Gulf Slope in the south, east to the Mississippi River.
- 4) *Desert*: The Desert lineage is found in the Chihuahan Desert of west Texas, southern New Mexico (including the Rio Grande River Valley), extreme southeastern Arizona and eastern Mexico, along the Mexican Plateau. May also occur in north central Arizona (Fig. 14a).
- 5) *Western*: The Western lineage occurs west of the Rocky Mountains, from the southern Great Basin in Nevada and Utah, southern Oregon south to Baja

California, and most of Sonora, Mexico, east to southeastern Arizona.

All lineages were strongly supported by both posterior probabilities ($P_p > 0.95$) and bootstrap proportions ($BS > 0.9$). The relationships among the lineages were also all supported at greater than 95% P_p and 90% BS , with the exception of the node subtending the Central lineage and the Western and Desert lineages, which received moderate support in the BI analysis (93% P_p) and weak support in the ML analysis (59% BS). Lineages of *L. getula* do not follow the currently designated subspecies taxonomy and appear to correspond to historical divergences at the Mississippi River (Western, Desert & Central vs. Eastern & Mississippi); the Rocky Mountains (Western & Desert vs. Central); the Cochise Filter Barrier (Western vs. Desert); and the Appalachian mountains/Chattahoochee River/Apalachicola River, here termed the Apalachee formation for ease of reference (Eastern vs. Mississippi). While some lineages (e.g., the Western and Eastern) exhibit strong concordance between the geographic mtDNA lineage and the currently subspecies described based on color pattern (see Fig. 13), others (e.g., the Central and Gulf lineages) do not. Thus, while morphological differentiation may be at least in part related to ecological divergence, the strength and underlying mechanisms of this pattern are unclear.

Divergence Dating and Historical Biogeography

Based on the prior distributions for the ages of the earliest known fossils of both taxa (Holman 2000; Parmley & Holman 1995), the dating analyses indicate that *L. getula* diverged from its sister taxon *Lampropeltis extenuata* during the Hemphillian of the late Miocene, approximately 6.54Ma (95% Highest Posterior Density = 4.20-8.93Ma). The

earliest divergence occurred at the Mississippi River approximately 4.91Ma (95% HPD = 2.63-7.32Ma) during the late Hemphillian of the early Pliocene. This initial divergence at the Mississippi River and an area of origin in the Central US corresponds with the area of origin for the tribe Lampropeltini, of which *L. getula* is a member (Pyron & Burbrink 2009a). Divergence between the western (Western & Desert) lineages and the Central lineage occurred during the Blancan of the early Pliocene (4.06Ma; 95% HPD = 1.86-6.12Ma). As fossils are known from Washington state dating to the Pliocene (Parmley & Walker 2003), we infer that this split occurred across the Rocky Mountains, and that the Cochise Filter Barrier divergence represents a subsequent west-to-east vicariance event. Thus, the parapatry of the Desert and Central lineages represents a zone of secondary contact. The Cochise Filter Barrier (2.16Ma; 95% HPD = 1.11-3.44Ma) and Apalachee (1.94Ma; 95% HPD = 0.75-3.35Ma) divergences occurred at roughly the same time at the Pliocene/Pleistocene boundary.

In terms of ancestral population structure, the Structured Model posits an initial divergence at the Mississippi River and subsequent divergences toward the West Coast (Central and Desert+Western, then Desert and Western clades) and the East Coast (Mississippi and Eastern clades). This structured tree was then tested against the Fragmented Ancestor model. Using Migrate-N v.2.4 (Beerli 2008), we calculated a maximum likelihood estimate (MLE) of $\Theta_{\text{Total}} = 0.1034$ (95% CI: 0.06-0.161). The MLE of Θ_{Total} equates to a Total N_e of 3 446 666. Based on values of M and Θ from Migrate-N (Beerli 2008), we calculated a mean probability of migration per individual per generation of 3.351×10^{-6} , and used this value in coalescent simulations. The number of deep coalescent events (nDC) for our ML tree fit into the Structured Model was 54. This

value was significantly less than results from the coalescent simulations under the Fragmented Ancestor tested against the Structured Model (mean nDC=175.506, SD=20.81539, $P=2.586 \times 10^{-9}$). Therefore, all subsequent tests of niche used the structure from the ML tree representing the Structured model.

Niche Modeling and Equivalency

All predictions for each of the five lineages differed significantly from random (binomial test, $p < 0.00001$) and exhibited high sensitivity and specificity (AUC/ROC values > 0.95 for all lineages). The predicted distributions for each lineage closely matched their known extent (Figs. 11, 13, 14). The use of museum records, which precludes further discovery of between-lineage dispersal, may cause an underprediction in niche overlap. However, overlap in predicted suitability is observed between all adjacent lineages, and ranges from 0.0006% to 37% of the area of the lineages (Table 2; Fig. 14). The variables with the greatest contribution to the models for each lineage were as follows: Western (BIO18: Precipitation of the Warmest Quarter; 44.7%), Desert (BIO2: Mean Diurnal Range [Temperature]; 35.1%), Central (BIO1: Annual Mean Temperature; 41.9%), Mississippi (BIO17: Precipitation of the Driest Quarter; 62.6%), and Eastern (BIO7: Temperature Annual Range: 23.1%).

Schoener's D and Warren et al.'s I values for the pairwise comparisons of interest were significantly lower than expected from a random distribution (Table 2) for all comparisons except for the Western and Desert lineages, rejecting the null hypothesis of niche equivalency for all adjacent lineages. The Western and Desert lineages are not identical ($D=0.65$, $I=0.523$), but the interpredicted suitability does not differ significantly

from the overlap between randomly assigned points. Given that these lineages are allo- or peripatric (Figs. 11, 13, 14), this is indicative of niche conservatism between these sister lineages. At the zone of secondary contact between the Desert and Central lineages, a small amount of interpredicted suitability in the north appears to be inhabited solely by the latter clade, whereas almost no overlap occurs along the western Gulf slope in the southern portion of the juncture, indicative of significant niche divergence (Table 2).

Discussion

Historical Biogeography of the Common Kingsnake

The common ancestor of the kingsnake originated in the late Miocene and has since diverged to form five lineages across major geographic barriers in North America. The initial divergence at the Mississippi River (~5.0Ma) is a pattern which has been documented in numerous species of both plants and animals (Soltis et al. 2006; Burbrink et al. 2000, 2008; Howes et al. 2006; Lemmon et al. 2007). Subsequently, three lineages diverged westward at ~4.0 MA (Central and Western/Desert) and 2.1MA (Western and Desert) and two eastward ~1.9 MA (Eastern and Mississippi). Divergences at the Cochise Filter Barrier (~2.1Ma) in the West and the Apalachee formation (~1.9 MA) in the East occurred more recently, and at a similar time near the Pliocene/Pleistocene boundary. This suggests that the congruent patterns of lineage divergence observed at these features may be the result of similar responses to physiographic and environmental shifts during the late-Pliocene and early-Pleistocene (Soltis et al. 2006; Castoe et al. 2007).

At the Cochise Filter Barrier, the separation of the Sonoran and Chihuahan desert provinces during the late-Pliocene (Morafka 1977) has been widely implicated in the

formation of geographic lineages across the western deserts as refugia formed on either side of the continental divide during the Pliocene and Pleistocene (Smith & Farrell 2005; Jaeger et al. 2005; Devitt 2006; Castoe et al. 2007; Mulcahy 2008). The observed divergence in the Western and Desert lineages of the kingsnake at ~2.1Ma is contemporaneous with the formation of Sonoran/Chihuahan/Mojave clades in cactus longhorn beetles (*Moneileima armatum*; Smith & Farrell 2005), western diamondback rattlesnakes (*Crotalus atrox*; Castoe et al. 2007) and desert spiny lizards (*Sceloporus magister*; Leaché & Mulcahy 2007). Although few studies have dated divergences at the Apalachee formation, most implicate the late-Pliocene and Pleistocene formation of glacial refugia in the southern Appalachians and Florida in the separation of lineages (Burbrink et al. 2000; Soltis et al. 2006; Pauly et al. 2007).

All five lineages occupy geographically distinct habitats according to niche modeling results and exhibit varying areas of predicted overlap. The ENMs of the sister Eastern and Mississippi lineages are found to be significantly different based on randomization tests, though the areas of predicted overlap are also associated with apparent sympatry of the lineages (Figs. 11, 13, 14). Predicted areas of overlap between the sister Western and Desert lineages are also associated with an area of sympatry (Figs. 11, 13, 14), though the ENMs for these lineages do not differ significantly which suggests a role for niche conservatism promoting speciation across the Cochise Filter Barrier (Fig. 11). The predicted overlap between the significantly different ENMs of Central and Mississippi lineages is not associated with widespread sympatry of the lineages, suggesting that the Mississippi river is a strong barrier to dispersal. The near total lack of predicted overlap between the Central and Desert lineages suggests a role for

niche divergence in promoting, or at least maintaining, allopatric population segregation along an environmental gradient.

Conservatism and Divergence in Ecological Niche

Rather than finding that a single process of niche evolution played a dominant role, all three modes of niche-barrier interaction are associated with lineage formation in the kingsnake. We find evidence for i) niche conservatism at the Cochise Filter Barrier, ii) niche divergence at the Apalachee Formation and the Mississippi River, and iii) niche divergence at the ecological transition between the adjacent Central and Desert/Western clades, which appears to lack a physical barrier. The sister lineage pairs (Western/Desert and Eastern/Mississippi) both appear to have separated along axes of precipitation and temperature, though the particular aspects of those variables is not consistent across the lineages. For a species occupying a range the width of a continent, it is not surprising to find a range of patterns suggesting different modes of lineage divergence with respect to ecological niche. Niche conservatism has been identified as the mechanism responsible for the formation of many organisms, such as species of plethodontid salamanders in temperate areas (Kozak & Wiens 2006; Shepard & Burbrink 2008, 2009). In contrast, niche divergence has been found to promote diversification in organisms such as tropical salamanders occurring along elevational gradients (Kozak & Wiens 2007) and ecological gradients in other ectothermic vertebrates (Graham et al. 2004; Raxworthy et al. 2007). However, to our knowledge, a role for both mechanisms in diversification and lineage formation in a single species has not been shown.

Although niche conservatism appears to be present between some lineages in *L. getula*, our results show that ecological niches have not been broadly conserved among lineages to a degree where adjacent lineages share identical ENMs. This suggests that environmental preferences are labile even on recent time scales, and species may evolve significant differences even between recently diverged sister lineage pairs as natural selection acts on populations in ecologically heterogeneous habitats (Wiens 2004). Indeed, ENM results predict almost complete ecological separation between the peripatric Western and Central lineages (Figs. 11, 14).

The indication from these results is that niche differentiation is based on the dominant ecological feature of the local environment. Thus, dry season precipitation exerts the strongest influence on the Mississippi clade which inhabits the mesic Mississippi River drainage, while annual temperature most strongly affects the Central lineage, which occurs as far north as Nebraska, in areas subject to extreme winters. However, even the non-differentiated Western and Desert lineages show influence from different, presumably locally adapted variables: daily temperature range for the Desert lineage and rainy-season precipitation for the Western lineage. Ultimately, while niche conservatism and divergence may both influence lineage formation, the particular niche differences between any given lineages may simply reflect historical contingencies rather than a unified pattern of ecological influence.

Although niches must always be conserved in the sense that descendant populations will inhabit similar geographic areas or ecological niches as their immediate ancestors (e.g., Wiens & Graham 2005; Losos 2008a,b; Wiens 2008b), our results provide an important perspective on the influence of niche conservatism on speciation.

The detection of niche equivalency between the sister Western and Desert lineages, which are distributed across a climatically unsuitable barrier, is indicative of the classic scenario for speciation through niche conservatism across a continuous landscape (Wiens 2004). However, the ecological divergence between the remaining lineages illustrates a more subtle point about niche conservatism. The maintenance of lineages in geographically distinct areas must be due, at least in part, to the conservation of niche preferences through natural selection against individuals that disperse out of the current niche (e.g. Holt & Gaines 1992; Wiens 2004). Indeed, phylogenetic niche conservatism has been shown to influence the continental distributions of many organisms (Ricklefs & Latham 1992; Wiens et al. 2006, 2009; Pyron & Burbrink 2009b). While niche conservatism may exert a powerful influence on the distribution of species, it is still possible for lineages to exhibit divergence in environmental preferences on short evolutionary time scales.

Conclusions

We find evidence that both niche conservatism and niche divergence have played roles in promoting and maintaining divergence between lineages of *L. getula*, as well as zones of secondary contact between lineages. While conservation of ancestral ecological conditions was detected between some lineages, our results indicate that ecological niche, or at least predicted habitat suitability based on expressed environmental preferences, is labile even on recent evolutionary timescales, and that niche expansion and divergence may occur rapidly even between sister lineages. Moreover, all of these processes are shown to have occurred and are associated with lineage formation in the Common

Kingsnake. While authors have recently argued about the basic definition of niche conservatism and the extent to which niches are conserved (Losos 2008a,b), it is apparent that it is not whether niches are conserved that is important for phylogenetic studies, but the extent to which niches are conserved and the impact that this has on our understanding of the processes which influence speciation (Wiens & Graham 2005; Wiens 2008b). The evolutionary history of organisms, particularly recently diverged species complexes, is likely a mixture of the inertial tendency of populations to maintain their current niche and the action of natural selection on populations which differ in habitat across ecological landscapes.

CHAPTER V: TAXONOMY (*LAMPROPELTIS GETULA*)

Systematics of the Common Kingsnake (*Lampropeltis getula*; Serpentes: Colubridae)

and the burden of heritage in taxonomy

The delimitation of species is a primary goal of systematic biology (Sites & Marshall 2004; Wiens 2007b), and species identification is a major application of molecular phylogenetics (Avice 2000; Wiens & Penkrot 2002; Lemmon et al. 2007; Mulcahy 2008). Recently, traditional taxonomy has been in upheaval, as the uncovering of cryptic phylogeographic lineage diversity has resulted in the discovery of many species which are morphologically similar, para- or peri-patric, and represent portions of wide-ranging species complexes that had long been perceived as single species (Wake 1997; Avice 2000; Burbrink et al. 2000; Wiens & Penkrot 2002; Sinclair et al. 2004). Additionally, historical inertia may promote the continued recognition of hazily defined species or subspecies whose phylogeny may be imprecisely known, resulting in a taxonomic ‘burden of heritage’ for systematists. We speculate that this is a combination of two factors. First is the necessity of continuing to recognize current taxonomic arrangements under the principle of priority of the International Code of Zoological Nomenclature, based on their historical primacy. Second is the tendency for the more obvious, but potentially less evolutionarily significant and phylogenetically less informative attributes such as color pattern variation to dominate the attention of investigators.

This may occasionally result in a tendency both to ascribe differences to visually distinct but genetically homogeneous populations, and to group as conspecific visually similar populations (see, in part, Burbrink et al. 2000; Baird et al. 2006). While such patterns may reveal intriguing sources of information regarding local adaptations and population interactions, their utility as a source of evolutionary or phylogenetic information is suspect at best. Therefore, any taxonomic scheme which incorporates

explicit phylogenetic hypotheses based on the evolutionary history of genetic loci is arguably superior to arbitrarily defined subspecies or species based on geographically inconsistent variation in color pattern or scalation (see Burbrink et al. [2000] and Mulcahy [2008]) or idiosyncratic subdivisions of smoothly grading morphoclines (see Crother et al. [2008] on *Sistrurus*).

We address these philosophical, phylogeographic, and taxonomic issues in the species *Lampropeltis getula*, the Common Kingsnake, a medium-sized constrictor that is one of the most widely occurring species in North America (Conant & Collins, 1998). The species also exhibits substantial color pattern variation, which has resulted in the description of at least seventeen different subspecies (Blanchard 1921; Blaney 1977; Conant & Collins 1998; Krysko 2001; Grismer 2003; Stebbins 2003; Krysko & Judd 2006). Recently, a study that sampled individuals from throughout their range revealed that population structure exists in the kingsnake that is only partially concordant with the previously recognized subspecies taxonomy (Pyron & Burbrink 2009c). Based on these results, a systematic revision of the group is warranted. We find evidence for the recognition of five distinct species in the *L. getula* species group. We also discuss the relevance that increasingly detailed, fine-scale phylogeographic DNA data may have on species-level taxonomy.

Species Accounts

Phylogeographic Analysis and Species Delimitation

Pyron & Burbrink (2009c) presented a phylogeographic analysis of the *L. getula* group based on 261 individuals sampled from throughout its range, sequenced for the

mitochondrial gene cytochrome *b* (1117bp). Analysis of the mtDNA data recovered five geographic lineages, each of which is associated with a distinct North American ecoregion. After diverging from *L. extenuata* during the late Miocene (~6.5 [4.2-8.9]Ma; Fig. 15; Pyron & Burbrink 2009a), the basal geographic break in *L. getula* occurred at the Mississippi River (4.9 [2.6-7.3]Ma; Figs. 15, 16), followed by subsequent divergences across the Rocky Mountains (4.1 [1.9-6.1]Ma), the Cochise Filter Barrier (2.16 [1.1-3.4]Ma), and the Apalachee Complex (1.94 [0.8-3.4]Ma; Pyron & Burbrink 2009c).

Our theoretical species concept is derived from the evolutionary species concept (ESC; Wiley 1978) and the general lineage concept of species (GLCS; de Queiroz 1998, 2007), implemented using the phylogenetic species concept (PSC; Cracraft 1989). We seek to identify uniquely evolving evolutionary lineages as species using operational criteria from Wiens & Penkrot (2002), Rissler & Apodaca (2007), and Bond & Stockman (2008). Based on the haplotype phylogeny of Pyron & Burbrink (2009c), we identify five geographically concordant lineages within the ‘focal species’ *L. getula*. By the criteria of Wiens & Penkrot (2002), the presence of several geographically distinct, reciprocally monophyletic lineages within the ‘focal species’ indicates the presence of multiple species hidden by the previous taxonomy. An apparent lack of distinct geographic structure within each lineage suggests widespread intra-species gene flow, indicating that no further taxonomic subdivision is possible. The five geographic lineages are all para- or allopatric and separated by putative barriers to gene flow, a primary criterion of Bond & Stockman (2008). Finally, adjacent lineages except the Western/Desert pair have significantly different predicted ecological niche preferences (Pyron & Burbrink 2009c).

However, the Western/Desert lineages are peripatric with only a small secondary contact zone at a putative genetic barrier, a major criterion of Rissler & Apodaca (2007).

Each of the geographic lineages corresponds roughly with one of five major previously recognized subspecies. Interestingly, and of particular importance regarding taxonomic status, these major subspecies were all originally described as distinct species. In the case of *L. g. californiae* (Blainville 1835; roughly co-terminous with the Western lineage), this recognition persisted for 101 years (Klauber 1936). In addition, *L. g. niger* (Yarrow 1882; encompassed by the Mississippi lineage), *L. g. holbrooki* (Stejneger 1902; comprises the Central and Mississippi [part] lineages), and *L. g. splendida* (Baird & Girard 1853; equivalent to the Desert lineage) were all originally described as separate species before being synonymized with *L. getula* (Blanchard 1921). Based on the unique evolutionary, ecological, and geographic identities of these lineages noted above, we suggest that these species designations be restored, indicating the distinctness of these lineages as independent entities (de Queiroz 1998). This taxonomy recognizes the five phylogeographic lineages as distinct species, each bearing the name of the nominate subspecific race. We retain no subspecies, based on the lack of any well-supported, geographically localized genetic variation that would be indicative of legitimate infraspecific lineages. Detailed histories of synonymy are available in Blanchard (1921), Blaney (1977), and at the JCVI Reptile Database (<http://www.reptile-database.org>).

***Lampropeltis getula* (Linnaeus 1766)**

(Figs. 15-17)

Eastern Kingsnake

Holotype: Unknown.

Type Locality: ‘Carolina’ (Linnaeus 1766), restricted to Charleston, SC by Klauber (1948)

Etymology: Specific epithet refers to the Getulian people of northern Morocco, whose tribal insignia bears a resemblance to the ‘chain’ pattern of the kingsnakes of the Eastern Seaboard of the United States.

Synonymy: This species comprises the previously recognized subspecies *L. g. getula*, *L. g. floridana* and *L. g. meansi*, as well as the historically recognized subspecies *L. g. goini* and *L. g. sticticeps*. The nominate subspecies was first designated by Cope (1875).

Diagnosis: The Eastern Kingsnake (*L. getula*) is a medium- to large-bodied constrictor, the largest in the genus *Lampropeltis* with a maximum total length of 208.3cm, though the average adult size range is 90-122cm (Conant & Collins 1998). Scales are smooth, anal plate single, and individuals typically exhibit 19-25 scale rows at midbody. Ventral scales number 200 to 223 in both sexes, while subcaudals number 45-58 in males and 37-55 in females (Blaney 1977). The Eastern Kingsnake ranges from New Jersey to the Florida keys in the east, and west to the western panhandle of Florida and southeastern Alabama (Fig. 16). The species *L. getula* can be distinguished from all other related snakes primarily on the basis of color pattern, which can be divided into two primary variants. From northern Florida to New Jersey, individuals typically exhibit a dark brown or black ground color, which is punctuated by 17-36 narrow crossbands of white, yellow, or reddish yellow (Blaney 1977), giving the appearance of a ‘chain’ pattern (Fig. 17). In peninsular Florida, the bands increase in both number (22-54) and width, and the ground color lightens considerably to a light brown color with yellow stippling (Blaney 1977;

Fig. 17). Isolated populations of other aberrant color pattern variants can be found in the panhandle of Florida (Krysko & Judd 2006). The shift between the Eastern Kingsnake and the Mississippi lineage is fairly abrupt, and previous authors have noted the narrow transition zone and apparent lack of morphological intermediacy in southern Alabama and central Georgia (Fig. 16, 17; Blanchard 1921; Blaney 1977; Mount 1980).

***Lampropeltis nigra* (Yarrow 1882)**

(Figs. 15-17)

Black Kingsnake

Holotype: USNM12149, collected by Robert Ridgway.

Type Locality: Wheatland, Knox Co. Indiana.

Etymology: Specific epithet refers to the predominantly black dorsal coloration of many specimens.

Synonymy: This species comprises the previously recognized subspecies *L. g. nigra* and *L. g. holbrooki* (part).

Diagnosis: The Black Kingsnake (*L. nigra*) is a large- to medium-bodied constrictor with an average adult size of 90-122cm, with larger individuals attaining maximum lengths of 147-183cm (Conant & Collins 1998). Scales are smooth, anal plate single, and individuals typically exhibit 19-25 scale rows at midbody. Ventral scale counts range from 197-222 in both species (fewer in the north), while subcaudals range from 45-59 in males and 37-51 in females (Blaney 1977). The Black Kingsnake can be distinguished from other species in the genus based on a combination of geography and color pattern. The Black Kingsnake ranges from southern Illinois to the Gulf coast along the

Mississippi River, and east to the Appalachian mountain and the Alabama River drainage in south Alabama (Fig. 16). Black Kingsnakes all exhibit a black ground color, typically with a black-and-white checkered venter, and rarely faint traces of dorsal crossbands (Blanchard 1921; Blaney 1977; Conant & Collins 1998). Each dorsal scale is punctuated by a yellow or white speckle near the center of the scale; this is strongest in the southern portion of their range and fades considerably in the north, where many adults may be almost completely black (Conant & Collins 1998; Fig. 17). The Black Kingsnake can be distinguished from the morphologically similar Central lineage on the basis of geography, as the Black Kingsnake is only found east of the Mississippi River (Fig. 16).

***Lampropeltis holbrooki* (Stejneger 1903)**

(Figs. 15-17)

Speckled Kingsnake

Holotype: Unknown

Type Locality: ‘Valley of the Mississippi’ (Holbrook 1842), restricted to Hot Springs, Arkansas (Schmidt 1953).

Etymology: Specific epithet is a patronym honoring John Edwards Holbrook, a prominent American herpetologist of the 19th century.

Synonymy: This species is essentially co-terminous with populations of the previously recognized subspecies *L. g. holbrooki* occurring west of the Mississippi River.

Diagnosis: The Speckled Kingsnake (*L. holbrooki*) is a medium- to large-bodied constrictor with a maximum adult size of 183cm and a mean adult range of 90-122cm (Conant & Collins 1998). Scales are smooth, anal plate single, and midbody scale rows

number 19-25 (Blaney 1977). Ventral scales number 197-222 in both genders, with subcaudals ranging from 46-59 in males and 37-51 in females (Blanchard 1921; Blaney 1977). The Speckled Kingsnake occurs west of the Mississippi River, from Iowa and Nebraska in the north to the Gulf Coast, and west to west-central Texas (Fig. 16). The majority of the range of *L. holbrooki* is characterized by the ‘speckled’ pattern, which consists of a black ground color, with a white or yellow speckle in the center of each scale, and very occasionally a faint trace of dorsal crossbanding (Fig. 17). Large geographical areas harboring at least superficial morphological intermediacy between the Speckled Kingsnake and the Desert lineage in west central Texas are apparently inhabited only by the Speckled Kingsnake, suggesting that such color pattern variation may be due to phenotypic responses to ecological gradation, rather than hybridization or introgression (Pyron & Burbrink 2009c). The precise western extent of the range of *L. holbrooki* is unclear, but ecological niche modeling predicts that the range extends approximately to the Pecos and Rio Grande River drainages (Fig. 14, 16).

***Lampropeltis splendida* (Baird & Girard 1853)**

(Figs. 15-17)

Desert Kingsnake

Holotype: USNM1726, collected by Col. J.D. Graham.

Type Locality: Sonora, Mexico (no further locality given).

Etymology: Specific epithet refers to the ‘splendid’ visage of the dorsal coloration.

Synonymy: This species is essentially co-terminous with the formerly recognized subspecies *L. g. splendida*.

Diagnosis: The Desert Kingsnake (*L. splendida*) is a medium- to large-bodied constrictor with an average adult size range of 90-114cm and a maximum size of 152cm (Conant & Collins 1998). Scales are smooth, anal plate single, with midbody scale rows typically numbering 23-25 (Blaney 1977). Ventral scales number 199-227 in males and 203-237 in females, while subcaudals range from 45-62 in males and 40-52 in females (Blaney 1977). The Desert Kingsnake can be distinguished from related species primarily on the basis of color pattern. The pattern of the Desert Kingsnake is characterized by a black or dark brown ground color with heavy yellow lateral and dorsolateral stippling. The remnant crossbands formed by this stippling yield a row of black or brown dorsal blotches or saddles, numbering 42-97. The head is typically black or dark brown, and the onset of the yellow dorsal patterning sometimes gives the appearance of a collar (Fig. 17; Blanchard 1921; Blaney 1977; Conant & Collins 1998). The Desert Kingsnake inhabits the Chihuahuan desert east of the Cochise Filter Barrier, from western Texas to extreme southeastern Arizona, north from central New Mexico in the Rio Grande River valley south to the south central portion of the Mexican Plateau (Fig. 16). Additionally, the ecological niche modeling results from Pyron & Burbrink (2009c) predict an area of habitat in north-central Arizona as suitable for *L. splendida* which is not predicted as suitable for the geographically adjacent California lineage (Fig. 16; Pyron & Burbrink 2009c). While kingsnakes are known from this region of Arizona (Stebbins 2003), it is not known to which species this population belongs. The Desert Kingsnakes may hybridize with the Western lineage in a narrow area in extreme southeastern Arizona and extreme southwestern New Mexico, where haplotypes co-occur and some apparent

hybrids have been found (Fig. 16; R.A. Pyron, *pers. obs.*), though morphological intermediacy is apparently not widespread (Conant & Collins 1998).

***Lampropeltis californiae* (Blainville 1835)**

(Figs. 15-17)

California Kingsnake

Holotype: Unknown, collected by M. Botta.

Type Locality: ‘California’ (Blainville 1835). Restricted to Fresno, California by Schmidt (1953).

Etymology: Specific epithet refers to the type locality.

Synonymy: This species is essentially co-terminous with the previously recognized subspecies *L. g. californiae*, and includes the subspecies *L. g. nigrita*.

Diagnosis: The California Kingsnake (*L. californiae*) is a medium- to large-bodied constrictor, with a mean adult size range of 76-122cm, and a maximum size of ~200cm (Stebbins 2003). In both genders, ventral scale counts range from 213-255, with 46-63 subcaudal scales in males and 44-57 in females (Blaney 1977). Scales are smooth and anal plate single, with 23-25 dorsal scale rows at midbody. The California Kingsnake exhibits one of the broadest ranges of any kingsnake species, occupying most habitats west of the continental divide at the Cochise filter barrier (Fig. 16). The species ranges from Oregon in the north, through the Great Basin in Nevada and Utah, east to extreme southwestern Colorado, south through the majority of California, Arizona and the Mexican states of Sonora, Baja California Norte, and Baja Sur (Fig. 16). The California Kingsnake can be distinguished from other species on the basis of color pattern, possibly

the most distinct of the group. Throughout the majority of their range, California Kingsnakes exhibit a black or dark brown ground color, with 21-44 broad crossbands of white or light yellow, which typically widen laterally. Along the Pacific coast from Los Angeles to San Diego counties, individuals can be found possessing a black or dark brown ground color and a single thin, white dorsal stripe beginning at the neck and continuing to the tail (Fig. 17). Finally, populations in the Mexican states of Sonora and Sinaloa may exhibit considerable ontogenetic darkening, with adults, and occasionally subadults and even juveniles turning jet black, with almost no trace of pattern (Blaney 1977; Stebbins 2003). To which species the Sonora populations belong is unclear; while we group them with *L. californiae* on the basis of geography, some authors have suggested that they resemble and hybridize with both *L. g. 'splendida'* and *L. g. 'californiae'* (Blanchard 1921; Blaney 1977).

Discussion

Given a prevailing philosophical climate which stresses the necessity for a taxonomic system that reflects the evolutionary history of a group (see Frost et al. 1992; Wiens & Penkrot 2002; de Queiroz 1998, 2007), modern phylogenetic methods have resulted in an upheaval in many groups as existing taxonomies are tested for the first time using molecular phylogenetic data to infer evolutionary histories. Continuing to recognize poorly defined infraspecific taxa while failing to recognize the presence of distinct lineages may cause a potentially severe underestimate of biodiversity due only to a taxonomic burden of heritage. Accurately documenting this diversity is a crucial goal of systematics, and one which is particularly important, not only for studies of species

richness (e.g., Pyron & Burbrink 2009b), but for studying and conserving biodiversity on a broad scale (Wiens 2007b). While the choice of genetic marker (such as mtDNA) may affect phylogenetic inference (Avice 2000; Burbrink & Castoe 2009; Edwards & Beerli 2000; Edwards & Bensch 2009; Brito & Edwards 2008; Zink & Barrowclough 2008;), the use of molecular phylogenetic data analyzed using rigorous statistical methodologies is preferable in any case to ad-hoc morphological diagnoses for species, or appeals to an existing status quo based solely on historical precedence.

The concordance between the original species descriptions in terms of geography and color pattern ranges is strong for some of the species (*L. californiae*), but weaker for others (i.e., *L. holbrooki* and *L. nigra*), where there is significant overlap in phenotype between deeply diverged lineages. There exist wide zones of morphological intermediacy between both the Desert and Speckled Kingsnakes and the Speckled and Black Kingsnakes, despite their apparent lack of female-mediated hybridization, significantly different ecological niche preferences, and relatively narrow zones of predicted niche overlap (Pyron & Burbrink 2009c). We hypothesize that color pattern evolution in these snakes may be driven by phenotypic responses to changes in ecological or environmental variables, or clinal variation thereof (i.e., from eastern mesic forests to western deserts), rather than by gene flow or hybridization, as has typically been assumed by other authors in the past (e.g., Blaney 1977; Conant & Collins 1998).

Here, we have shown that the original description of at least five distinct species in the *L. getula* complex yields a far more useful and accurate picture of the evolutionary history and current genetic structure of the group than the seventeen subspecies described over the last 75 years. Indeed, the most morphologically and geographically distinct

form, *L. californiae* (Blainville 1835), was recognized as a distinct species for over a century before being demoted to a subspecies (Klauber 1936). While hybridization may occur between some of the adjacent lineages, recent research suggests that this may be common among recently diverged species (e.g., Gibbs et al. 2006; Leaché 2009; Niemiller et al. 2008; Nosil 2008), and the apparent lack of any broad regions of sympatry between the species suggests that they are not experiencing widespread introgression (Fig. 16; Pyron & Burbrink 2009c). Recognition within the *L. getula* complex of five distinct species, *L. getula*, *L. nigra*, *L. holbrooki*, *L. splendida*, and *L. californiae*, provides a phylogenetically robust taxonomic description of the Common kingsnake group, while retaining the historical connection to the original descriptions of those taxa extending back over 250 years.

CONCLUSIONS

Summary & Future Directions

Several interesting evolutionary processes operating in the Lampropeltini have been uncovered by this research, aided by an organismal phylogeny containing all currently recognized species (Pyron & Burbrink 2009a). An extratropical species richness peak can apparently be explained by the biogeographic origin of the lampropeltinines in temperate regions of North America allowing more time for the development of species richness, along with the phylogenetic conservation of this ancestral niche preference limiting dispersals to tropical areas (Pyron & Burbrink 2009b). A phylogenetic correlation of numerous putatively unrelated traits is explained by underlying relationships between those traits and body size, revealing that ecomorphological diversification in the Lampropeltini occurred primarily along a body size axis. This also suggests that body size may be a primary determinant of potential morphological diversity when multiple traits are tied to body size (Pyron & Burbrink 2009c). Finally, phylogeographic analysis of a widespread lampropeltinine species revealed the presence of cryptic lineage diversity. Analysis of the niche preferences of these lineages suggests that allopatric lineage formation was promoted by both phylogenetic niche conservatism and niche divergence. This indicates that ecological niches may be labile on recent evolutionary timescales and that speciation may take place in both ecological and geographic dimensions simultaneously (Pyron & Burbrink 2009d, e).

Considering the results of the phylogenetic analyses presented here, it is clear that significant revisions to our understanding of both phylogeny and taxonomy may be for many groups, as the need for simple inference of organismal phylogeny has not yet been filled. For instance, the ongoing suggestion from phylogeographic studies is that lineage diversity continues to be underestimated, even in well-studied groups such as the

lampropeltinines. The Common Kingsnake group (*Lampropeltis getula*) consists of at least five lineages that merit recognition as species, while taxa such as the Milksnake (*L. triangulum*) may consist of up to eight distinct lineages (Ruane et al. unpubl. data).

In the roughly twenty years since phylogeographic analyses have become common, approximately 100 snake species have been studied, primarily with single mitochondrial loci (Burbrink & Castoe 2009) and their possible attendant problems (Edwards & Beerli 2000). Current estimates suggest that there are approximately 3202 species of snake (JCVI Reptile Database: <http://www.jcvi.org/reptiles/>). At the past rate of ~5 species/year, it would take approximately 620 years before all species are investigated on even a cursory, mitochondria-based level. Even if 100 species per year are investigated, it will still be over thirty years before a barely sufficient picture of the genetic diversity of the extant snakes can be captured. This also assumes that this diversity will still exist in the future, given the magnitude of the current biodiversity crisis and the state of ecological ruin in which much of the planet lies.

At our current state of knowledge, it is possible already to undertake the fundamental investigations into organismal biology that molecular systematics and statistical phylogenetics allow. It is also clear that much work still needs to be done to generate robust estimates of the evolutionary relationships and the genetic diversity of the extant biodiversity of the planet. In addition, more radical developments in analytical methods, including statistical phylogeographic frameworks (Knowles & Maddison; Carstens & Richards 2007), coalescent methods for assessing simultaneous divergence (Hickerson et al. 2006), and genealogical methods for resolving species tree/gene tree incongruence (Edwards 2009) will further revolutionize evolutionary biology.

TABLES

Table 1 Dietary composition, morphological measurements, and mimetic color pattern class for the 31 recognized species of Lampropeltini, and the OW outgroup *Coronella austriaca*.

Species	Diet %	SVL	Gape	PC1	Mimic	References
<i>Arizona elegans</i>	0.51	67.54	213.03	0.95	No	1, 2
<i>Bogertophis rosaliae</i>	0.00	100.92	482.03	-0.81	No	3, 1
<i>Bogertophis subocularis</i>	0.04	105.50	520.94	-1.20	No	1
<i>Cemophora coccinea</i>	0.77	36.92	72.18	2.66	Yes	1, 9
<i>Coronella austriaca</i>	0.92	41.20	123.82	2.10	No	4
<i>Lampropeltis alterna</i>	0.83	53.01	256.93	1.06	Yes	5, 19
<i>Lampropeltis calligaster</i>	0.14	76.31	273.32	0.45	No	6
<i>Lampropeltis elapsoides</i>	0.90	36.60	76.46	2.73	Yes	7-9
<i>Lampropeltis extenuata</i>	1.00	33.30	35.32	3.34	No	9
<i>Lampropeltis getula</i>	0.59	91.09	388.35	-0.44	No	1, 10
<i>Lampropeltis mexicana</i>	0.75	52.15	248.92	0.98	Yes	11, 12, 19
<i>Lampropeltis pyromelana</i>	0.59	72.44	252.95	0.69	Yes	1, 13, 20
<i>Lampropeltis ruthveni</i>	0.75	63.30	210.16	0.98	Yes	** , 19
<i>Lampropeltis triangulum</i>	0.13	67.85	202.77	1.09	No	14
<i>Lampropeltis zonata</i>	0.84	66.39	188.93	1.15	Yes	1, 15, 20
<i>Pantherophis allegheniensis</i>	0.07	113.64	605.91	-1.77	No	1
<i>Pantherophis bairdi</i>	0.00	84.00	423.51	-0.44	No	1
<i>Pantherophis emoryi</i>	0.14	80.82	390.63	-0.23	No	1
<i>Pantherophis guttatus</i>	0.14	96.26	387.30	-0.55	No	1
<i>Pantherophis obsoletus</i>	0.07	114.39	752.62	-2.44	No	1
<i>Pantherophis slowinskii</i>	0.14	82.96	359.32	0.07	No	1
<i>Pantherophis spiloides</i>	0.07	114.63	560.10	-1.52	No	1
<i>Pantherophis vulpinus</i>	0.00	112.41	585.22	-1.76	No	1
<i>Pituophis catenifer</i>	0.04	103.31	556.93	-1.36	No	1, 16
<i>Pituophis deppei</i>	0.00	98.13	496.49	-0.98	No	1
<i>Pituophis lineaticollis</i>	0.00	121.17	663.10	-2.17	No	1
<i>Pituophis melanoleucus</i>	0.26	134.47	834.37	-3.21	No	1
<i>Pituophis ruthveni</i>	0.18	139.83	863.27	-3.60	No	17
<i>Pituophis vertebralis</i>	0.00	107.21	516.18	-0.98	No	16
<i>Pseudelaphe flavirufa</i>	0.10	116.50	541.32	-1.52	No	17
<i>Rhinocheilus lecontei</i>	0.73	58.69	155.99	1.60	Yes	18, 21
<i>Senticolis triaspis</i>	0.00	81.22	302.20	0.39	No	1, 17

¹ Rodriguez-Robles & de Jesus-Escobar 1999; ² Rodrigues-Robles et al. 1999a; ³ Schulz 1996; ⁴ Luiselli et al. 1996; ⁵ Werler & Dixon 2000; ⁶ Fitch 1999; ⁷ Palmer & Braswell 1995; ⁸ H.W. Greene unpubl. data; ⁹ Conant & Collins 1998; ¹⁰ K.T. Wiseman & H.W. Greene unpubl. data; ¹¹ Gehlbach & Baker 1962; ¹² Smith 1944; ¹³ A.T. Holycross & C.W. Painter unpubl. data; ¹⁴ Brown 1979; ¹⁵ Greene & Rodriguez-Robles 2003; ¹⁶ Rodriguez-Robles 2002; ¹⁷ Rudolph et al. 2002; ¹⁷ Schulz 1996; ¹⁸ Rodriguez-Robles & Greene 1999; ¹⁹ Garstka 1982; ²⁰ Stebbins 2003; ²¹ Savage & Slowinski 1992

Table 2 Area of predicted habitat suitability for the five inferred lineages of *Lampropeltis getula* and niche overlap calculations for pairwise comparisons of adjacent clades.

Lineage	Area	$D (\bar{X}, S.D., p)$	$I (\bar{X}, S.D., p)$
Eastern	823590km ²	--	--
Mississippi	1085876km ²	--	--
Central	1892157km ²	--	--
Desert	726695km ²	--	--
Western	1225441km ²	--	--
E vs. M	279184km ²	0.265 (0.866, 0.031, < 0.001)	0.523 (0.898, 0.022, < 0.001)
C vs. M	304064km ²	0.172 (0.811, 0.025, < 0.001)	0.441 (0.860, 0.017, < 0.001)
C vs. D	1139km ²	0.036 (0.312, 0.119, 0.01)	0.333 (0.542, 0.086, 0.008)
D vs. W	78893km ²	0.120 (0.275, 0.149, 0.150)	0.428 (0.502, 0.100, 0.227)

Area calculated as the total number of 30 arc second pixels predicted as suitable using a binary threshold, multiplied by 0.86 (30 seconds of arc=0.86km²). Niche overlap calculations for pairwise comparisons of adjacent clades. Overlap is the total area of the zone of predicted overlap in environmental suitability from the ENMs. Values in parentheses give the mean and standard deviation of the null distribution of D and I and the probability of the observed values from the niche equivalency test of Warren et al. (2008) implemented in ENMTools.

FIGURES

Figure 1

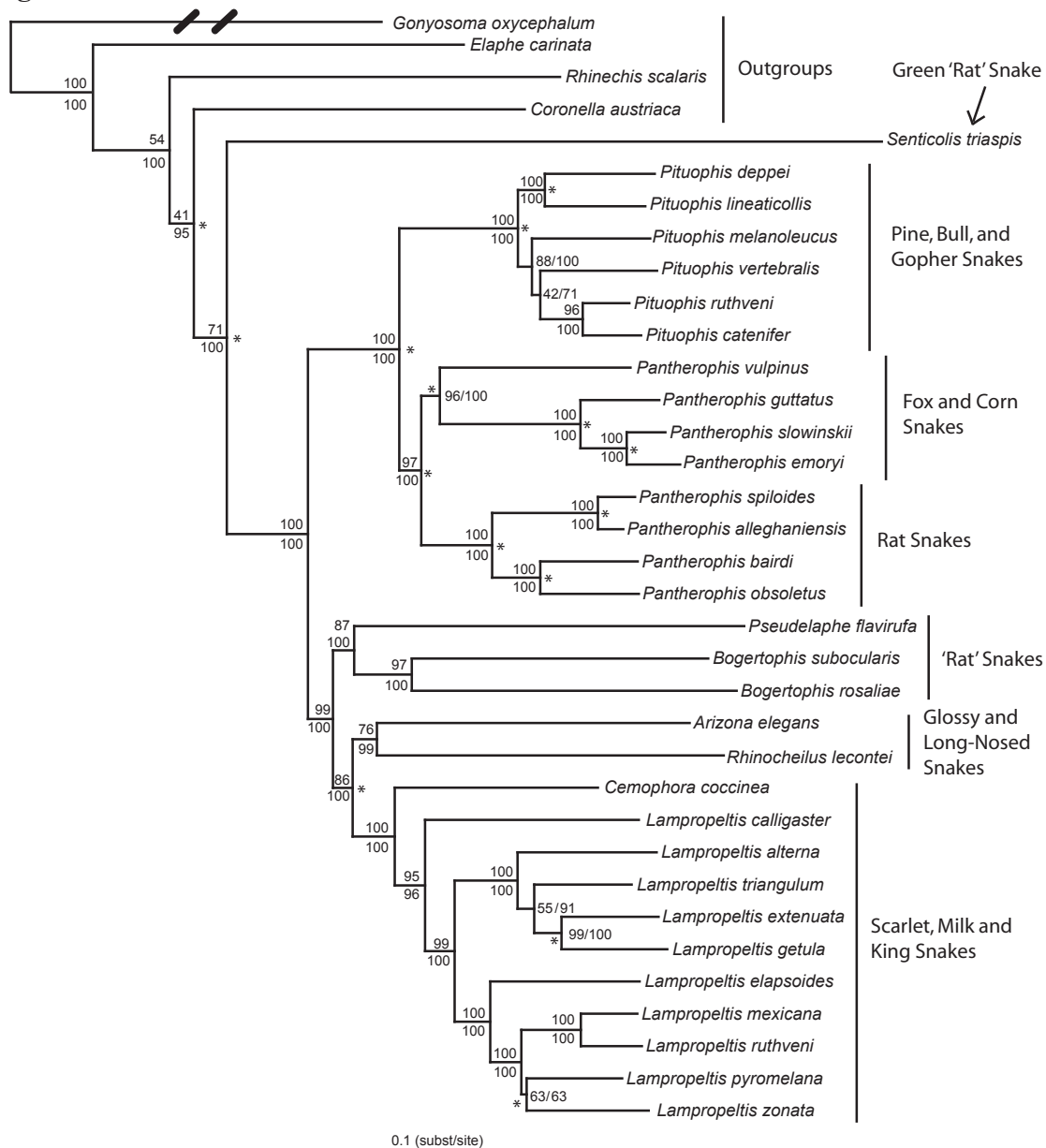


Figure 2

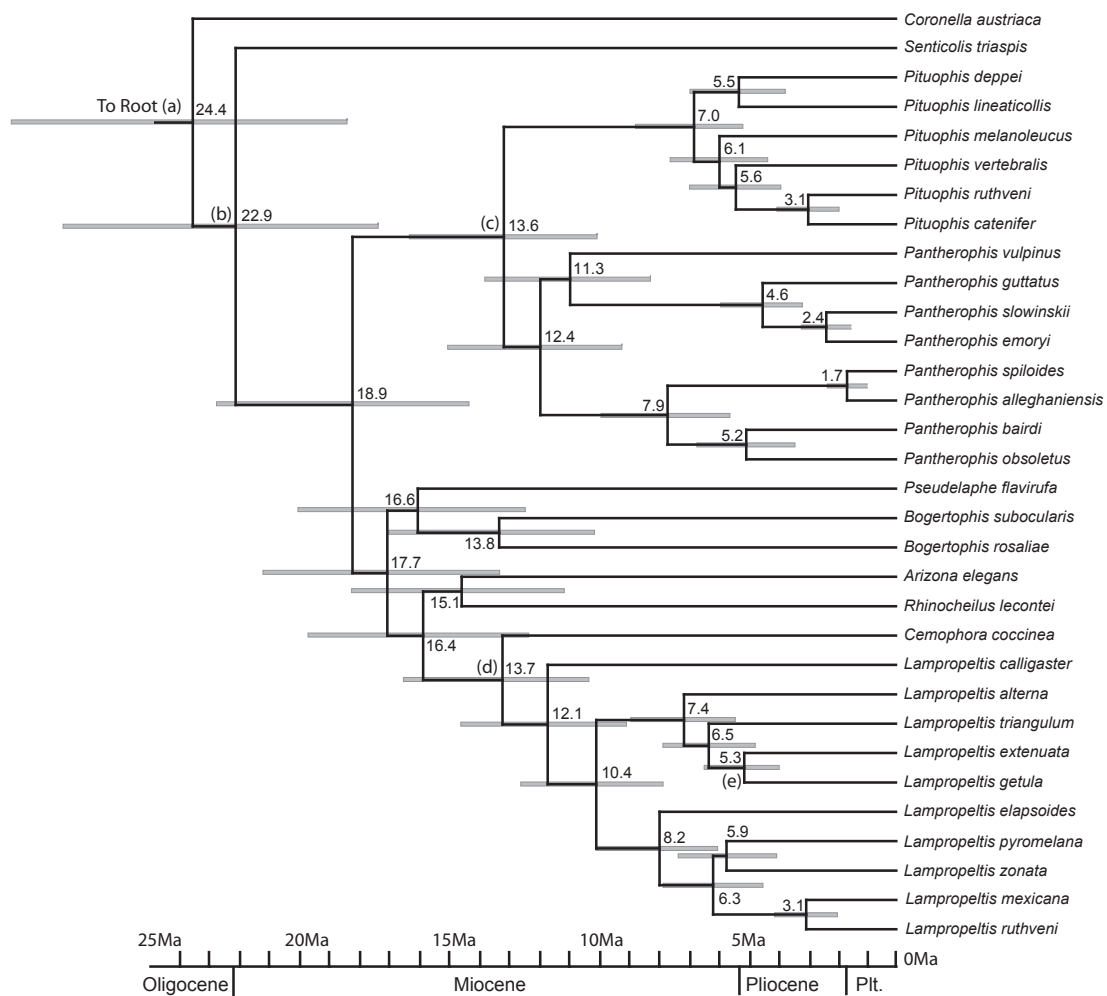


Figure 3

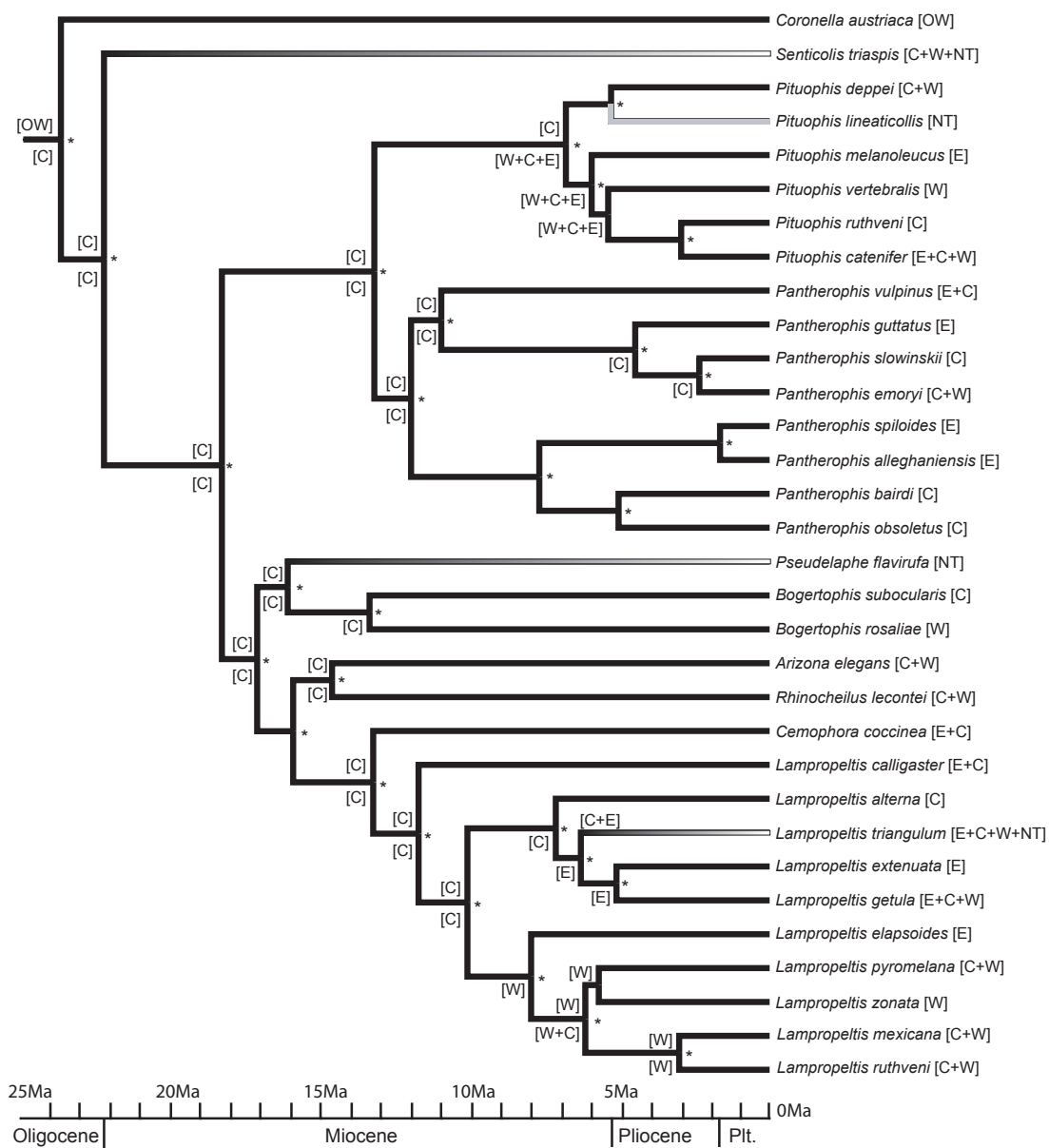


Figure 4

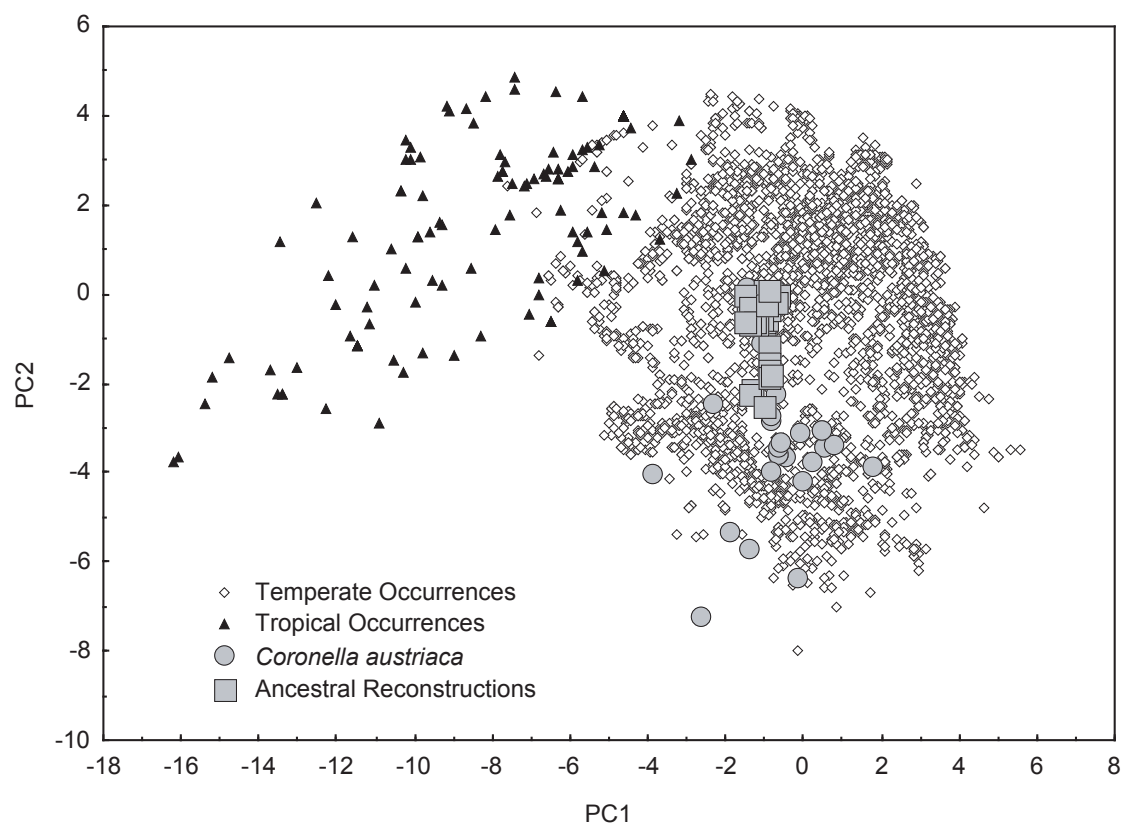


Figure 5

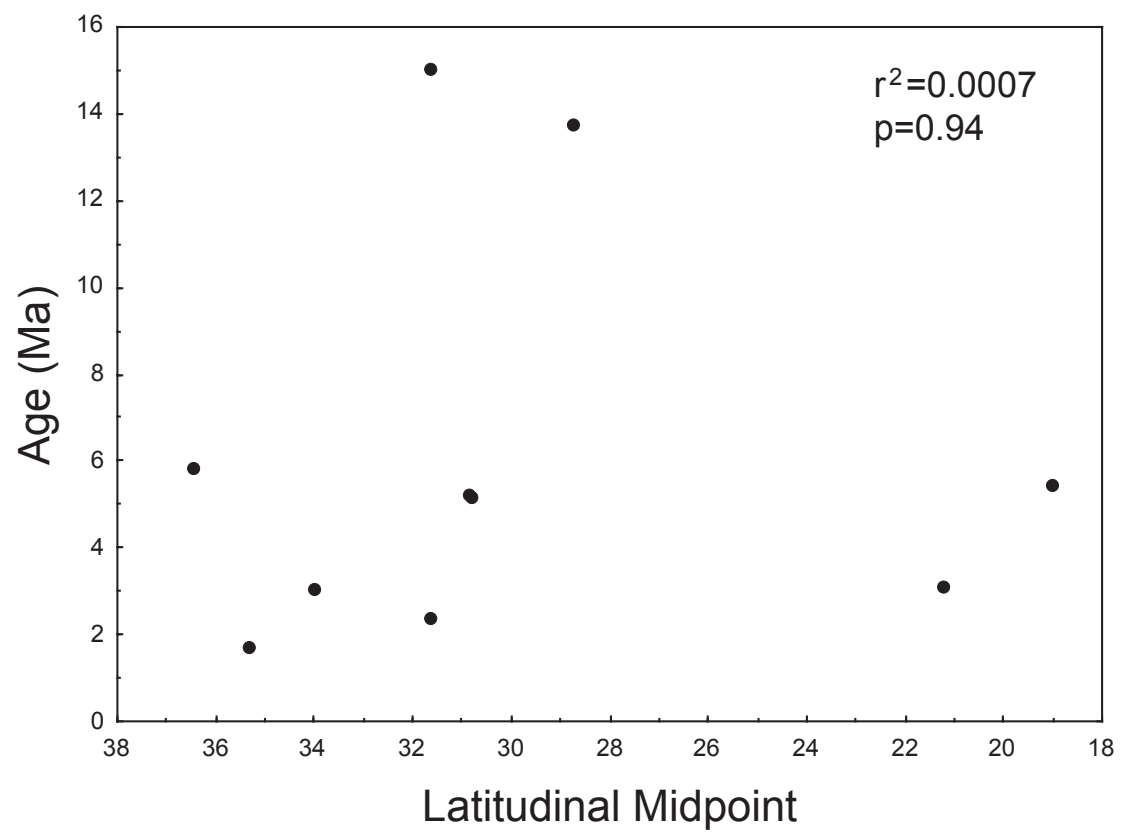


Figure 6

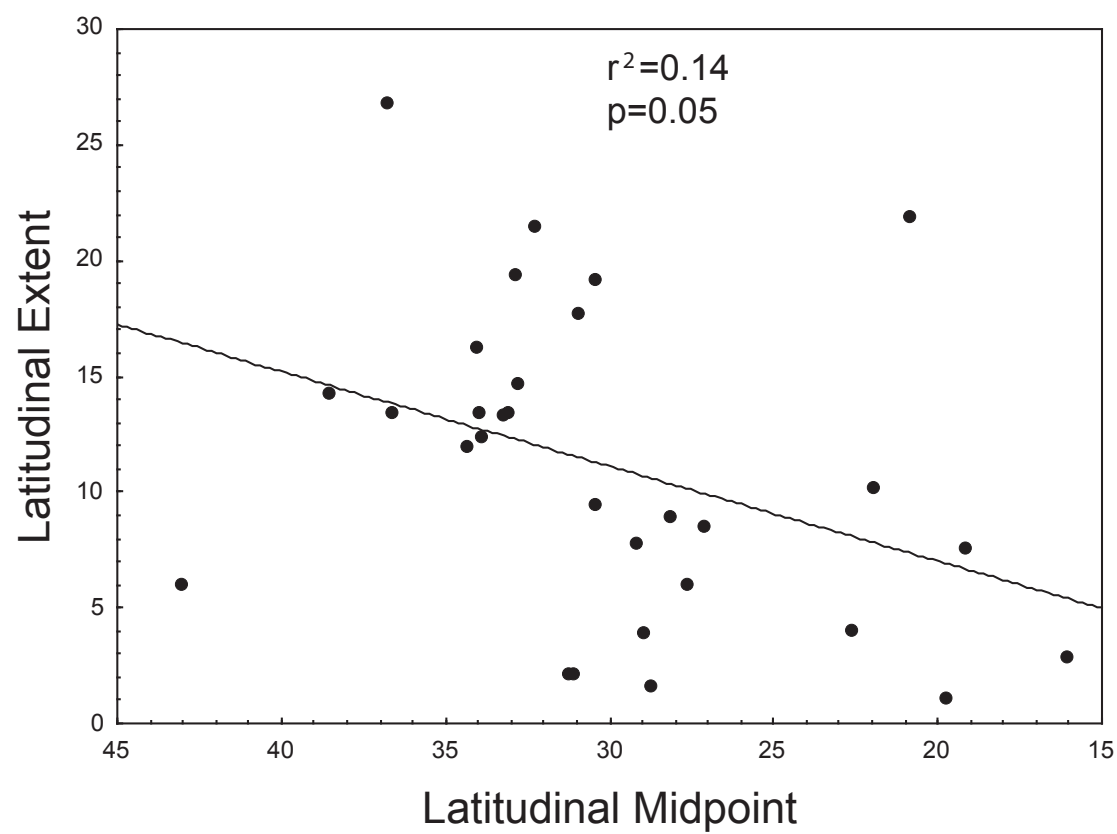


Figure 7

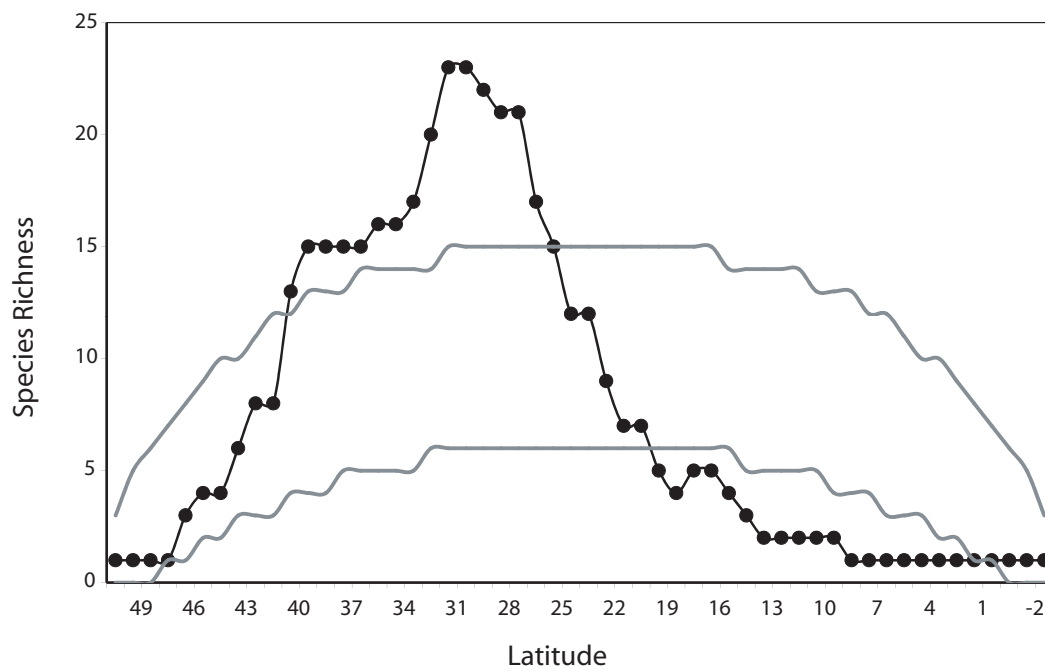


Figure 8

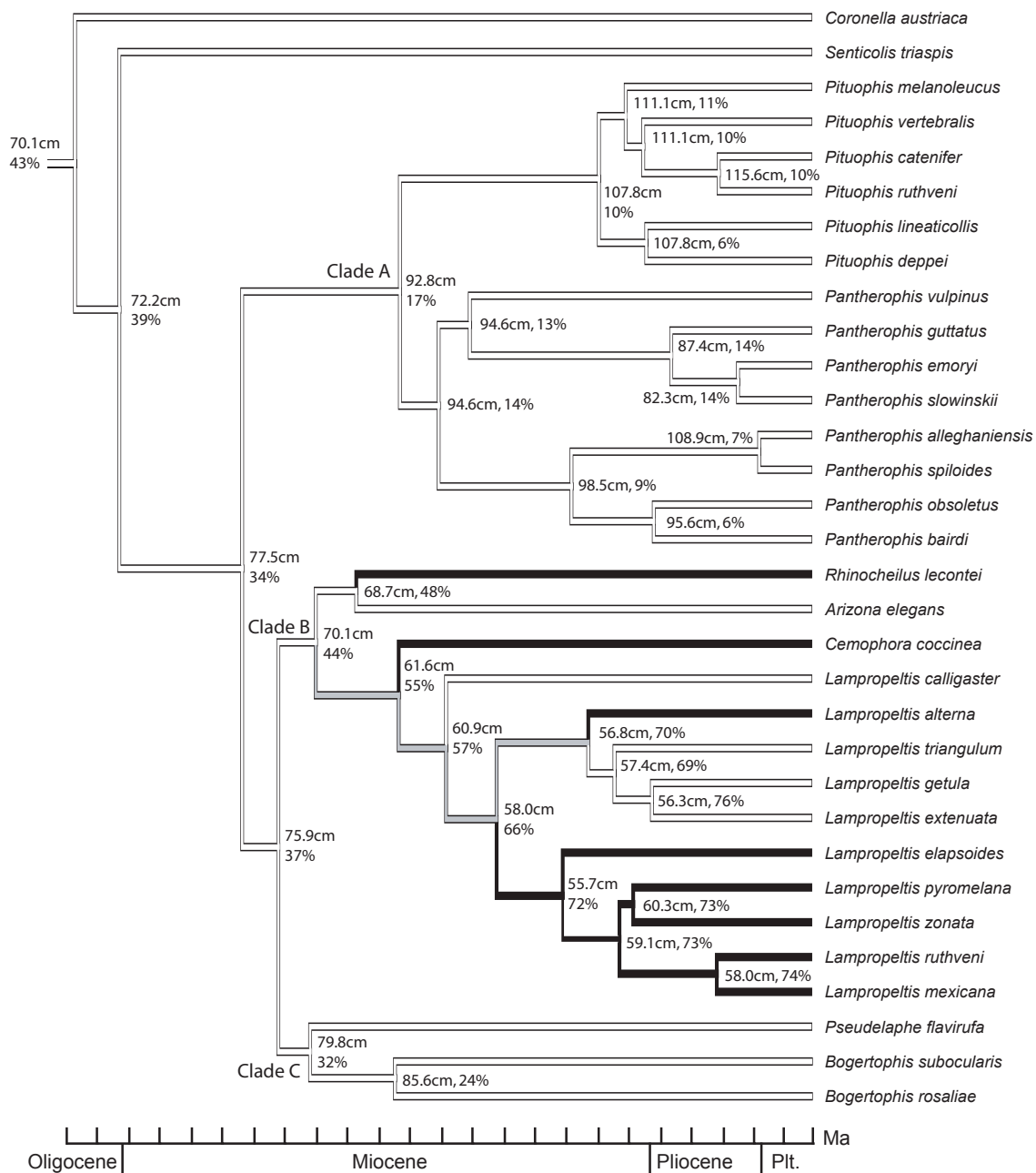


Figure 9

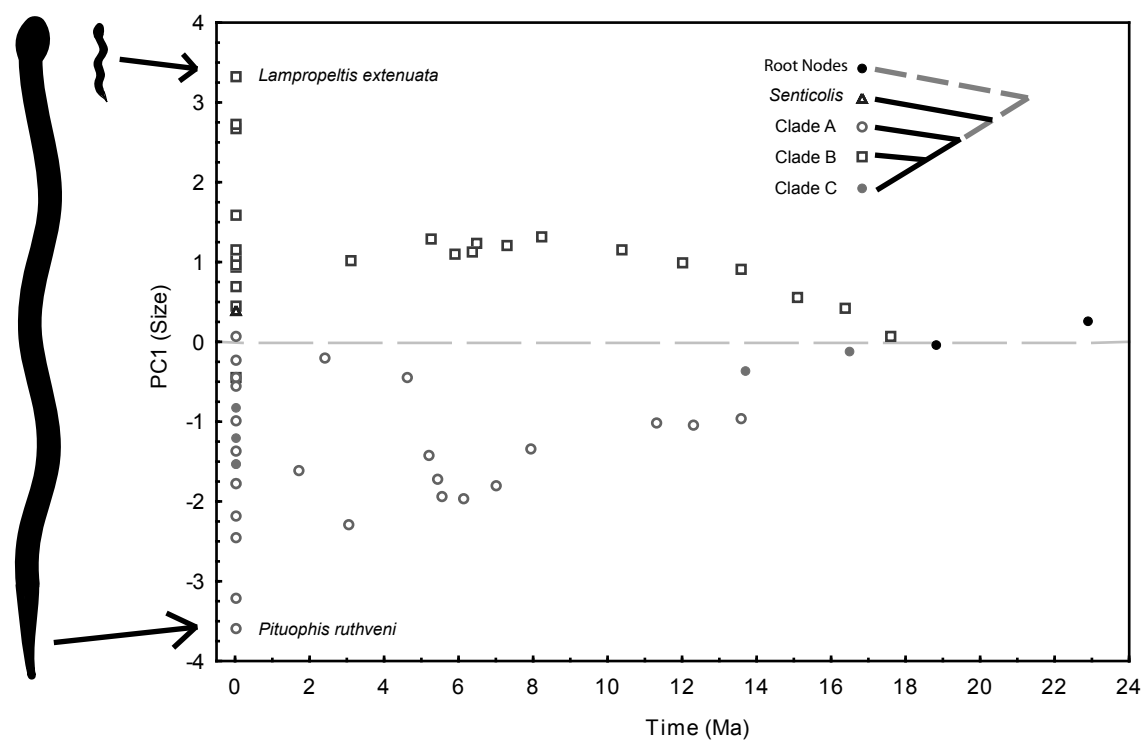


Figure 10

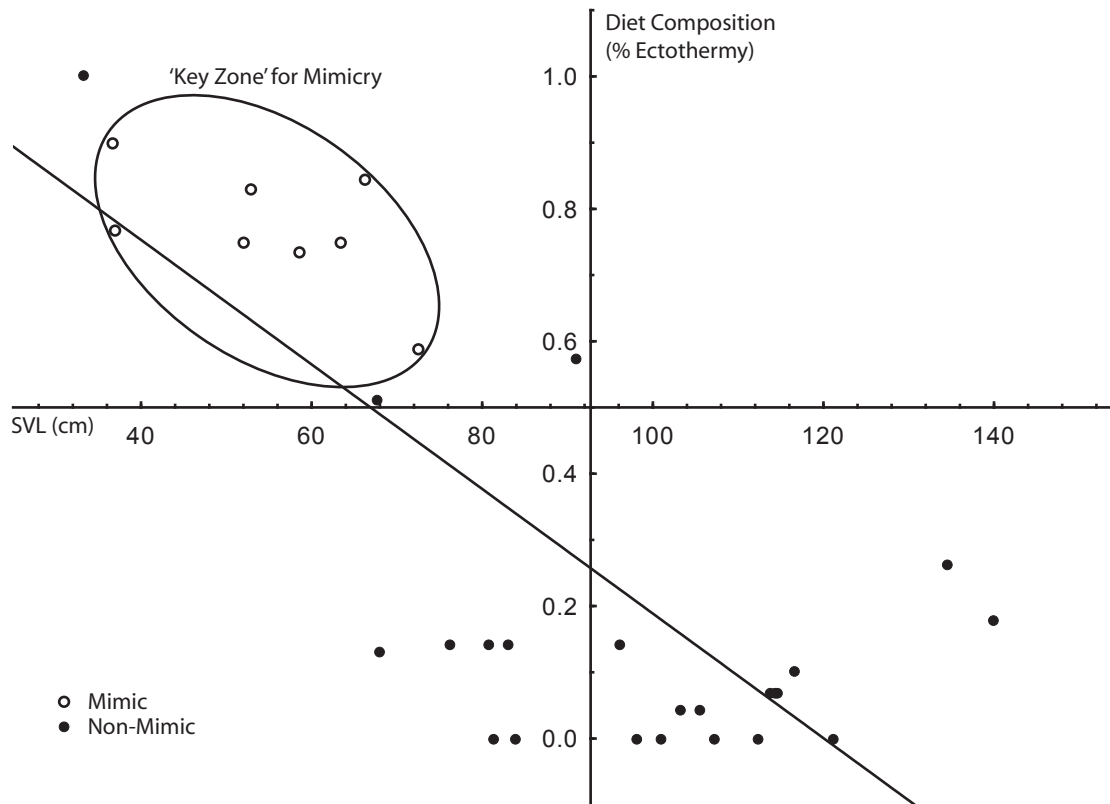


Figure 11

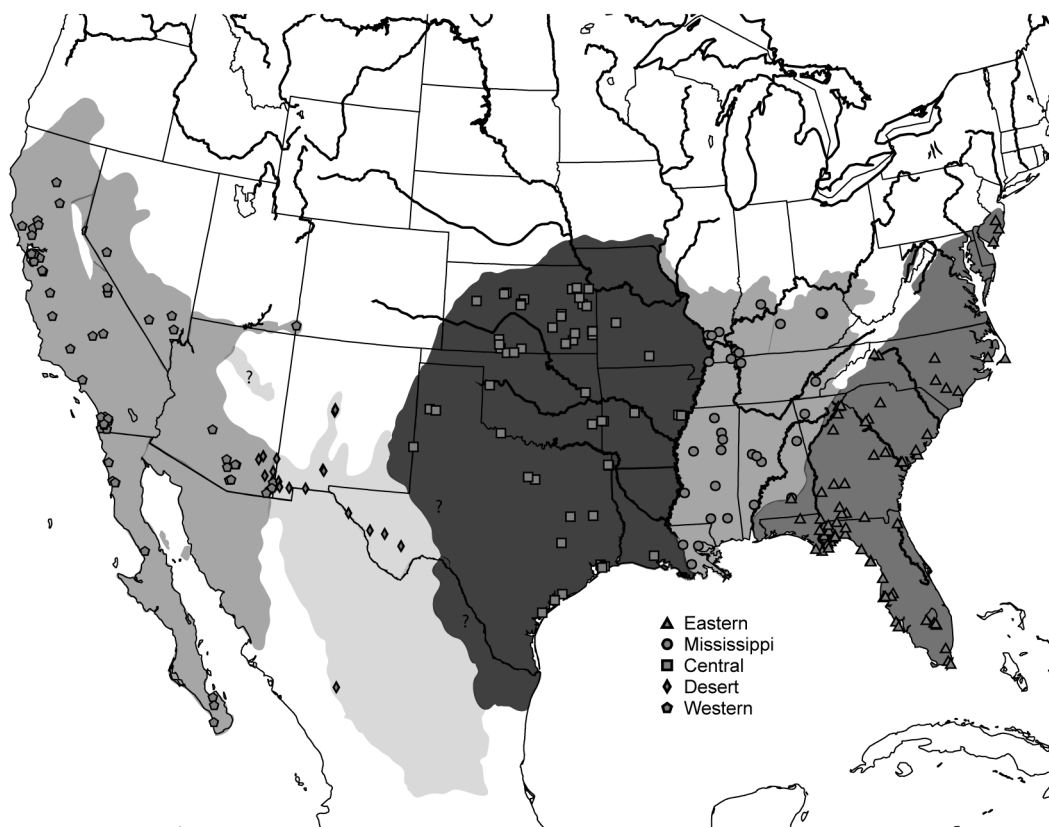


Figure 12

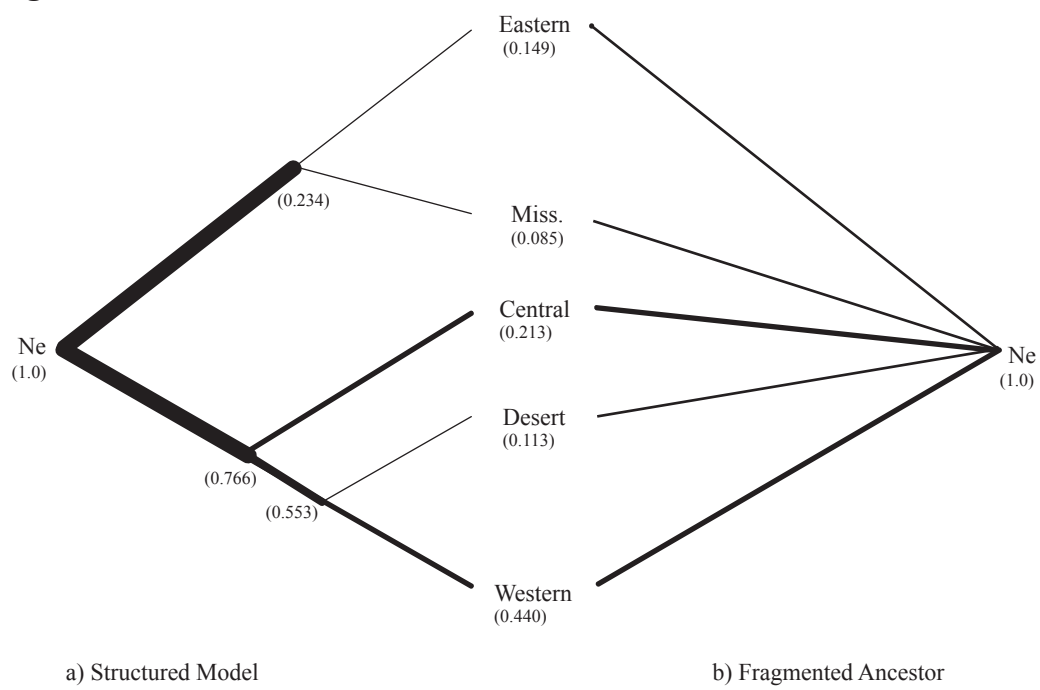
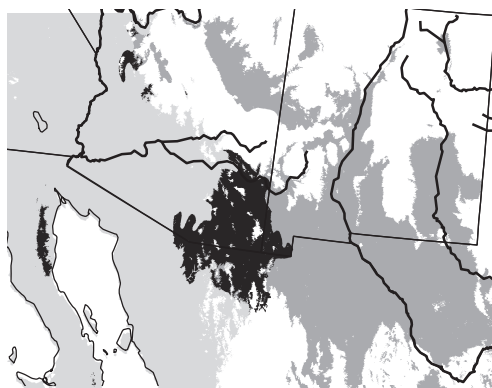
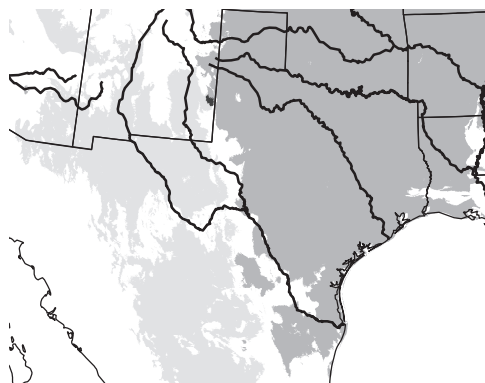
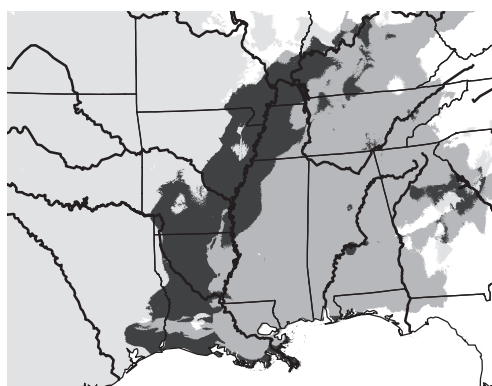


Figure 14

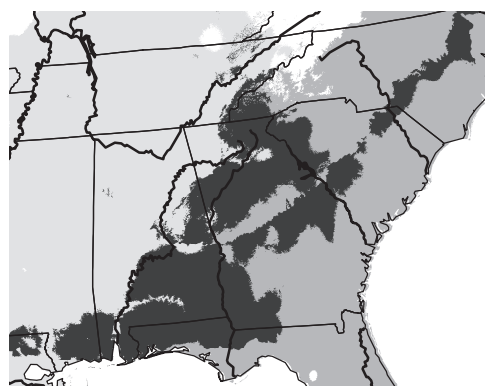
a) Western vs. Desert



b) Desert vs. Central



c) Central vs. Mississippi



d) Mississippi vs. Eastern

Figure 15

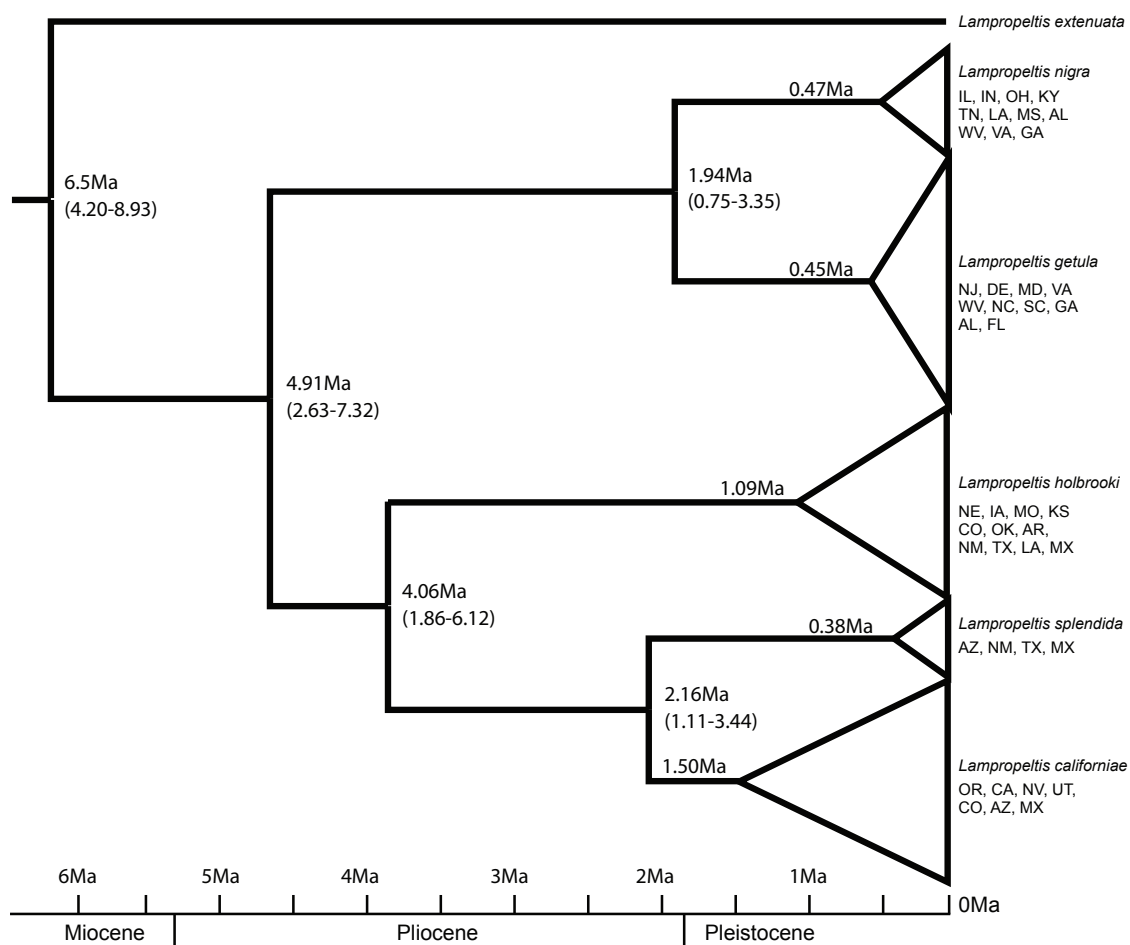


Figure 16

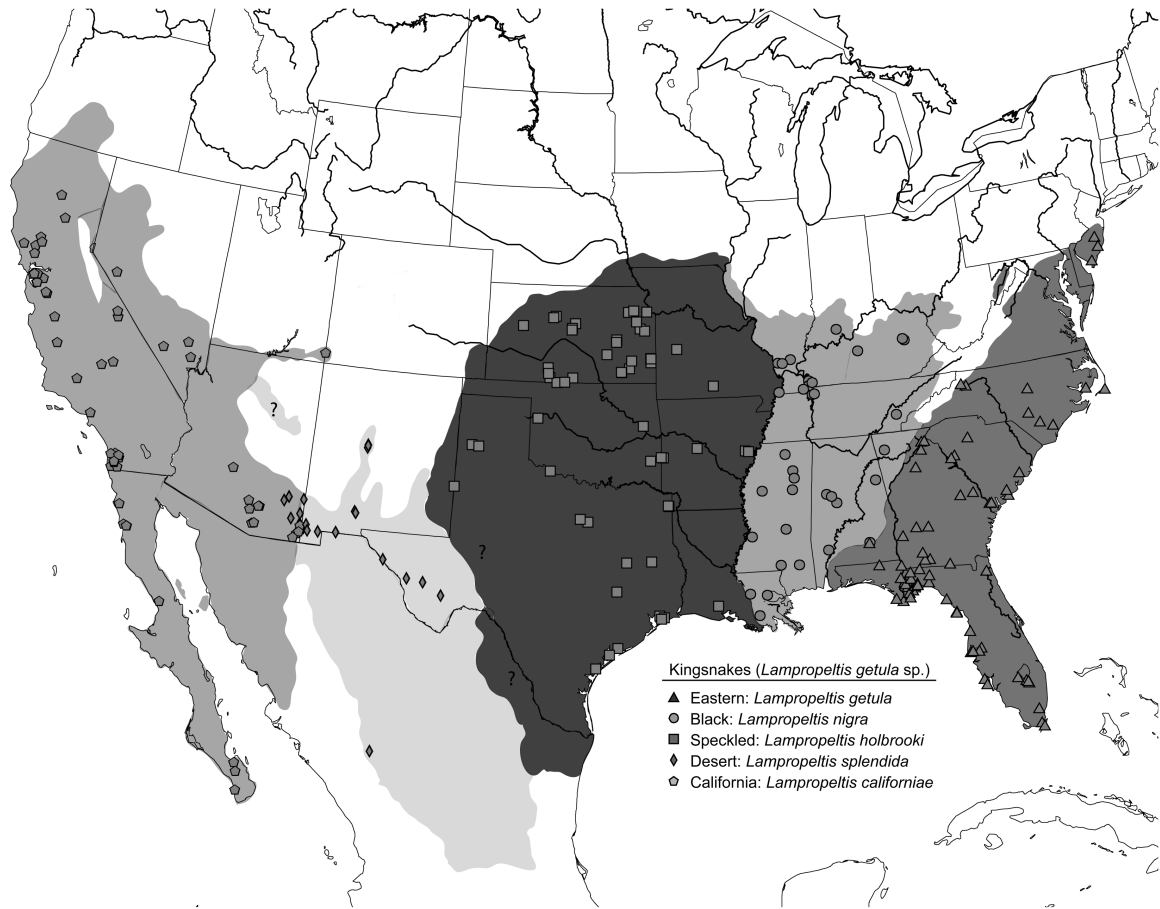


Figure 17

Lampropeltis getula

L) Chain pattern

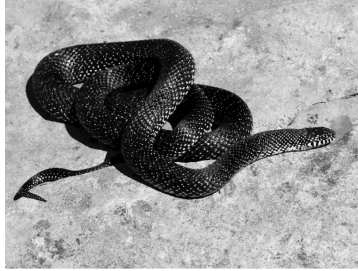
R) 'Floridana' pattern



Lampropeltis nigra

L) Black pattern

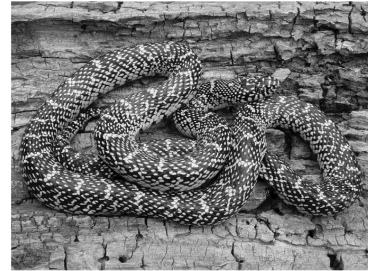
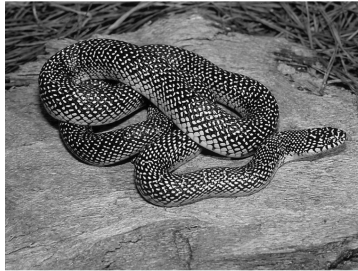
R) Speckled pattern



Lampropeltis holbrooki

L) Speckled pattern

R) 'Desert Intergrade'



Lampropeltis splendida

L) Desert pattern

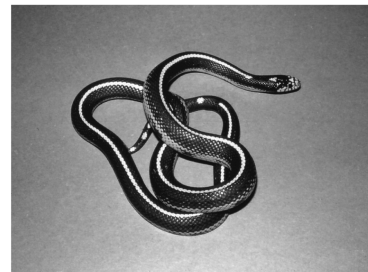
R) Darker adult



Lampropeltis californiae

L) Banded pattern

R) Striped pattern



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