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Phylogeographic analysis of the cornsnake (*Elaphe guttata*) complex as inferred from maximum likelihood and Bayesian analyses

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Abstract

Most phylogeographic studies have used maximum likelihood or maximum parsimony to infer phylogeny and bootstrap analysis to evaluate support for trees. Recently, Bayesian methods using Markov chain Monte Carlo to search tree space and simultaneously estimate tree support have become popular due to its fast search speed and ability to create a posterior distribution of parameters of interest. Here, I present a study that utilizes Bayesian methods to infer phylogenetic relationships of the cornsnake (*Elaphe guttata*) complex using cytochrome *b* sequences. Examination of the posterior probability distributions confirms the existence of three geographic lineages. Additionally, there is no support for the monophyly of the subspecies of *E. guttata*. Results suggest the three geographic lineages partially conform to the ranges of previously defined subspecies, although Shimodaira–Hasegawa tests suggest that subspecies-constrained trees produce significantly poorer likelihood estimates than the most likely trees reflecting the evolution of three geographic assemblages. Based on molecular support, these three geographic assemblages are recognized as species using evolutionary species criteria: *E. guttata*, *Elaphe slowinskii*, and *Elaphe emoryi* [phylogeographic, maximum likelihood, maximum parsimony, bootstrap, Bayesian, Markov chain Monte Carlo, cornsnake, Cytochrome *b*, geographic lineages, *E. guttata*, *E. slowinskii*, and *E. emoryi*].

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1. Introduction

Phylogeographic studies of widely distributed reptile taxa have resulted in the detection of distinct lineages likely to represent species, identified geographical features that reduce gene flow between populations, and allowed the proposal of taxonomies that reflect evolutionary history (Burbrink, 2001; Burbrink et al., 2000; Leache and Reeder, 2002; Rodríguez-Robles, 1999; Rodríguez-Robles, 2000; Zamudio et al., 1997). A marked genetic break (reflected in phylogenetic history) has been identified for multiple species at the Mississippi and Apalachicola Rivers (Avise, 1996; Burbrink et al., 2000; Leache and Reeder, 2002; Mayden, 1988; Walker et al., 1998; Wiley and Mayden, 1985). These dispersal barriers

may also limit genetic interchange for other unstudied taxa with similar distributions and habitat constraints.

The cornsnake (*Elaphe guttata*) is of particular interest here, as the range of this taxon is continuous across multiple aquatic barriers in the US, including the Mississippi River. It is predicted that a phylogeographic split at the Mississippi River should be noticeable if populations have existed on opposite sides of this River for a long time. Additionally, *E. guttata* is generally restricted to drier pine and grassland habitats and is often not found in mesic forests. Therefore, the flanking riparian forest along the Mississippi River should also strengthen this river barrier to cornsnake dispersal. As a result of these natural history observations, this makes the cornsnake an ideal candidate to determine if phylogeographic patterns reflect a split at the Mississippi River.

Found throughout the pine forests of the southeastern US and the prairie/semi-desert regions of the Southwest (Fig. 1), the complex of *E. guttata* is composed of five differently colored subspecies: *E. guttata guttata*, *Elaphe guttata rosacea*, *Elaphe guttata emoryi*,

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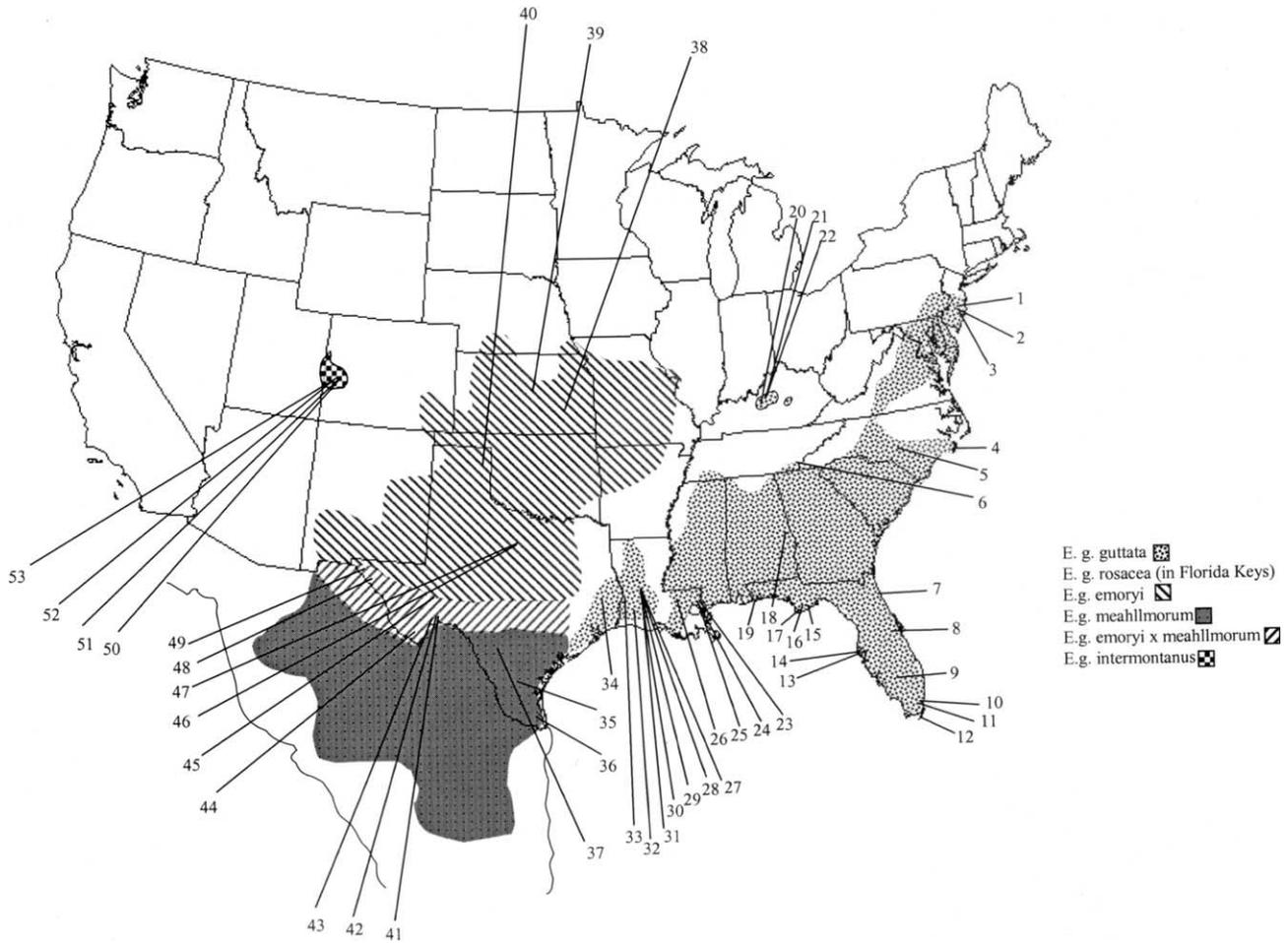


Fig. 1. Distribution of the subspecies of *Elaphe guttata* across North America with the localities of specimens sampled (Appendix A). *Elaphe guttata rosacea* is found in the Florida Keys and is represented by specimen number 12 on this map.

Elaphe guttata intermontanus, and *Elaphe guttata meahllmorum* (Schultz, 1996) (Fig. 1). The nominate form, *E. g. guttata*, occurs in the pine forests of the southeastern US and is distinguished by having an orange, reddish-brown, or gray ground color with 25–38 reddish-brown or orange dorsal blotches and lateral blotches bordered in black. This subspecies may have a black and white checker-board pattern or black ventral surface (Schultz, 1996). Similar in appearance to *E. g. guttata*, *E. g. rosacea*, of the Florida Keys, is smaller and displays a distinct reduction in dark pigment, and usually possesses 35–50 dorsal blotches. The more robust western prairie/semi-desert US subspecies, *E. g. emoryi*, has a brown or gray ground color, with 27–73 brown, gray, or olive dorsal blotches and similarly colored lateral blotches (Schultz, 1996). The ventral surface may be checkered or spotted. Of questionable taxonomic status are *E. g. intermontanus* and *E. g. meahllmorum*. The range of *E. g. intermontanus* is disjunct from populations of *E. g. emoryi* and restricted to western Colorado and eastern Utah. This form is slightly smaller than *E. g. emoryi* (Schultz, 1996). Finally, *E. g. meahllmorum*, restricted to southern Texas and Mexico, is distinguished

as having fewer than 44.5 blotches, whereas, *E. g. emoryi* usually displays greater than 44.5 blotches (Smith et al., 1994). If any of the subspecies of *E. guttata* represent distinct evolutionary lineages, then their histories may form distinct partitions in the phylogeny.

Phylogenetic estimates of population relationships of cornsnakes using DNA sequences from the mitochondrial gene cytochrome *b* were produced with maximum likelihood (ML) analyses. To determine support for these estimates, Bayesian analyses were employed. Typically, computer-intensive non-parametric bootstrap methods using maximum parsimony or ML models have been applied to estimate support for branches (Larget and Simon, 1999). Alternatively, several authors have used Bayesian inference to generate support for phylogenetic relationships (Huelsenbeck and Ronquist, 2001; Larget and Simon, 1999; Mau, 1996; Mau and Newton, 1997; Mau et al., 1999). Markov chain Monte Carlo (MCMC) with the Metropolis–Hastings algorithm was used to sample posterior probability space by these authors. This method has several advantages over traditional bootstrapping methods (Geyer, 1991; Huelsenbeck and Ronquist, 2001; Larget and Simon, 1999; Mau

et al., 1999): prior beliefs about the behavior of individual parameters can be incorporated into the model before a search of parameter space, the determination of support for branches using MCMC accompanies tree estimation, and the production of each tree provides a sample for the posterior probability distribution. Finding a single tree is not the goal of Bayesian search, but rather the production of a summary of the parameters of interest (particularly tree topology) as described by their marginal posterior distribution. This is in contrast to estimating uncertainty using the bootstrapping methods of ML and MP, where an optimal tree must first be assembled, bootstrap samples generated from the data, and trees subsequently re-estimated from each bootstrap sample (Hillis and Bull, 1993; Mau et al., 1999). Using the Metropolis-coupled MCMC allows the user to run multiple chains simultaneously. Additionally, these chains can swap states which potentially minimizes the chance of any chain becoming stuck on local optima (Huelsenbeck and Ronquist, 2001). Consequently, these attractive features of Bayesian inference lend themselves to analyzing this molecular data set, which is composed of many closely related samples.

2. Materials and methods

2.1. Data collection

Liver or shed skin was used as a source of mitochondrial DNA from 53 specimens of *E. guttata* and one specimen of each outgroup (*Elaphe obsoleta* and *Elaphe vulpina*). Tissues were obtained from across the range (Fig. 1) of *E. guttata* (Appendix A). Specimens were identified to subspecies by examination of bodies or by locality of capture within the range of a particular subspecies. Tissues were digested in 500 μ l of STE (0.1 M NaCl, 0.05 M Tris-HCl, and 0.001 M EDTA pH 7.5) with 25–50 μ l of Proteinase K (25 μ l for liver, 50 μ l for shed skin) and incubated at 55 °C for 2–24 h (generally overnight for shed skin). Undigested liver was removed following incubation. To increase DNA yield from shed skin, the undigested shed skin was included in the first round of DNA extraction and removed in the following rounds. Digestion of all tissue types was followed by four extractions with phenol/CHCl₃ (PCI). DNA was precipitated from the aqueous PCI layer using refrigerated absolute ethanol. After 1 h at –20 °C, samples were spun, dried, and resuspended in pure H₂O.

Cytochrome *b* was amplified from 3 μ l of template DNA in 50 μ l reactions using the following protocol: An initial template denaturation for 3 min at 94 °C followed by 40 cycles at 94 °C for 30 s (denature), 47 °C for 30 s (primer annealing), and 72 °C for 1 min (extension) and a final extension of 72 °C at 10 min after the previous 30 cycles. The following oligonucleotides were used to

amplify the 700+ base pairs of cytochrome *b*: light strand primer L4910B (5'GACCTGTGATMTGAAA AACCAYCGTT3') and heavy strand primer H15720 (5' TCTGGTTTAATGTGTTGTGGT3').

PCR products were cleaned using the QIAquick PCR purification kit (QIAGEN, Valencia, CA) and cycle sequenced using the Big Dye Terminator Sequence kit (Perkin-Elmer, Norwalk, CT). The cycle sequencing reaction was executed using the following protocol: 45 cycles at 96 °C for 10 s, 48 °C for 5 s, and 60 °C for 4 min. Sequencing primers were identical to amplification primers. Nucleotide sequences were ascertained using an ABI model 377 DNA Sequencer (Applied Biosystems, Norwalk, CT).

To reduce the chance of mistaking a nuclear pseudogene for the true mitochondrial cytochrome *b*, long-range PCR was used to amplify a 13.5 kb piece of DNA that includes the cytochrome *b* gene. The cytochrome *b* sequence obtained from this larger piece of mtDNA was compared to the sequence of the individually amplified cytochrome *b* sequence from the same specimen.

2.2. Phylogenetic analyses

DNA sequences were aligned by eye using Sequencher 4.1.2. (Gene Codes Corporation, 2000). To determine the appropriate model of sequence evolution and statistically compare successively nested more parameter-rich models for this data set, the program MODELTEST (Posada and Crandall, 1998) was used. The chosen model was applied to the data matrix to produce ML estimates using PAUP* version 4.0b2 (Swofford, 1999). Mr. Bayes 2.0 (Huelsenbeck and Ronquist, 2001) was used to generate a posterior probability distribution using Metropolis-coupled MCMC under the appropriate model. No a priori assumptions about the topology of the tree were made and all Metropolis-coupled MCMC searches were provided with a uniform prior. Three separate runs were performed to help assure that each of the searches were mixing well or searching randomly across the tree space landscape. Each search was run for four million generations and every 1000th tree was sampled. Burn-in, or the time for each parameter to reach stationarity, was determined when visual inspection indicated that the log-likelihood values reached an asymptote over a large number of generations. Posterior probabilities for each branch were calculated using the trees visited by the Markov chains after burn-in.

The ML trees with highest ln *L* score were compared to two alternative tree hypotheses: (1) trees constrained to reflect the monophyly of the three conventional subspecies taxonomy (*E. g. guttata*, *E. g. rosacea*, and *E. g. emoryi*) and (2) constraint trees that reflect the monophyly of the five subspecies (*E. g. guttata*, *E. g. rosacea*, *E. g. emoryi*, *E. g. intermontanus*, and *E. g. meahlmo-*

rum). Since the identities and the validity of *E. g. intermontanus* and *E. g. meahllmorum* are questionable (especially with some *E. g. meahllmorum* specimens found in areas intergrading into *E. g. emoryi*) these subspecies were classified as the currently accepted *E. g. emoryi* in the treatment that constrains the traditional three subspecies taxonomy. Maximum likelihood parameters were estimated using this data set separately on the three and five subspecies trees. Subsequent ML searches were performed until trees with the highest likelihood were produced under the two respective taxonomic constraint

systems. Since the unconstrained ML trees were not specified a priori, the use of the Kishino–Hasegawa likelihood test to compare competing topologies is inappropriate (Goldman et al., 2000; Hasegawa and Kishino, 1989; Kishino and Hasegawa, 1989; Shimodaira and Hasegawa, 1999). Instead, the Shimodaira–Hasegawa test (SH test) (Goldman et al., 2000; Shimodaira and Hasegawa, 1999) was used with full likelihood optimizations and 1000 bootstrap replications (PAUP* version 4.0 b.2). The probability of support for the monophyly of each subspecies was assessed by examin-

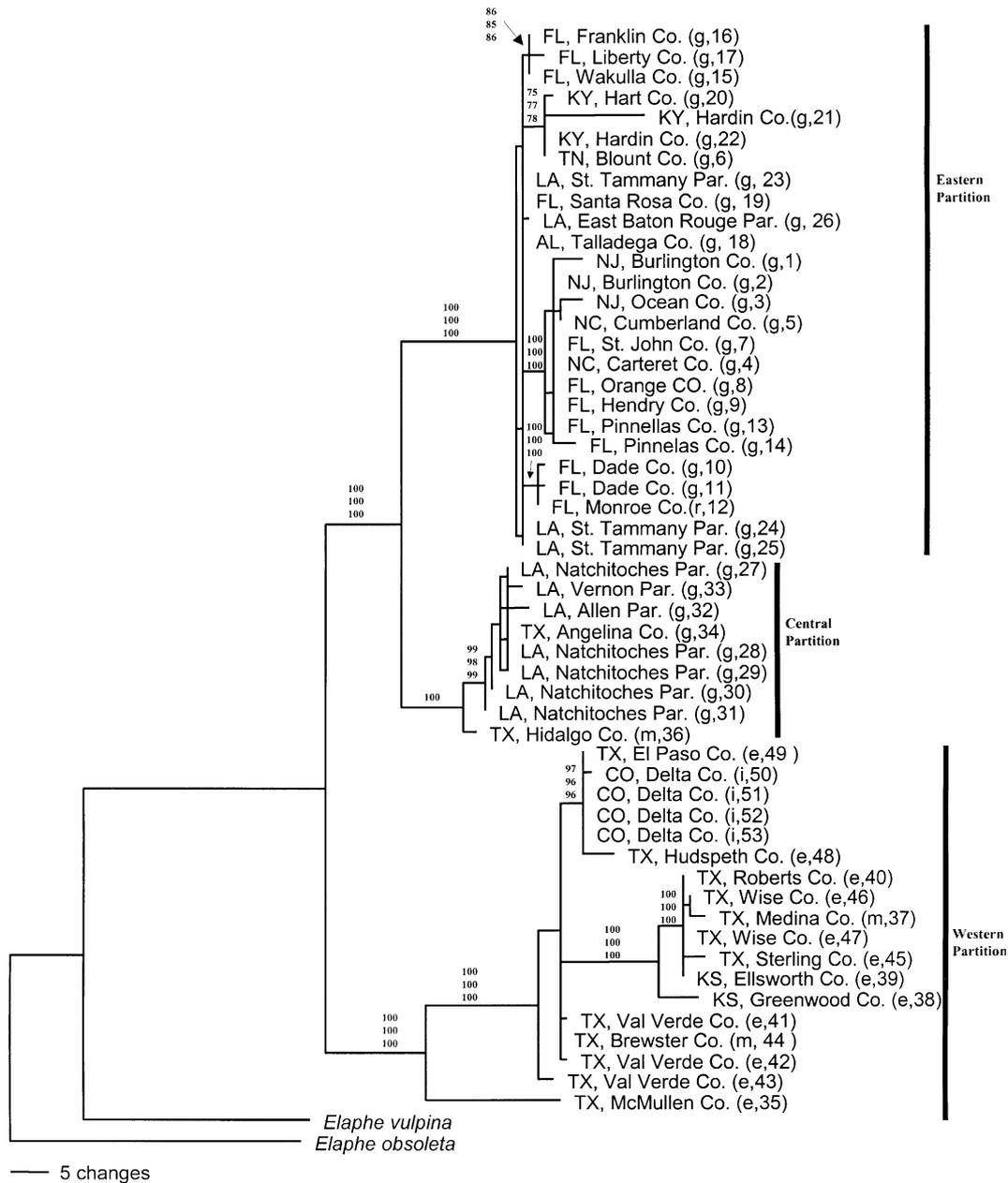


Fig. 2. Consensus of nine maximum likelihood trees showing localities of samples of *Elaphe guttata* with posterior probability support values > 74% listed above branches. These three values represent the percentage of support for each branch determined from each of the three separate searches, listed from top to bottom, respectively. Letters in parentheses represent the following subspecies: g = *guttata*, r = *rosacea*, e = *emoryi*, m = *meahllmorum*, and i = *intermontanus*. Numbers in parentheses represent the localities listed in Fig. 1 and Appendix A.

ing the posterior probability distribution generated from the post-burn-in Bayesian searches. The number of times a bipartition is recovered in the posterior probability distribution is directly proportional to the probability of support for that bipartition.

3. Results

Sequences for all individuals ranged from 560 to 760 bp of the total 1117 bp of the cytochrome *b* gene for *E. guttata*. All sequences had an open reading frame with no internal stop codons and no gaps were detected. The amplified fragments likely originated from the mitochondria and were not pseudogenes due to the fact that sequences obtained from the cytochrome *b* section of the 13.5 kb mitochondrial amplification were identical to the 700+ bp amplifications.

MODELTEST (Posada and Crandall, 1998) determined that the HKY85 (Hasegawa et al., 1985) model with γ -distributed rates is the statistically appropriate model for the data set. The value for the γ -shaped parameter with four discrete rate categories was 0.334693 and the transition/transversion ratio was 3.582 with the following nucleotide frequencies: *A* = 0.32430, *C* = 0.26919, *G* = 0.11331, and *T* = 0.29320. The maximum likelihood search using those parameters found nine trees with a $\ln L$ of -2631.61072 (Fig. 2). A majority rule consensus of these nine trees clearly shows three large geographical partitions of *E. guttata*; a group partitioned east of the Mississippi River (eastern partition), a group bound between the Mississippi River in the east and the central plains/deserts in the west (central partition), and a western assemblage existing in the plains and desert (western partition).

An inspection of plots for $\ln L$ tree stationarity of all three Metropolis-coupled MCMC searches revealed that burn-in occurred between 10,000 and 20,000 generations (Fig. 3B). To buffer these estimates, the credible posterior probability distribution was created from all tree samples taken after 40,000 generations. The mean $\ln L$ score of all post-burn-in tree topologies for search 1, 2, and 3 were practically identical: -2691.878 , -2691.860 , and -2691.883 , respectively. Support for this tree was assessed by examining the number of times each branch was recovered from the posterior probability of all post-burn-in trees. Branches found in greater than 74% of all posterior distribution samples for each of the three searches are plotted on the ML tree (Fig. 2). The three geographic assemblages (eastern partition, central partition, and western partition) were found in 100% of the trees sampled from the posterior probability distribution for all searches. Other partitions appear at 98–100% within these primary geographic assemblages. These smaller well-supported assemblages appear to be geographically clustered as well (Fig. 2).

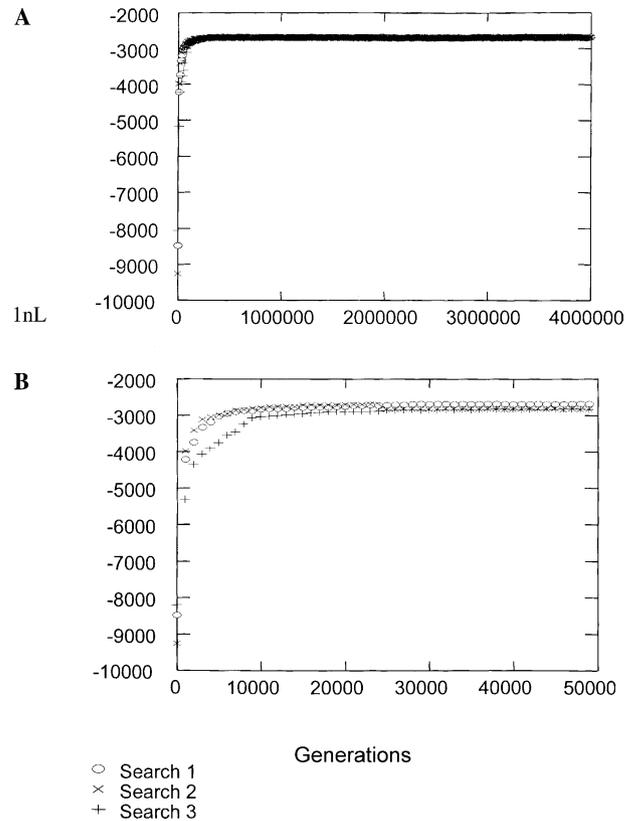


Fig. 3. Burn-in plots of tree topology likelihoods versus generation number for: (A) all generations; and (B) magnified view for the first 50,000 generations presented to help visualize the range where burn-in occurs.

The posterior probability distributions from each of the three Bayesian searches failed to produce trees supporting the monophyly of any subspecies. The most likely subspecies trees constrained to reflect the monophyly of *E. g. guttata*, *E. g. rosacea*, and *E. g. emoryi* produced a significantly lower likelihood score (-2874.0116) than the nine most likely trees (-2631.61072) as demonstrated by the Shimodaira–Hasegawa test ($P < 0.002$). The most likely constraint trees with five monophyletic subspecies (*E. g. guttata*, *E. g. rosacea*, *E. g. emoryi*, *E. g. intermontanus*, and *E. g. meahllmorum*) also yielded lower likelihood scores (-2946.74539) than the maximum likelihood tree with no constraints ($P < 0.002$).

4. Discussion

Maximum likelihood produced phylogeographic estimates of *E. guttata* that revealed the presence of three large geographic assemblages: the eastern partition, central partition, and western partition. Support for the assemblages was derived from the posterior probability distribution obtained using Bayesian methods of searching by Metropolis-coupled MCMC without an a priori

defined tree. Three separate searches of four million generations each produced a posterior probability distribution of topologies in 42 h (using Mr. Bayes 2.0; Huelsenbeck and Ronquist, 2001 on a PC running Linux at 1.3 MHz). Every tree within the posterior probability distribution supported the monophyly of the three major geographic assemblages discovered in the ML searches. Due to the phylogenetic concordance between ML and Bayesian methods and the character support values obtained from the posterior probability distribution of these independent searches, it seems likely that the three assemblages are quite credible using cytochrome *b*.

The Bayesian methods employed here produced support for three assemblages that appear to be separated by geographic barriers or habitat differences of known importance in the distribution of other species. Although the eastern and central partitions of *E. guttata* are generally found in sandy pine forests and grass flatwoods, their ranges are severed by the Mississippi River with the accompanying wide mesic hardwood riparian forest. The Mississippi River is a known phylogeographic barrier (Blair, 1958, 1965; Burbrink et al. 2000; Leache and Reeder, 2002; Leache and Reeder, 2002; Mayden, 1988; Walker et al., 1998; Wiley and Mayden, 1985) and has probably disrupted gene flow in many other terrestrial species with trans-Mississippi ranges. Unlike certain other taxa distributed throughout the southeastern US, no genetic break was discovered within the eastern partition at the Apalachicola River (Avice, 1992, 1996; Birmingham and Avice, 1986; Blaney, 1971; Burbrink et al., 2000; Lawson, 1987; Neill, 1957; Swift et al., 1985). The central partition seems to follow the western extent of the eastern pine and pine-hardwood forests of the US and individuals probably do not occur in the prairie/semi-desert habitats found farther west. This apparent western edge of the range of the central partition into the Austroriparian habitats of east Texas coincides with the western limit of the ranges of several snake taxa: *Cemophora coccinae copei*, *Farancia abacura*, *Lampropeltis triangulum amaura*, *Masticophis flagellum flagellum*, *Nerodia erythrogaster*, *Nerodia flavigaster*, *Nerodia fasciata confluens*, *Pituophis ruthveni*, *Regina rigida*, *Storeria occipitomaculata*, *Agkistrodon contortrix contortrix*, and *Sistrurus miliarius* (Werler and Dixon, 2000). The largest and most basal phylogenetic split occurs between the combined eastern/central assemblages of the eastern US forests and members within the western assemblage found in the drier plains/desert regions of the west. By definition, the split between these two groups is older than the genetic break at the eastern and central partitions at the Mississippi River.

4.1. Taxonomic implications

The discordance between the currently accepted subspecies and the most likely trees produced here is of tax-

onomic interest. The constrained subspecies trees have statistically lower likelihood values than the ML tree reflecting the three geographic assemblages, and no subspecies were discovered to be monophyletic when examining the posterior probability distributions. However, most members of the eastern partition were defined as *E. g. guttata*. Although only one specimen of *E. g. rosacea* from the Florida Keys was examined, it is clearly a member of the eastern partition and always groups with the most proximal mainland specimens (Dade, FL). This agrees with previous studies that have demonstrated that specimens from mainland Florida often resemble *E. g. rosacea*, and specimens from the Florida Keys often resemble *E. g. guttata*, indicating that there has been recent gene flow. (Duellman and Schwartz, 1958; Thomas, 1974; Mitchell, 1977). These studies have recommended that *E. g. rosacea* not be recognized as a distinct form. The generally bright color patterns indicative of *E. g. guttata* and *E. g. rosacea*, with the exception of certain northern populations, are usually indicative of members found in the eastern partition. The gray or brown ground color of specimens from the central partition often resembles the ground color of specimens identified as *E. g. emoryi*. Based on the intermediacy of ground color, it was thought that members from western Louisiana may represent remnant intergrades between *E. g. guttata* and *E. g. emoryi* (Thomas, 1974). However, using evidence from scale morphology and color pattern, Vaughan et al. (1996) determined that these central partition specimens are more similar to the eastern *E. g. guttata*. Phylogenetically, specimens from the central assemblage are more closely related to members of the eastern assemblage. The sample of *E. g. meahllmorum* originating from that collected in the Tamaulipan habitat of Hidalgo, Texas was collected within the putative range of the western partition. This specimen is a taxonomic mystery because it clusters just outside of the cohesive central partition members. Moreover, specimens morphologically defined as *E. g. guttata* in Texas by Vaughan et al. (1996), which most likely represent members of the central partition, have been found near the Tamaulipan habitat of south Texas (Werler and Dixon, 2000). It is possible that the specimen from Hidalgo, TX may represent yet another undiscovered partition or that there is remnant gene flow among members of the central and western partitions. All specimens from the western partition have traditionally been identified as *E. g. emoryi*. There is no statistical support for a monophyletic *E. g. intermontanus*. Moreover, three of the four specimens of *E. g. intermontanus* have identical sequences to that of a specimen of *E. g. emoryi* originating from El Paso, TX. This agrees with the decision of Smith et al. (1994) to regard *E. g. intermontanus* as a junior synonym of *E. g. emoryi*. Finally, *E. g. meahllmorum* does not form a monophyletic group with respect to *E. g. emoryi* and should also be recognized as a junior synonym of *E. g. emoryi*.

The implication of the phylogenetic estimate presented here on the species taxonomy of *E. guttata* must be addressed. An important function of systematics is to determine which evolutionary lineages represent species (DeQueiroz, 1998). Several authors have argued that mtDNA should not be used alone to delimit species (Moritz, 1994; Moritz et al., 1992; Sites and Crandall, 1997) and that mtDNA only yields estimates of female gene flow (Avise, 1994). These arguments have effectively been countered by several authors (Moore, 1995, 1997; Wiens and Penkrot, 2002). The effective population size of mtDNA is 1/4 that of the effective population size of nuclear DNA and, therefore, should coalesce four times faster than nuclear genes. This rapid rate of mtDNA coalescence should readily allow for the detection of recently evolved species (Moore, 1995; Wiens and Penkrot, 2002). This property makes mitochondrial markers a valuable tool in determining species limits with great efficiency and probability of success (Wiens and Penkrot, 2002). Wiens and Penkrot (2002) have formalized a set of criteria for concluding if mitochondrial lineages represent species. First, ingroup lineages must be exclusive of the outgroup. Second, lineages within the species complex must be geographically exclusive of one another. Determining if each basal lineage is concordant with a particular geographic area and exclusive (i.e., species) is applicable to the research presented here. Although heavy sampling of the outgroup taxa (*E. obsoleta* and *E. vulpina*) was not used to determine if the ingroup (*E. guttata*) was exclusive with respect to those outgroup taxa, the approach of Wiens and Penkrot (2002) is still applied. These outgroup taxa are genetically and morphologically distinct from the ingroup taxa and haplotypes are not shared between them. The three lineages of *E. guttata* are each geographically confined to discrete areas, generally exclusive of one another, and most likely represent three distinct species. There are several allopatric populations that have diverged so recently that little or no mitochondrial sequence variation can be found between them and the basal partitions (i.e., specimens from Kentucky in the eastern partition and Colorado specimens in the western partition). Although these isolated populations are separated from the large continuous partitions and may represent independent evolutionary lineages, they will not be considered species here because they have not been appreciably examined morphologically or molecularly. Although the three partitions appear to be independent evolutionary lineages occupying distinct geographic areas, one specimen (Hidalgo, TX) of the central partition was located outside of the presumed geographic area and habitat identified for other members of that partition. It is unknown if this is the result of gene flow between the central and western partition, the range of the central partition actually extends farther west and south, or the specimen is a

member of yet another undiscovered partition. Specimens presumed to be from the central partition are morphologically distinct from members of the western partition within Texas. Moreover, there appears to be a significant gap in the ranges of these two partitions in Texas (Dixon, 1987; Vaughan et al., 1996; Werler and Dixon, 2000). The oldest names of type specimens within the eastern and western partitions happen to be the names of the dominant subspecies within those areas. Therefore, the eastern and western partitions should have their subspecific names elevated to *E. guttata* and *E. emoryi*, respectively. There are no previously named type specimens definitely found within the range of the central partition. Bonnaterre (1790) possibly described a specimen from Louisiana and named it *Coluber maculatus*, although it was not determined from which side of the Mississippi River the specimen was secured or if the sample was actually obtained from Louisiana specifically (the specimen was obtained prior to the Louisiana Purchase and the currently defined boundaries of the State of Louisiana). Smith et al. (1994) determined that Bonnaterre (1790), without a type specimen, may have examined and described the color pattern of another species of snake (*Lampropeltis calligaster*) and copied the ventral and caudal counts from Catesby's (1743) pre-Linnean description of *E. guttata* from Carolina. Smith et al. (1994) relegated the name of *C. maculatus* to the status of junior synonym of *E. g. guttata* based on Catesby's Carolina description of that taxon. It appears that the central partition represents a distinct lineage and is given the name *E. slowinskii* in honor of Joseph B. Slowinski. Although more detailed morphological analyses are required in light of the phylogenetic estimates presented here, a brief description of the three species within the *E. guttata* complex follows.

4.2. *Elaphe guttata*

Coluber guttatus Linnaeus (1766, p. 385). Type locality: "Carolina." Subsequently restricted to Charleston, Charleston, SC by Dowling (1952). (Holotype: Zoological Museum of Uppsala 147).

Coluber maculatus Bonnaterre (1790, p. 19);
Coluber compressus Donndorf (1798, p. 206);
Coluber carolinianus Shaw (1802, p. 460);
Coluber molossus Daudin (1803, p. 269);
Coluber pantherinus Daudin (1803, p. 318);
Coluber floridanus Harlan (1827, p. 360);
Coluber guttatus sellatus, Cope (1888, p. 387);
Coluber rosaceus Cope (1888, p. 388).

Standard English name. Red Cornsnake

Distribution. This species is found throughout the southeastern US, ranging from the New Jersey Pine Barrens to the Florida Keys following the eastern edge of Appalachian Mountains and the Atlantic coastal

plains (Fig. 4) (Schultz, 1996). The range continues west to the Mississippi River in southern Louisiana. Specimens displaying the typical *E. guttata* color pattern have been found in Louisiana west of the Mississippi River in Point Coupee Par. (LSUMZ 8669), Iberia Par. (LSUMZ 79045), and West Baton Rouge Par. (LSUMZ 40526). It appears this taxon ranges west of the Mississippi River to the banks of the Atchafalaya River (Dundee and Rossman, 1989), the original outlet of the Mississippi River and possibly the true western boundary between *E. guttata* and *E. slowinskii*. *Elaphe guttata* species ranges northward to the Cumberland Plateau from the Tennessee River to the Mississippi River. Large gaps in the range of *E. guttata* occur in Kentucky, and possibly northern North Carolina, Virginia, Maryland, and New Jersey. Sequences from one of the disjunct Kentucky specimens are identical to an individual sequenced from Tennessee.

Description. This medium-sized snake averages from 90 to 120 cm and has 203–245 ventral scales and 47–84 subcaudals (Schultz, 1996). They possess 25–38 reddish-brown or orangish rectangular blotches with black margins on a red, orange, yellowish, brown, or gray ground color (Christman, 1980; Mitchell, 1977). Speci-

mens from the northern part of the range often have a dull gray ground color. In addition, specimens from southern Florida may have a very light gray ground color with very dark dorsal blotches. Lateral dorsal blotches of the same color as the dorsal blotches are found from the neck to the cloaca. The venter has a stark black and white checker pattern and two black lateral lines along the tail. However, the belly may be entirely black in northern populations. Most individuals have a spear-shaped mark on the parietal and frontal scales, extending to the neck. In addition, a mask is always present, extending across the prefrontals and preoculars and continuing through the eyes and 7th and 8th supralabials to the neck. Specimens from south Florida and the Florida Keys, previously referred to as *E. g. rosacea*, are often smaller on average (60–90 cm) with higher number of dorsal blotches (35–60), and have a noticeable reduction of black pigment throughout the body.

4.3. *Elaphe slowinskii* sp. nov.

Elaphe slowinskii Type locality: Longleaf Pine Highway, 0.5 miles west of Hwy 119, Natchitoches Parish,

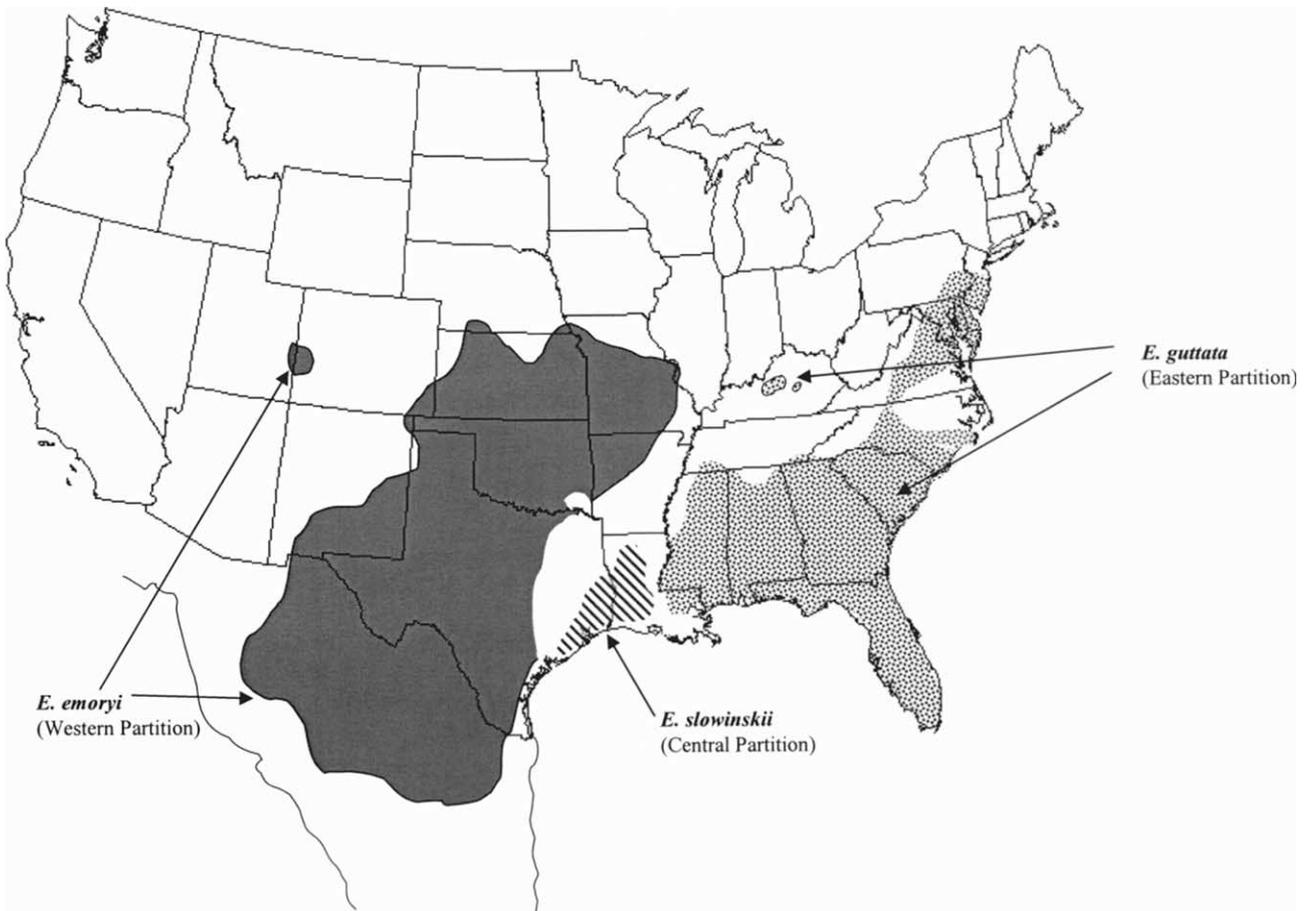


Fig. 4. Proposed distribution of the three species (partitions) within the *Elaphe guttata* complex.

Louisiana (Holotype: LSUMZ 55922; LSUMNS Tissue No. H-2036, specimen secured by J. Boundy and J. Slowinski on 6 May 1993).

Holotype description. LSUMZ 55922 is an adult male with a snout to vent length of 93 cm and a tail length of 19.2 cm. This specimen has 219 ventral scales, 72 subcaudal scales, eight supralabial scales on both sides of the head, 11 infralabial scales on both sides of the head, and 46 dorsal blotches. The ground color is dark grey and the dorsal blotches are dark brown with a thin black margin. The ventral ground color is white and has a dark black checker-board pattern. The ventral side of the tail displays two lateral stripes with a white center. The head shows the typical pattern found in the nominate form.

Standard English name. Slowinski's Cornsnake

Description. This species is found throughout the pine/oak upland habitat of western and northwestern Louisiana and eastern Texas (Fig. 4). A narrow gap in the distribution exists between this species (referred to as *E. g. guttata* in Werler and Dixon, 2000) and *E. emoryi* in eastern Texas. No record exists south of the Brazos River in Texas. Thomas (1974) and Smith et al. (1994) claimed that specimens from Arkansas and Missouri appeared similar to specimens in western Louisiana. Tissues were unavailable from these areas and therefore the partition identity of these populations could not be determined. If the populations in southern Arkansas are determined to be *E. slowinskii*, then they represent an isolated population from *E. slowinskii* that is nearly contiguous with *E. emoryi*. The range of *E. slowinskii* is presented here (Fig. 4) as occurring only in western Louisiana and eastern Texas (Fig. 4).

Description. Since this species is first described in this paper, a number of morphological characters were scored and general color pattern information was gathered for this taxon and compared to specimens of *E. guttata* in eastern Louisiana and *E. emoryi* in Texas (Appendix B). This comparison is made to aid in the identification of specimens captured near the ranges where these taxa approach one another.

Elaphe slowinskii is very similar to *E. guttata* in body shape and head markings, but is distinguished from the nearby southern *E. guttata* by lacking the brighter yellow and red dorsal color patterns (Vaughan et al., 1996). They generally have a grayish, brown, or tan ground color with dark red, maroon, or brownish blotches. In some specimens, the dark brown ground color may obscure the dorsal color pattern. The belly pattern may be checkered, but often displays smaller and more evenly paired rows of ventral blotches with some degree of peppering across the cream ventral ground color. The black margin surrounding the dorsal blotches at midbody tends to occupy only 25–50% of each scale in *E. slowinskii*, but usually occupies an entire scale in *E. guttata*. Some specimens of *E. slowinskii* appear similar

to *E. emoryi*. However, most *E. emoryi* tend to be gray with dark gray or olive colored blotches and often have more sparsely patterned bellies than *E. slowinskii* (Vaughan et al., 1996).

Although the range of values for the following characters overlap among taxa, they are nevertheless significantly different and trends among species are evident ($p < 0.000$ between all taxa as determined using ANOVA with a Bonferroni correction using SYSTAT 8.0; SPSS, 1998). The number of combined dorsal body and tail blotches in *E. slowinskii* (mean = 48, range = 42–56, $n = 17$) are greater than that of *E. guttata* (mean = 38, range = 34–47, $n = 18$) and less than that of *E. emoryi* (mean = 58, range = 48–65, $n = 19$). Vaughan et al. (1996) compared the number of combined body and tail blotches of specimens from east Texas (*E. slowinskii*) with specimens of *E. emoryi* from northwest, south, central, and west Texas and found that the number of these blotches in *E. slowinskii* were significantly different from *E. emoryi* and ranged from 44 to 59 (mean = 50.7). The width of dorsal blotches at midbody, measured in number of scales, in *E. slowinskii* (mean = 13, range = 10.5–15, and $n = 17$) is less than *E. guttata* (mean = 16.0, range = 13–19, and $n = 18$) and greater than *E. emoryi* (mean = 12, range = 9–15, and $n = 19$). The length of dorsal blotches at midbody, measured in number of scales, in *E. slowinskii* (mean = 4, range = 3–5, and $n = 17$) is less than *E. guttata* (mean = 6, range = 5–7, and $n = 18$) and greater than *E. emoryi* (mean = 4, range = 2.5–5.5, and $n = 19$).

The following \log_{10} transformed mensural characters did not vary significantly between taxa: head length, snout to vent length, tail length, head width, snout width, and muzzle length. However, the \log_{10} transformed diameter of the eye was significantly different between *E. slowinskii* and *E. emoryi*, indicating that *E. emoryi* has a larger eye relative to body size.

Etymology. This specimen is named in honor of Joseph B. Slowinski, who passed away on 12 December 2001 while collecting reptiles and amphibians in Myanmar. Joe's passion for investigating snake biology was contagious and unmatched in the field of herpetology. His expertise for solving problems in systematics and phylogenetics was superior. His friendship and warm personality will always be missed.

4.4. *Elaphe emoryi*

Scotophis emoryi Baird and Girard (1853, p. 157). Type locality: 20 miles southwest of Ozona, Crockett County, TX. (Holotype: Lost from the United States National Museum collection (Dowling, 1951)).

Scotophis calligaster Kennicott (1859, p. 88);

Coluber rhinomegas Cope (1860, p. 255);

Coluber laetus Boulenger (1894, p. 49);

Elaphe laeta intermontanus Woodbury and Woodbury (1942, p. 139);

Elaphe quivira Burt (1946, p. 116) (*nomen nudum*);

Elaphe guttata meahllmorum Smith et al. (1994, p. 259).

Standard English name. Great Plains Ratsnake

Distribution. This most western member of the *E. guttata* complex occurs in central and western Texas south into the Mexican States of San Luis Potosi, Tampico, Durango, and Chihuahua, and continues north into New Mexico, Colorado, Nebraska, southern Missouri, and Arkansas (Fig. 4). Disjunct populations occur in western Colorado and eastern Utah. Another isolated population occurs east of the Mississippi River in southern Illinois. Members of the rare and threatened Illinois population could not be obtained but, based on color pattern, appear very similar to *E. emoryi*.

Description. Compared to other members of the *E. guttata* complex, *E. emoryi* is more robust and has a larger head that is distinctly offset from the neck (Schultz, 1996). Adults of this species reach a total length of 80–150 cm and have 197–236 ventral scales and 58–83 subcaudal scales, with 27–73 dorsal blotches (Schultz, 1996). The dorsal ground color is gray, olive, or brown with brown or olive dorsal and lateral blotches with black borders. The pattern on the head is very similar to the other two species, whereas the belly may be only sparsely patterned (particularly in the southern part of the distribution) with small triangular or semi-circular blotches (Schultz, 1996).

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Appendix A. Tissue vouchers

Numbers in parentheses proceeding state and county collecting localities refer to specimens listed in Figs. 2 and 3. Letter abbreviations in parentheses preceding tissue voucher numbers refer to H or B = The Louisiana State University Museum of Natural Science Frozen Tissue Collection, TCWC = The Texas Corporative Wildlife Collection, and CAS = California Academy of Sciences.

(1) NJ: Burlintgon (H-18460), (2) NJ, Burlington (H-18462), (3) NJ, Ocean (H-16050), (4) NC: Carteret (B15P8), (5) NC: Cumberland (H-18463), (6) TN: Blount (H-16045), (7) FL: St. John (H-18461), (8) FL: Orange (H-18465), (9) FL: Hendry (H-3258), (10) FL: Dade (H-215-37), (11) FL: Dade (H-7478), (12) FL: Monroe (B14P137), (13) FL: Pinnellas (B15P27), (14) FL: Pineallas (H-8423), (15) FL: Wakulla (H-16041), (16) FL: Franklin (H-18458), (17) FL: Liberty (H-7354), (18) AL: Talladega (H-15043), (19) FL: Santa Rosa (H-2447), (20) KY: Hart (H-18459), (21) KY: Hardin (H-18464), (22) KY: Hardin (H-18465), (23) LA: St. Tammany Par. (B14P72), (24) LA: St. Tammany Par. (B14P73), (25) LA: St. Tammany Par. (H-16057), (26) LA: East Baton Rouge Par. (H-7489), (27) LA: Natchitoches Par. (H-14706), (28) LA: Natchitoches Par. (H-2036), (29) LA: Natchitoches Par. (H-15042), (30) LA: Natchitoches Par. (H-2944), (31) LA: Natchitoches Par. (H-14705), (32) LA: Allen Par. (H-16130), (33) LA: Vernon Par. (H-16044), (34) TX: Angelina (TCWC 7593), (35) TX: McMullen (H-16132), (36) TX: Hidalgo (H-14726), (37) TX: Medina (B13P147), (38) KS: Greenwood (H-16135), (39) KS: Ellsworth (H-16135), (40) TX: Roberts (H-16051), (41) TX: Val Verde (H-16042), (42) TX: Val Verde (H-2239), (43) TX: Val Verde (H-16136), (44) TX: Brewster (H-15968), (45) TX: Sterling (H-16133), (46) TX: Wise (TCWC 4536), (47) TX: Wise (TCWC 4537), (48) TX: Hudspecth (H-16137), (49) TX: El Paso (H-16131), (50) CO: Delta (H-18351), (51) CO: Delta (H-18352), (52) CO: Delta (H-18353), (53) CO: Delta (18354).

Appendix B. Specimens examined

All specimens examined morphologically from the following state and county collecting localities were obtained from the Louisiana State University Museum of Natural Science (LSUMZ).

Elaphe guttata Louisiana: East Baton Rouge Par.: LSUMZ 1534, 3812, 11471, 11472, 23927, 27712, 38963, and 55587. East Feliciana Par.: LSUMZ 24961, 76962, and 75963. Iberville Par.: LSUMZ 11470. St. Helena Par.: LSUMZ 12258. St. Tammany Par.: LSUMZ 20156, 43669, and 57650. West Feliciana Par.: LSUMZ 11475, 80797.

Elaphe slowinskii Louisiana: Allen Par.: LSUMZ 57630. Bienville Par.: LSUMZ 58442. Natchitoches Par.: LSUMZ 19194, 38958, 55922, 57069, 58444, 58469, 58513, 58999, 59000, 59634, 59696, 81140, and 83713. Sabine Par.: LSUMZ 35320. Texas: Jasper: LSUMZ 23500.

Elaphe emoryi Texas: Atascosa: LSUMZ 23198. Bexar: LSUMZ 23126. Brewster: 80990. Comal: LSUMZ 261804. Dallas: LSUMZ 24801. Dimmit: LSUMZ 23167. Duval: LSUMZ 23168. Edwards: LSUMZ 56293. Hidalgo: LSUMZ 59062, 73867. Jim Hogg: LSUMZ 39562. Kennedy: LSUMZ 13146. Maverick: LSUMZ 75977. Medina: LSUMZ 23197. Starr: LSUMZ 46932. Uvalde: LSUMZ 73867. Willacy: LSUMZ 36311. Val Verde: LSUMZ 56131, and 81138.

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