

Morphological systematics of kingsnakes, *Lampropeltis getula* complex (Serpentes: Colubridae), in the eastern United States

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Abstract

Kingsnakes of the *Lampropeltis getula* complex range throughout much of North America. Using morphology and color pattern, Blaney made the last revision of this species complex nearly 30 years ago and recognized seven subspecies. Furthermore, Blaney hypothesized that populations in the eastern United States consist of two closely related taxa, *L. g. getula* & *L. g. floridana*, which are morphologically divergent from all other subspecies. At the same time, Means hypothesized that an undescribed taxon existed in the Eastern Apalachicola Lowlands in the Florida panhandle. To test these hypotheses as well as help better understand phylogenetic relationships, we examine morphological characters and color pattern of *L. getula* throughout its range, particularly those populations in the eastern United States, and make comparisons to molecular data. We find that populations in the eastern United States represent a well-supported monophyletic group. Although some infraspecific clades (i.e., subspecies) within the *L. getula* complex may be weakly supported by homoplasious characters, at least one synapomorphy supports the monophyly of each group, including the two currently recognized subspecies in the eastern United States and the unnamed entity in the Eastern Apalachicola Lowlands, described herein as *L. g. meansi*. Justification for naming this natural clade at the infraspecific level (rather than species level) is provided. Furthermore, this panhandle clade is diagnosed by more synapomorphies than any other currently recognized taxon of *L. getula*, and overlaps in distribution with numerous other endemic plants and animals. All molecular analyses produced very similar tree topologies as our morphological dataset.

Key words: Apalachicola, Florida, morphology, phylogenetics, reptile, snake

Introduction

Kingsnakes of the *Lampropeltis getula* complex (Linnaeus) range throughout much of temperate and subtropical North America, from Oregon to the Mexican Plateau in the west

and from southern New Jersey to southern Florida in the east (Krysko 2001). Based on morphology and color pattern, Blaney (1977) made the last revision of this species complex and recognized seven subspecies of *L. getula* throughout its range: *L. g. californiae* (Blainville), *L. g. floridana* Blanchard, *L. g. getula* (Linnaeus), *L. g. holbrooki* Stejneger, *L. g. nigra* (Yarrow), *L. g. nigrita* Zweifel & Norris, and *L. g. splendida* (Baird & Girard). Furthermore, Blaney (1977) hypothesized that populations in the eastern United States represent a distinct clade consisting of *L. g. getula* and *L. g. floridana*, which are morphologically divergent from all other recognized subspecies.

Lampropeltis g. getula occurs from southern New Jersey to northern peninsular and panhandle Florida (Blaney 1977; Conant & Collins 1998; Krysko 1995, 2001; Means & Krysko 2001; Tennant 1997). Its dorsal pattern is solid black to chocolate brown with 19–32 narrow (1.5–2.5 dorsal scale rows wide) crossbands and a lateral chain pattern (Blaney 1977; Krysko 1995, 2001; see Fig. 2 in Means & Krysko 2001). *Lampropeltis g. floridana* occurs from central to southern peninsular Florida (Blanchard 1919, 1920; Blaney 1977; Krysko 1995, 2001; Means & Krysko 2001; Tennant 1997). Its dorsal pattern has > 34 narrow (1.5 dorsal scale rows wide) crossbands, a degenerate lateral chain pattern and undergoes various degrees of ontogenetic interband (= interspaces between light crossbands) lightening, giving it a yellowish speckled appearance in the adult stage (Blanchard 1919, 1920; Blaney 1977; Krysko 1995, 2001; see Fig. 3 in Means & Krysko 2001). Additionally, Means (1977) hypothesized that an unnamed taxon existed in the Eastern Apalachicola Lowlands in the Florida panhandle.

To test Blaney's (1977) and Means' (1977) hypotheses, as well as help better understand phylogenetic relationships, we examine morphological characters and color pattern of *Lampropeltis getula* throughout its range, particularly those populations in the eastern United States, and compare these data to molecular data. Our interpretation of infraspecific taxa, along with their geographic ranges, is presented using the Apomorphic Species Concept (= Phylogenetic Species Concept *sensu* Donoghue 1985; Mishler 1985; Mishler & Brandon 1987; Mishler & Theriot 2000).

Material and methods

We examined external morphology and color pattern from 52 snakes in the *Lampropeltis getula* complex (Appendix 1; Fig. 1), including 10 from the Florida peninsula, 9 from the Eastern Apalachicola Lowlands, 13 from the region surrounding the Eastern Apalachicola Lowlands, 12 from the Atlantic Coast north of Florida, and 1–2 individuals of each of the five remaining recognized subspecies west to northern Mexico and California. Although we have examined the morphology of more than 1,000 specimens, these operational taxonomic units (OTUs) are representatives that were carefully selected in order to include the total pattern variation of each recognized taxon. In order to facilitate comparison of morphological and molecular phylogenetic results, most of these individuals were the

same as those used in DNA analyses (paper in progress, but *see* Krysko 2001; Krysko & Franz 2003). For some missing localities, replacement specimens with a similar morphology and locality were used.

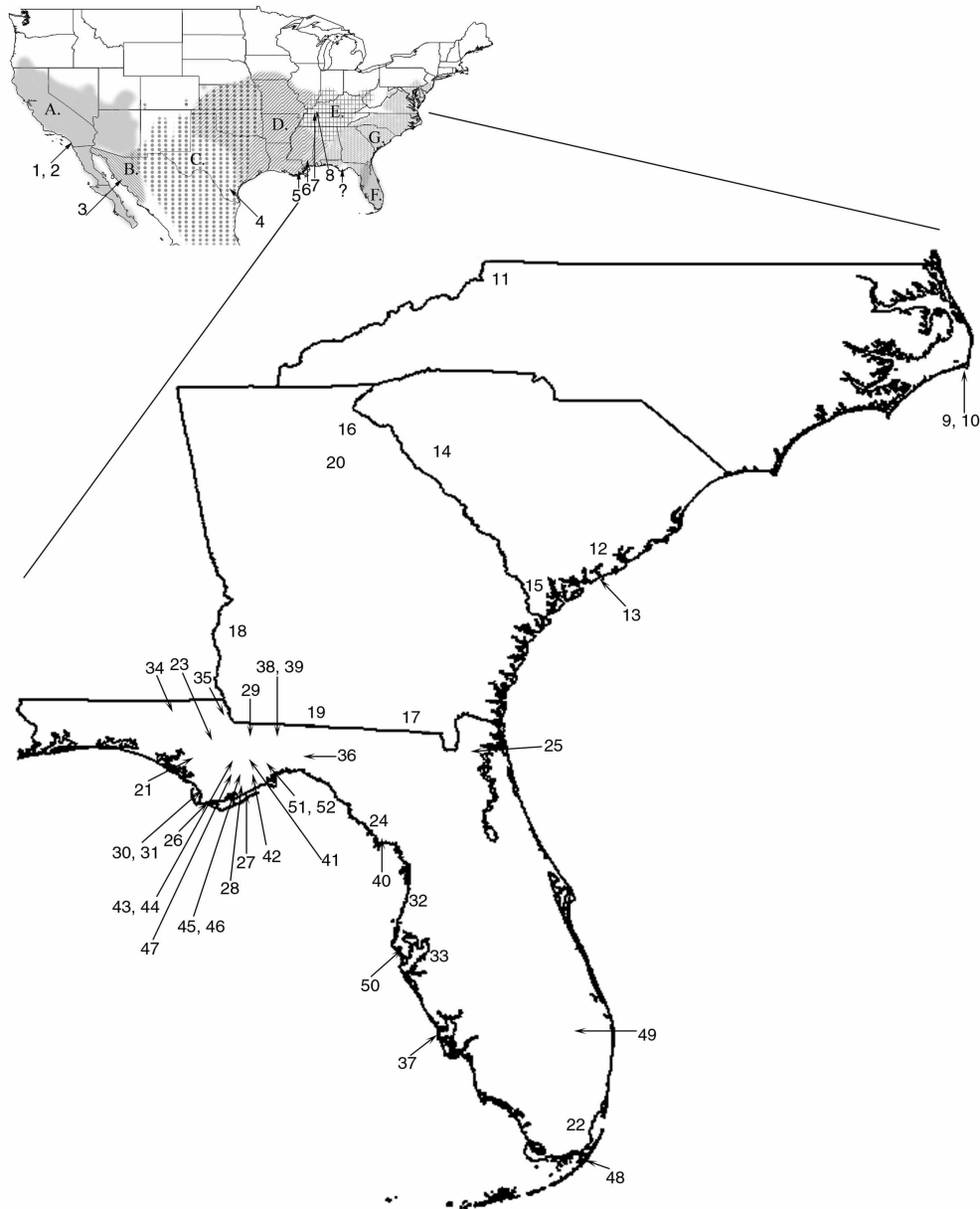


FIGURE 1. Map of United States showing localities of *Lampropeltis getula* samples used for morphological analysis. Numbers refer to samples in Appendix 1. Letters refer to recognized subspecies: A. *L. g. californiae*, B. *L. g. nigrita*, C. *L. g. splendida*, D. *L. g. holbrooki*, E. *L. g. nigra*, F. *L. g. floridana*, and G. *L. g. getula*. Question mark refers to Eastern Apalachicola Lowlands population.

Morphological characters. Supralabial, infralabial, loreal, temporal, ocular and ventral scales were examined, and because of the extensive amount of variation and overlap between recognized taxa these characters were determined to be phylogenetically uninformative and omitted from the analyses. Twelve variable and potentially phylogenetically informative characters (Table 1) were used in cladistic analyses and plesiomorphic (0) and apomorphic (1–4) conditions (Appendix 2, Table 1) were determined using the five western and midwestern taxa as functional outgroups (Maddison *et al.* 1984). Although the majority of the remaining characters relate to color pattern, such characters have been used successfully to illustrate phylogenetic relationships of infraspecific taxa using cladistic analyses (Hammond 1990[1991]). Because not every snake could be examined throughout its entire life, coding for juvenile and adult characters was inferred from other specimens collected in close geographic proximity. Characters are listed and discussed below. When ordering of characters is used in the analysis it follows the progression stated for each character, which is justified under each character.

Dorsal scale rows (DSR). Maximum number at midbody (Fig. 2). Individuals examined had either 21, 23, or 25 DSR (Table 1).

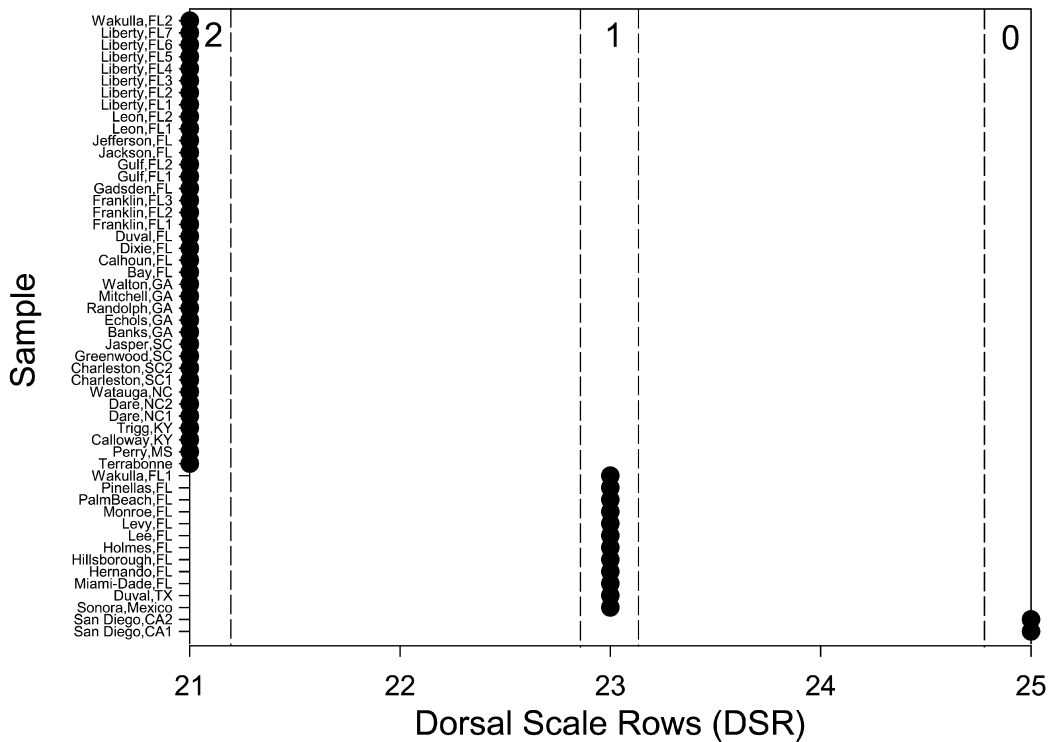


FIGURE 2. Dorsal scale rows (DSR) at midbody in *Lampropeltis getula* complex. Note plesiomorphic (0) and apomorphic (1–2) conditions.

Ventral pattern as juvenile. Primary ventral patterns are illustrated in Fig. 3. The ventral pattern is typically A = ringed, in extreme western North America or B = light with

dark lateral margins, in the San Diego region of California (A and B = *Lampropeltis getula californiae*), D = tight checkerboard, south into northern Mexico and east to peninsular Florida (= *L. g. nigrita*, *L. g. splendida*, *L. g. holbrooki*, *L. g. nigra*, and *L. g. floridana*), E = loose checkerboard, north along the Atlantic United States border (*L. g. getula*), F = loose checkerboard with interspersed bicolored scales, in the Eastern Apalachicola Lowlands and surrounding Florida panhandle, and G = bicolored, in the Eastern Apalachicola Lowlands of Florida. There is a morphological progression from A and B to D to E to G (Table 1). Pattern C is discussed below.

Ventral pattern as adult. Although the ventral pattern does not typically change ontogenetically, snakes from northern Mexico (= *Lampropeltis getula nigrita*) gain dark pigment until becoming completely black (Fig. 3C). There may be considerable variation within a single clutch of eggs in *L. g. nigrita*, where some newborns might exhibit a tight checkerboard ventral pattern (Fig. 3D) like those of neighboring populations of *L. g. splendida*, while other siblings might exhibit a nearly completely black venter (Fig. 3C). After only a few periods of ecdysis following hatching, all ventral and dorsal pattern remnants are usually lost. Because this evidence suggests that the ventral pattern may be ontogenetically controlled and there appears to be a progression like that in the juvenile stage described above, this character was treated as an ordered transformation series (Table 1).

Dorsal pattern as juvenile. Primary dorsal patterns are illustrated in Fig. 3. The dorsal pattern is typically A = ringed, in extreme western North America or B = light striped, in the San Diego region of California (A and B = *Lampropeltis getula californiae*), D and E = narrow banded, south into northern Mexico and east to the Atlantic Coast (= *L. g. nigrita*, *L. g. splendida*, *L. g. holbrooki*, *L. g. nigra*, *L. g. floridana*, and *L. g. getula*), F = wide banded, in the Eastern Apalachicola Lowlands and surrounding Florida panhandle, and dark striped (see Fig. 5D in Krysko & Franz 2003 and Fig. 20 in Means & Krysko 2001) and G = patternless, in the Eastern Apalachicola Lowlands. See above for explanation regarding *L. g. nigrita*, and Means & Krysko (2001) regarding dark striped Eastern Apalachicola Lowlands. There is a morphological progression from A and B to D and E to F to G (Table 1).

Ontogenetic change in dorsal pattern. The dorsal pattern changes in certain geographic areas, including the juvenile's light bands becoming black (Fig. 3C) in northern Mexico (= *Lampropeltis getula nigrita*) and in the midwestern United States on the western side of the Appalachian Mountains (= *L. g. nigra*), black interbands becoming lightened laterally in the Texas area (= *L. g. splendida*), or lightened over the entire dorsum east to Florida (= *L. g. holbrooki* and *L. g. floridana*). *Lampropeltis g. getula* from the Outer Banks, Dare County, North Carolina, as well as snakes in the Apalachicola region of Florida also undergo interband lightening over the dorsum.

TABLE 1. Morphological characters of kingsnakes in the *Lampropeltis getula* complex used for cladistic analyses. Note plesiomorphic (0) and apomorphic (1–4) conditions.

#	Character	Character State (Coding)
1	<i>Dorsal scale rows</i> (Fig. 2).	25 (0), 23 (1), 21 (2)
2	<i>Ventral pattern as juvenile</i> (Fig. 3).	(Ordered transformation series) Ringed or light with dark lateral margins (0), tight checkerboard (1), loose checkerboard (2), loose checkerboard with interspersed bicolored scales (3), bicolored (4)
3	<i>Ventral pattern as adult</i> (Fig. 3).	(Ordered) Ringed or light with dark lateral margins = A, tight checkerboard = B, solid dark = C, loose checkerboard = D, loose checkerboard with interspersed bicolored scales = E, bicolored = F 3a. A (0), not A (1) 3b. C (1), not C (0) 3c. A, B or C (0); D, E or F (1) 3d. A, B, C or D (0); E or F (1) 3e. A, B, C, D or E (0); F (1)
4	<i>Dorsal pattern as juvenile</i> (Fig. 3).	(Ordered) Ringed or light striped (0), narrow banded (1), wide banded (2), dark striped (3), patternless (4)
5	<i>Ontogenetic dorsal pattern change.</i>	Ringed or light striped with no change (0), banded with no change (1), bands becoming solid dark (2), interband lightening laterally (3), interband lightening over dorsum (4)
6	<i>Band or ring formation as juvenile</i> (Fig. 3).	(Ordered) Ringed around body or light striped (0), forked laterally (1), fused laterally and/or dorsally (2)
7	<i>Placement of light pigment within band or ring scales as juvenile</i> (Fig. 4A).	(Ordered) Entire scale (0), centered (1), anterior (2)
8	<i>Placement of ontogenetically lightened pigment within dark interband or ring scales</i> (Fig. 4B).	(Ordered) No lightening (0), centered (1), anterior (2)
9	<i>Red tipping of dorsal scales as juvenile.</i>	Absent (0), present (1)
10	<i>Number of light bands or rings as juvenile</i> (Fig. 5).	A (0), B (1), C (2), D (3)
11	<i>Fraction of light pigment within band or ring scales as juvenile</i> (Fig. 6).	1.00 (0), 0.50 (1), 0.33 (2)
12	<i>Band or ring width as juvenile</i> (Fig. 7).	A (0), B (1), C (2), D (3), E (4)

Band or ring formation as juvenile. Formations include 1) ringed around body or light-striped, in extreme western North America, or light-striped, in the San Diego region of California (Fig. 3A, B = *Lampropeltis getula californiae*), 2) narrow bands that fork laterally, east to the Atlantic Coast (Fig. 3E = *L. g. splendida*, *L. g. nigrita*, *L. g. holbrooki*, *L. g. nigra*, *L. g. floridana*, and *L. g. getula*), and 3) bands that fuse laterally and/or dorsally, in the southwestern and Eastern Apalachicola Lowlands (Fig. 3F, G). There is a progression from 1 to 2 to 3 (Table 1).

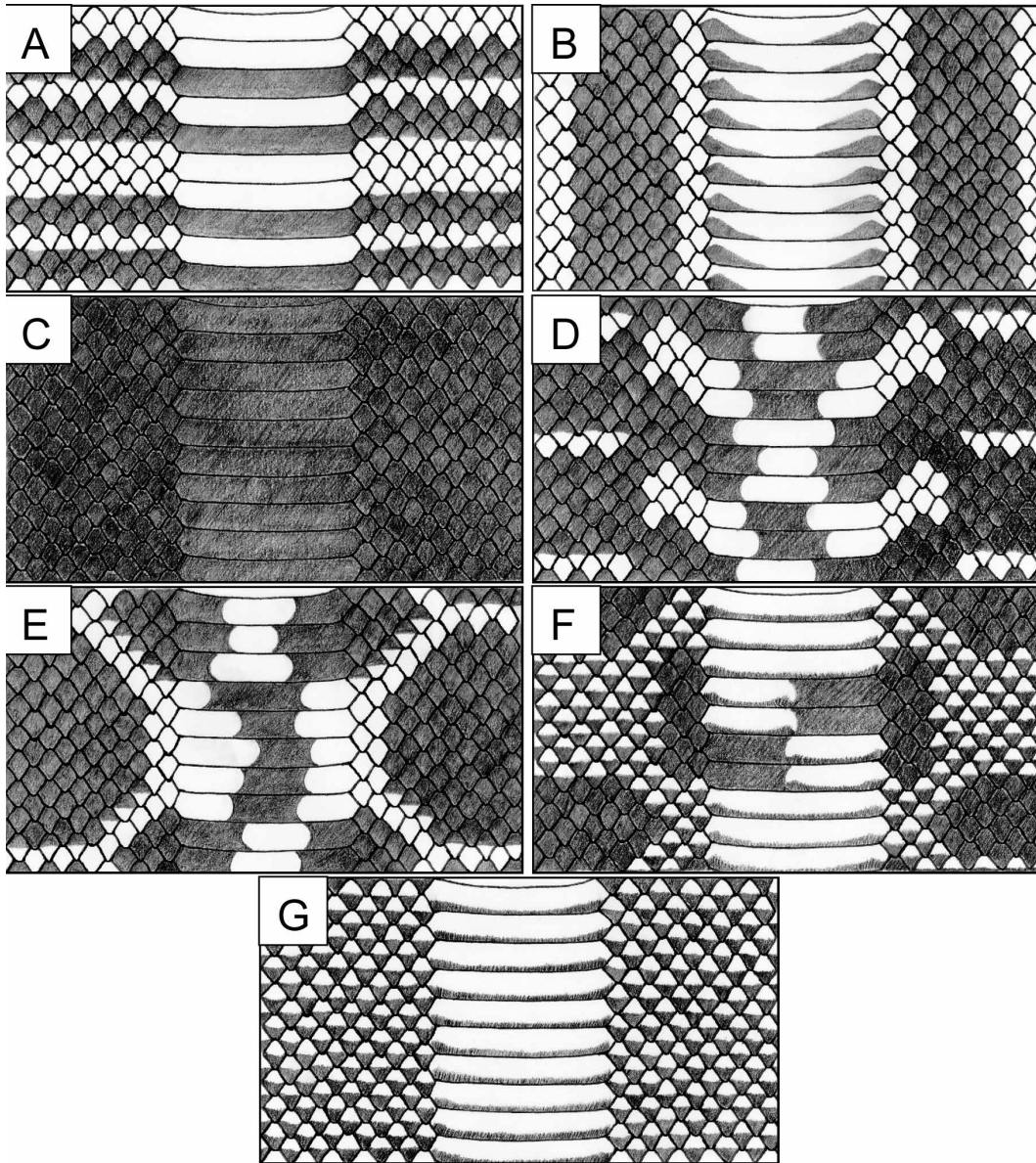


FIGURE 3. Primary dorsal and ventral patterns in the *Lampropeltis getula* complex. Note that dorsal or ventral patterns might be referred to separately in text.

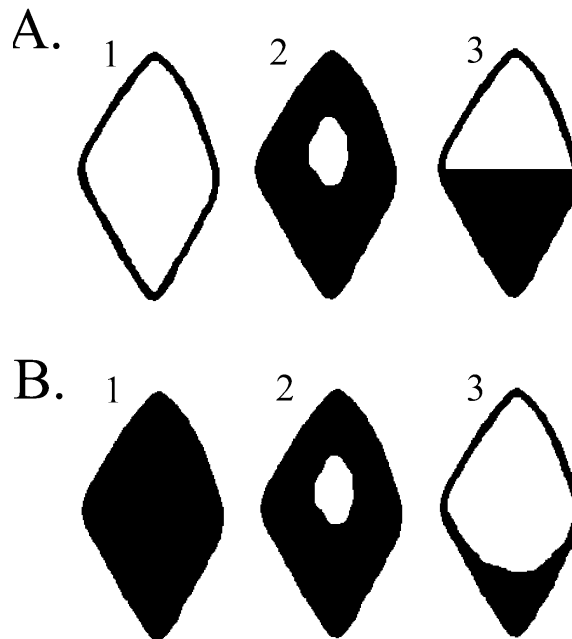


FIGURE 4. A) Placement of light pigment within light band or ring scales in the *Lampropeltis getula* complex; Pigment is either located 1 = on the entire scale, 2 = in center, or 3 = anteriorly. B) Placement of ontogenetically lightened pigment within dark interband or ring scales in the *Lampropeltis getula* complex; There is either 1 = no pigment, or light pigment is located 2 = in center, or 3 = anteriorly. See Table 1 for character state codings.

Placement of light pigment within band or ring scales as juvenile. Pigment is either 1) located on the entire scale, 2) centered, or 3) anterior (Fig. 4A). There is a progression from 1 to 2 to 3 (Table 1).

Placement of ontogenetically lightened pigment within dark interband or ring scales. There is either 1) no light pigment, or light pigment is 2) centered, or 3) anterior (Fig. 4B). There is a progression from 1 to 2 to 3 (Table 1).

Red tipping of dorsal scales as juvenile. Although previously reported for only southern peninsular Florida populations (Neill 1954), neonate *Lampropeltis getula* from the entire eastern United States populations may exhibit reddish coloration within light crossband scales. There may be considerable variation within a single clutch of eggs as different proportions of siblings may or may not exhibit this trait. It appears that the brightest red bands usually change ontogenetically into an off-white, beige, or dull brown color (K.M. Enge & H. Sherman pers. comm.). Bands without reddish scales usually remain brilliant white or yellow.

Number of light bands or rings as juvenile. On body starting one head-length posterior to the head and ending above the cloaca (Fig. 5): A = 0, B = 1, C = 16–34, and D = 44–65. Note that the light striped individual (San Diego, CA¹) has no bands (Figs. 3B, 5) and many individuals from the Eastern Apalachicola Lowlands are considered to have only one band (*see* Fig. 20 and explanation in Means & Krysko 2001).

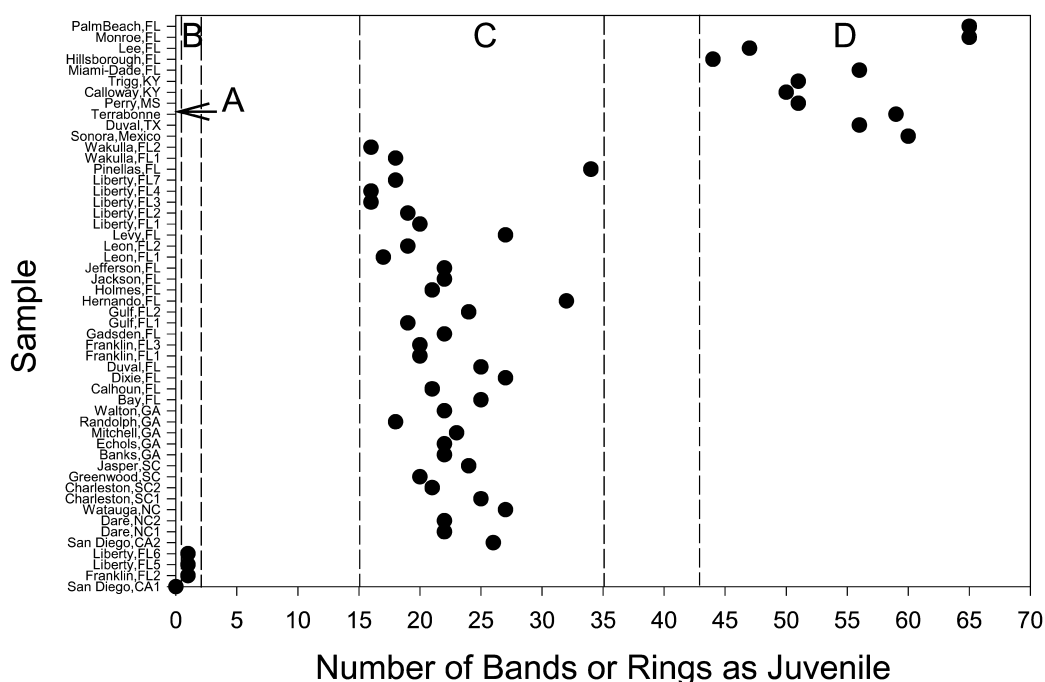


FIGURE 5. Number of light bands or rings in juveniles of the *Lampropeltis getula* complex. See Table 1 for character state coding.

Fraction of light pigment within band or ring scales as juvenile. As a fraction, light pigment incorporates entire = 1.00, half = 0.50, or one-third = 0.33 of entire scale (Fig. 6).

Band or ring width as juvenile. Mean scale width (= mid-dorsal scale rows) on body: A = 0, B = 0.33, C = 1.5–2, D = 2.5–8, E = 200 (Fig. 7). Character state delimitation of D is somewhat subjective because it incorporates a relatively wide range of values, however, this variation is found almost exclusively in the Eastern Apalachicola Lowlands and adjacent region. Note that the light striped individual (San Diego, CA¹) has no bands.

Cladistic Analyses. Relationships among individuals (Appendix 2) are investigated with the maximum-parsimony (MP) method using PAUP* (ver. 4.08b, Swofford 2000). MP analyses were constructed using delayed transformation (DELTRAN) with an heuristic search using 1000 repetitions of random stepwise additions with tree-bisection-reconnection (TBR) branch swapping, with limits set to 25 trees (30 steps) per random addition replicate. Both unordered and ordered character analyses were performed (Table 1). Confidence limits for phylogenetic groupings in both approaches were assessed with bootstrapping (Felsenstein 1985), with full heuristic search using 1000 repetitions of random stepwise additions with TBR and limits set to 5 trees per random addition replicate. Nonparametric bootstrapping generally yields conservative measures of the probability that a group represents a true evolutionary clade (Hillis & Bull 1993; Rodriguez-Robles & De Jesus-Escobar 1999; Rodriguez-Robles *et al.* 1999; Zharkikh & Li 1992).

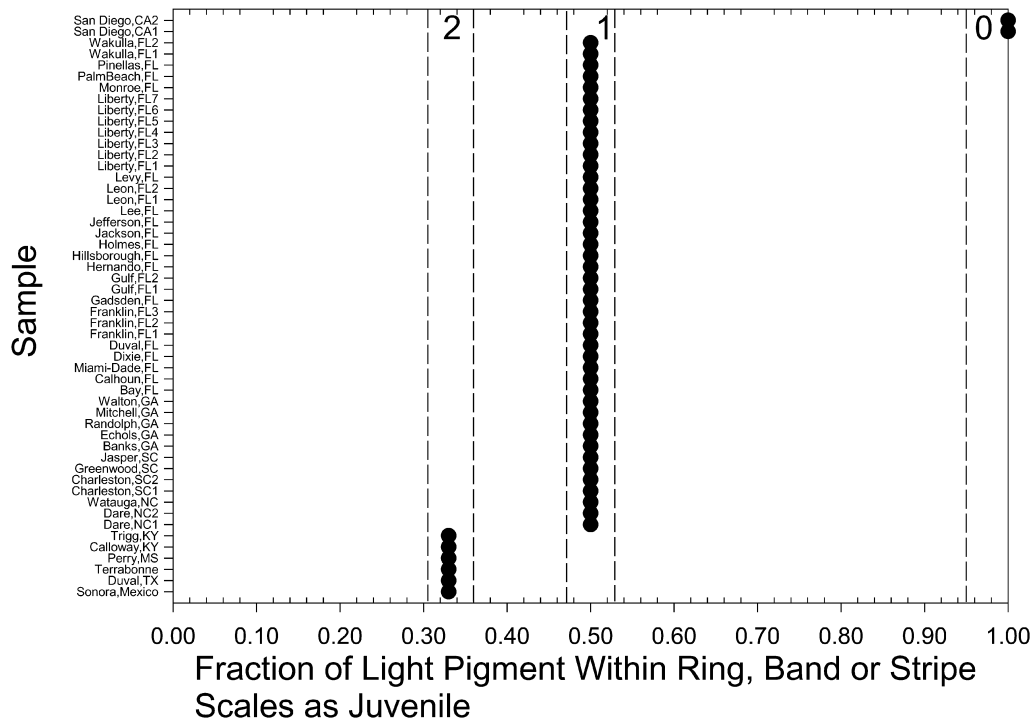


FIGURE 6. Fraction of light pigment within band or ring scales in juveniles of the *Lampropeltis getula* complex. Note plesiomorphic (0) and apomorphic (1–2) conditions.

Molecular Analyses. Mitochondrial DNA (mtDNA) was sequenced from a total of 64 snakes. These include 55 individuals from 3 eastern U.S. areas: 1) 15 from Atlantic coast, 2) 25 from panhandle Florida, and 3) 15 from peninsular Florida (Appendix 3). Additionally, mtDNA was sequenced from 5 midwestern and 3 western United States individuals along with one currently recognized congener, *Lampropeltis elapsoides* (G. Harper pers. comm.; also see Armstrong *et al.* 2001), which were used as outgroups to the eastern United States populations. In order to facilitate comparison of molecular and morphological phylogenetic results, most of these individuals were the same as those used in morphological analyses (Table 1).

Laboratory Techniques. Mitochondrial DNA samples were obtained from blood, muscle tissue, shed skins, and bone. Between 0.5 and 1.0 ml of blood was taken from the caudal vein of live specimens and stored in lysis buffer (100 mM Tris-HCl, pH 8; 100 mM EDTA, pH 8; 10 mM NaCl; 1.0% sodium dodecyl sulfate) in approximately 1:10 blood to buffer ratio (White & Densmore 1992). Muscle tissue was taken from salvaged dead-on-road (DOR) specimens and stored in SED buffer (saturated NaCl; 250 mM EDTA, pH 7.5; 20% DMSO; Amos & Hoelzel 1991, Proebstel *et al.* 1993). DNA isolations were obtained following protocols of Hillis *et al.* (1990) for blood and muscle tissue, Clark (1998) for shed skins, and Iudica *et al.* (2001) for bone.

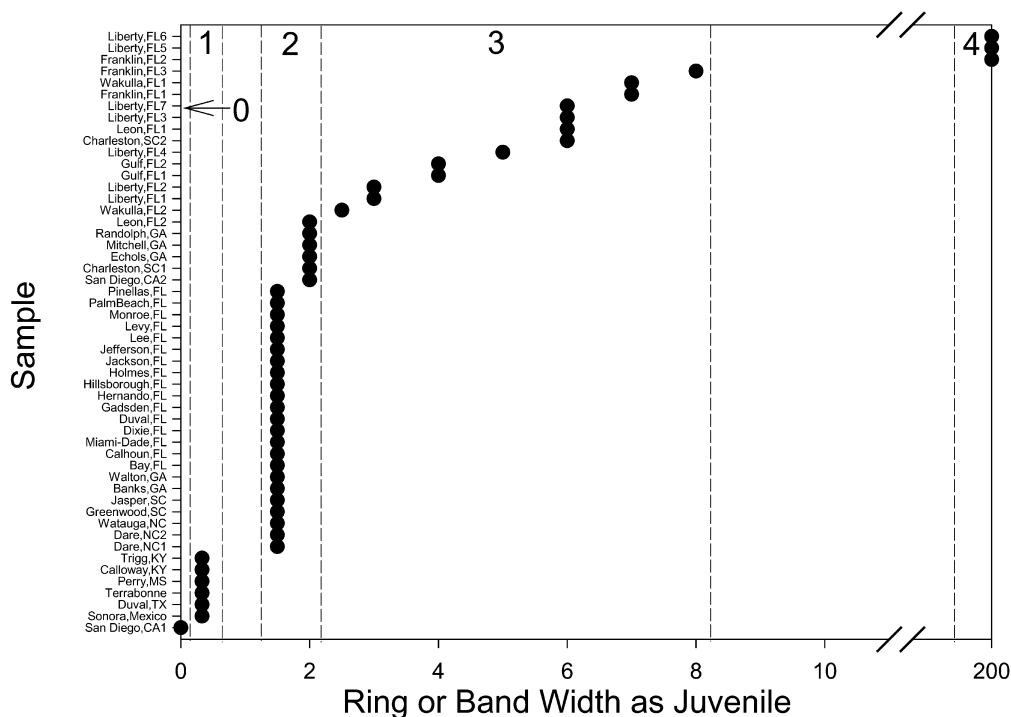


FIGURE 7. Band or ring width in juveniles of the *Lampropeltis getula* complex. Note plesiomorphic (0) and apomorphic (1–4) conditions.

Using total cellular DNA as a template and polymerase chain reaction (PCR) methodology (Saiki *et al.* 1988), we amplified and sequenced mtDNA from the cytochrome *b* (*cyt b*) gene and the nicotinamide adenine dinucleotide dehydrogenase subunit 4 (ND4) region. Cytochrome *b* was sequenced using the primers LGL765 (Bickham *et al.* 1995) and H15919 (Fetzner 1999). For degraded samples, we used *cyt b* primer CYB 2 (Kessing *et al.* 1989), along with designed internal primers using OLIGO software (ver. 4.06): CYB 1L, CYB 2L, CYB 1H, CYB 2H (*see* Table 2 and Fig. 8 in Krysko & Franz 2003). PCR was conducted in a Biometra thermal cycler in 50 μ l reactions: 25.9 μ l H₂O, 5.0 μ l 10 x PCR reaction buffer (Sigma[®]), 8.0 μ l deoxynucleotide triphosphates (800 μ M), 6.0 μ l MgCl₂ (25 mM, Sigma[®]), 1.2 μ l each primer (10 μ M), 0.2 μ l *Taq* DNA polymerase (Sigma[®], 5 U / μ l), and 2.5 μ l template DNA. PCR parameters included initial denaturing at 96°C for 3 min, followed by 45 cycles of amplification: denaturing at 95°C for 25 sec, annealing at 53°C for 1 min, and extension at 72°C for 2 min, followed by a final extension at 72°C for 5 min (J.W. Fetzner pers. comm.). The ND4 region included a section of the 3' end of the ND4 gene, and 3 transfer ribonucleic acids (tRNA^{His}, tRNA^{Ser}, tRNA^{Leu}), which were sequenced using the primers ND4 and Leu (Arevalo *et al.* 1994, Rodriguez-Robles & De Jesus-Escobar 1999), along with designed internal primers using OLIGO software (ver. 4.06): ND4 1L, ND4 1H (*see* Table 2 and Fig. 8 in Krysko & Franz 2003). Designed internal primers are noted above. PCR was

conducted in 50 µl reactions as above. PCR parameters included initial denaturing at 96°C for 2 min, followed by 45 cycles of amplification: denaturing at 95°C for 10 sec, annealing at 52°C for 25 sec, and extension at 72°C for 45 min, followed by a final extension at 72°C for 7 min (Rodriguez-Robles & De Jesus-Escobar 1999).

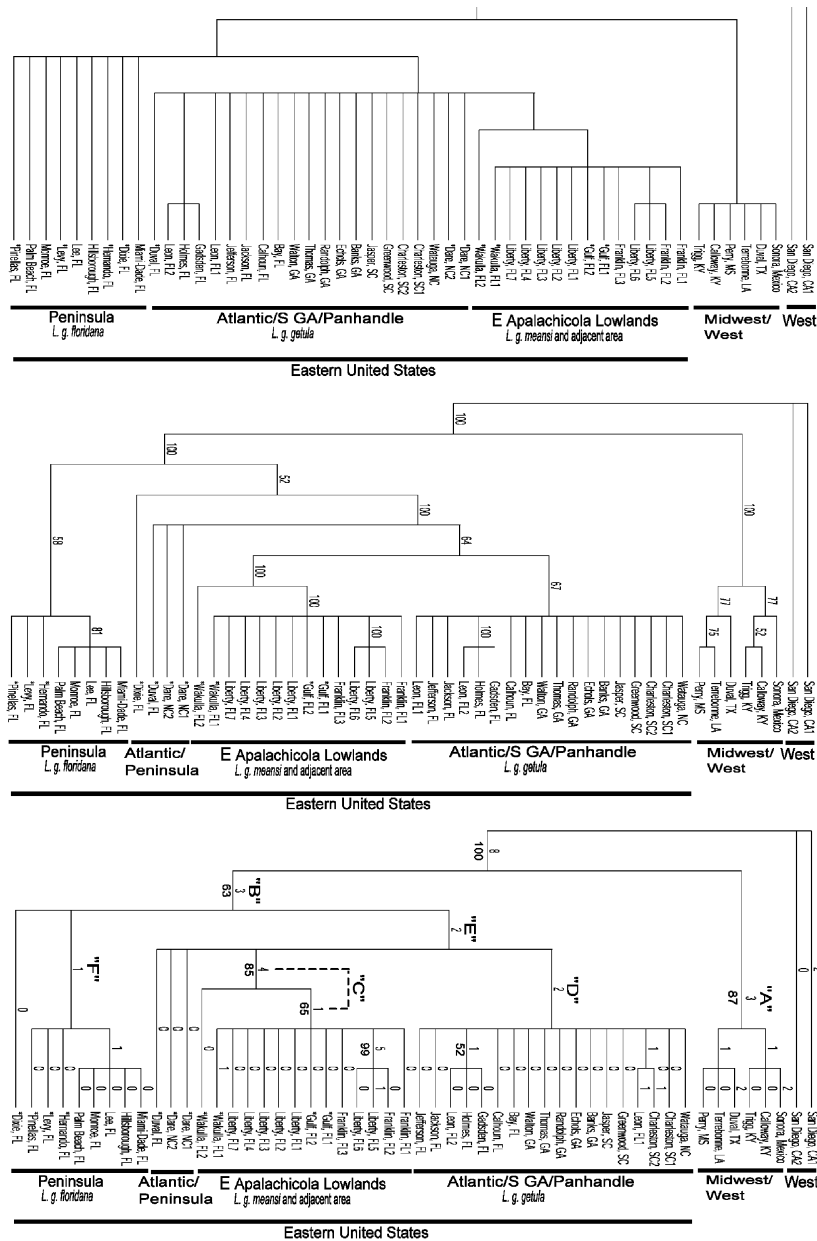


FIGURE 8. Strict consensus (left), majority rule (center), and random representative of 69 equally parsimonious trees (right) from unordered maximum-parsimony analysis of morphological data in the *Lamproveltis getula* complex. Majority rule (center) illustrates the percentage that nodes are found. Randomly selected tree (right) illustrates the number of steps (above) and bootstrap values (> 50%, below). Putative morphological intermediates are indicated with an asterisk next to sample.

Five μl of each PCR product were electrophoresed on a 1% agarose gel, visualized with ethidium bromide staining, and compared with a DNA standard. Double-stranded PCR products were cleaned with 30,000 MW Millipore filters. Cleaned PCR products were sequenced with Big Dye[®] terminator reagents (Applied Biosystems, Norwalk, CT) according to manufacturer's instructions, except that reactions were scaled down to 1/8 volume in 20 μl reactions: 1 μl of terminator mix, 3.5 μl 5x buffer (400 mM Tris, pH 9.0, 10 mM MgCl_2), 1 μl primer (10 μM), and H_2O (13.5–10.5 μl) and PCR products (1–4 μl) for a total volume of 20 μl . Single stranded sequence products were analyzed with automated DNA sequencers (Applied Biosystems models 373 and 377). New haplotypes were confirmed by comparing complimentary DNA strands, and ambiguities that could not be resolved were resequenced. Initial sequences were screened for the presence of mitochondrial-like pseudogenes (from the nuclear genome) using patterns of nucleotide substitution, stringency tests, and primer redesign (Zhang & Hewitt 1996). Sequence files from the automated sequencer were assembled and edited as necessary with Sequencher (ver. 3.1, Genes Codes Corp., Ann Arbor, MI) and aligned manually.

TABLE 2. Shared haplotypes of *Lampropeltis getula* (paper in progress, but see Krysko 2001, Krysko & Franz 2003). Note that first sample listed for each haplotype was used in phylogenetic analyses, while others afterward were omitted. For accession numbers and locality see Appendix 3.

Haplotype	Samples
C	Liberty County, FL ³ ; omitted: Liberty County, FL ⁴
J	Calhoun County, FL ¹ ; omitted: Bay County, FL, Calhoun County, FL ² , Leon County, FL ¹ , and Franklin County, FL ²
S	Wakulla County, FL ¹ ; omitted: Wakulla County, FL ²
bb	Dare County, NC ² ; omitted: Mitchell County, GA, and Randolph County, GA
cc	Watauga County, NC; omitted: Charleston County, SC ¹ , Dare County, NC ¹ , and Dare County, NC ³
hh	Lee County, FL; omitted: Pinellas Co, FL ²
jj	Palm Beach County, FL; omitted: Hendry County, FL
tt	Stewart County, TN; omitted: Calloway County, KY

Phylogenetic Analyses. Relationships among mtDNA haplotypes were estimated with MP using PAUP* (ver. 4.0b8; Swofford 2000) and Bayesian criteria (BA) using Mr Bayes (ver. 3.1.1; Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Redundant haplotypes (n = 14) were excluded from analyses (Table 2). MP cladograms (strict and majority-rule consensus) were constructed using delayed transformation (DELTRAN) with an heuristic search using 1000 repetitions of random stepwise additions with TBR branch swapping, saving all trees per random addition replicate. Equal weighting of

transitions (TS) and transversions (TV) was used. Support for phylogenetic groupings in MP was assessed with bootstrapping (Felsenstein 1985), with full heuristic search using 1000 repetitions of random stepwise additions with TBR and limits set to 5 trees per random addition replicate. BA phylogenies were constructed using four independent runs with four chains each run for 3 million generations using the best fit model GTR+G (MrModeltest v.2.2, Nylander 2004). Output files were analyzed and the first 300,000 generations were discarded as burn-in. All four runs had identical tree topologies and highly similar posterior probability values, thus the trees from all runs were combined to obtain the final tree.

Results

Cladistic Analyses. MP analysis using unordered characters (Table 1) resulted in 69 most parsimonious trees of 44 steps (CI = 0.795, RI = 0.954) from 12 parsimony-informative characters. Strict and majority rule consensus trees were produced (Fig. 8). The midwestern/western and eastern United States samples form separate monophyletic groups. The eastern United States clade is further divided into three subclades, including the two recognized subspecies (*Lampropeltis getula getula* and *L. g. floridana*) and an unnamed group of snakes in the Eastern Apalachicola Lowlands. Throughout the text we refer to this third subclade as “Eastern Apalachicola” because it consists mostly of snakes from this area, along with fewer putative morphological intermediates (or hybrids) from the adjacent region. Wagner (1980) suggested removing all recognized intermediate phenotypes or hybrids from analyses, however McDade (1992) illustrated that if these individuals were included it would not result in any dramatic or negative conclusions as long as the individuals are closely related. Although morphological intermediates were included in both morphological and molecular analyses, removing them would likely strengthen support for our three monophyletic subclades in the eastern United States. The strict consensus tree illustrates the Eastern Apalachicola Lowland subclade, nested within an Atlantic Coast, southern Georgia, and Florida panhandle (Atlantic/S GA/Panhandle) subclade (= *L. g. getula*). Florida peninsula populations (*L. g. floridana*) are most closely related to the Atlantic/S GA/Panhandle subclade. A randomly chosen representative of the 69 shortest trees was selected, illustrating the number of character differences between individuals and bootstrap support above 50% (Fig. 8). Major nodes are statistically supported: western clade (100% = *L. g. californiae*), midwestern/western clade (87%, = *L. g. nigrita*, *L. g. splendida*, *L. g. holbrooki*, and *L. g. nigra*), and eastern United States clade (63%). Most nodes within the eastern United States clade are not as well supported, having fewer character differences, thus illustrating their close relationships. The Eastern Apalachicola Lowlands samples exist within the only well-supported subclade (85%). The MP trees demonstrate the relationships of the outgroups with 100% bootstrap support, where the midwestern/western clade (= *L. g. nigrita*, *L. g. splendida*, *L. g. holbrooki*, and *L. g. nigra*) is the sister group to the eastern clade rather than the western clade (= *L. g. californiae*).

MP analysis using ordered multi-state characters (Table 1) resulted in 171 most parsimonious trees of 46 steps (CI = 0.761, RI = 0.952) from 12 parsimony-informative characters. Strict and majority rule consensus trees (Fig. 9) were produced and yield results congruent with those of the unordered MP analysis. Although this analysis produces more equally parsimonious trees of greater number of steps, it gives more statistical support of the ingroup (eastern United States) relationships.

Character Evolution. Although many characters used in our analyses were homoplasious, synapomorphies were identified supporting the monophyly of particular clades. There were three characters supporting the monophyly of *L. g. holbrooki*, *L. g. nigra*, *L. g. nigrita*, and *L. g. splendida* in the midwestern/western clade (node “A”, Fig. 8), including centered light pigment within band scales as juvenile (character 7-1, Table 1; Fig. 4A-2), light pigment incorporating 33% of band scales as juvenile (character 11-2, Table 1; Fig. 6), and 33% mean band width as juvenile (character 12-1, Table 1; Fig. 7). Centered ontogenetically lightened pigment within dark interband scales (character 8-1, Table 1; Fig. 4B-2) is the only character supporting the monophyly of *L. g. splendida* and *L. g. holbrooki* from Duval County, TX, Terrebonne Parish, LA, and Perry County, MS, within the midwestern/western clade (Fig. 8). Ontogenetically darkened dorsal and ventral patterns in the adult stage (characters 3b-1, 5-2, Table 1; Fig. 3C) were the two characters supporting the monophyly of *L. g. nigrita* from Sonora, Mexico (Fig. 8). However, it is noted that an ontogenetically darkened dorsal pattern (character 5-2, Table 1; Fig. 3C) is homoplasious, because it is also found on the western side of the Appalachian Mountains (= *L. g. nigra*). Three characters support the monophyly of the eastern United States clade with *L. g. floridana*, *L. g. getula*, and Eastern Apalachicola Lowlands populations (node “B”, Fig. 8), including anterior light pigment within band scales as a juvenile (character 7-2, Table 1; Fig. 4A-3), red tipping of dorsal scales as juvenile (character 9-1, Table 1), and light pigment incorporating 50% of band scales as a juvenile (character 11-1, Table 1; Fig. 6). Five synapomorphies support the monophyly of the Eastern Apalachicola Lowlands populations with morphological intermediate snakes from the adjacent areas (nodes “C”, Fig. 8), including a ventral pattern of loose checkerboard with interspersed bicolored scales as a juvenile (character 2-3, Table 1; Fig. 3F), ventral patterns of loose checkerboard with interspersed bicolored scales or bicolored as an adult (character 3d-1, Table 1; Fig. 3F, G), wide banded dorsal pattern (character 4-2, Table 1; Fig. 3F), band formation fused laterally (*see* Fig. 20 in Means & Krysko 2001) and/or dorsally as a juvenile (character 6-2, Table 1; Fig. 3G), and band width of 2.5–8 DSR as a juvenile (character 12-3, Table 1; Fig. 7). Six autapomorphies are found within the Eastern Apalachicola Lowlands populations (Fig. 8), including bicolored ventral patterns as a juvenile and an adult (characters 2-4, 3e-1, Table 1; Fig. 3G), dark striped (*see* Fig. 20 in Means & Krysko 2001) and patternless dorsal patterns as a juvenile (characters 4-3, 4-4, Table 1; Fig. 3G), one light dorsal band as a juvenile (character 10-1, Table 1; Fig. 5), and band width of entire body length as a juvenile (character 12-4, Table 1; Fig. 7). Two synapomorphies

including a loose checkerboard, loose checkerboard with interspersed bicolor scales or bicolored ventral patterns (character 3c-1, Table 1; Fig. 3E, F, G), and laterally forked banded dorsal pattern (character 6-1, Table 1; Fig. 3E). The monophyly of *L. g. floridana* from the peninsula (node “F”, Fig. 8) is weakly supported by a single homoplasious character of a band width of 1.5 DSR (character 12-2, Table 1; Fig. 7).

Molecular Analyses. All molecular analyses yielded very similar tree topologies as in our morphological analyses. MP analysis using combined genes with equally weighted TS:TV resulted in 1169 most parsimonious trees of 364 steps (CI = 0.799, RI = 0.854). A randomly selected representative of these shortest trees was created illustrating the number of base differences between haplotypes and bootstrap support above 50% (Fig. 10). BA analysis resulted in very similar tree topologies as in MP (Fig. 11), but with elevated posterior-probabilities at some ingroup nodes. The western, midwestern, and eastern United States samples formed separate and extremely well-supported (100%) monophyletic groups, with relatively large genetic breaks between them (Fig. 10). The eastern United States clade is further divided into 3 subclades: Peninsula, Atlantic, and Eastern Apalachicola/Peninsula/southern Georgia (E Apalachicola/Panhandle/S GA). Nodes within the eastern United States clade are less well-supported, and there are fewer base differences between subclades and individuals, illustrating their close relationships to each other.

Discussion

Based on a previous cladistic analysis, Keogh (1996) found that a terrestrial behavior was the single synapomorphy defining the monophyly of *Lampropeltis getula* throughout its wide range. In this study, although some clades (or currently recognized subspecies) within the *L. getula* complex may be weakly supported by homoplasious characters, at least one synapomorphy supports the monophyly of each major clade. Midwestern/western snakes (*L. g. holbrooki*, *L. g. nigra*, *L. g. nigrita*, and *L. g. splendida*) represent sister taxa to the eastern United States clade (*L. g. floridana*, *L. g. getula*, and Eastern Apalachicola Lowlands snakes) (Figs. 8, 9). Genetic data illustrate very similar tree topologies (Figs. 10, 11; paper in progress, but *see* Krysko 2001, Krysko & Franz 2003) as those generated in our morphological results (Figs. 8, 9), but include only midwestern snakes (*L. g. holbrooki* and *L. g. nigra*) as sister taxa to the eastern United States clade. These different datasets support the two nearly identical hypotheses of Blanchard (1921) and Blaney (1977), in which the eastern United States populations were derived from midwestern/western populations. Additionally, both datasets support Blaney’s (1977) hypothesis that populations in the eastern United States represent a distinct and well-supported monophyletic group, suggesting that they be recognized as a distinct species. Although Blaney (1977) hypothesized that only two evolutionary entities (*L. g. getula* and *L. g. floridana*) exist in the eastern United States, these morphological and molecular datasets consistently yield three natural groups or subclades. These three subclades

correspond to the two currently recognized subspecies, *L. g. getula* and *L. g. floridana*, and a third group consisting of unnamed snakes from the Eastern Apalachicola Lowlands. The constant detection of this unnamed group supports Means' (1977) hypothesis that these populations represent a natural group and distinct biological entity. However, the circumscription of three identified subclades is occasionally problematic because of morphological intermediates (or hybrids) formed through interbreeding with adjacent populations.

Krysko (1995, 2001) stated that an intergradation zone between *Lampropeltis getula floridana* and *L. g. getula* occurs from Pinellas County in the central Florida peninsula northeast to Duval County in the northern peninsula. Molecular results group all morphological intermediates from this zone within the peninsula subclade (= *L. g. floridana*) (Figs. 10, 11), and all but two samples (Dixie and Duval counties) in our morphological analyses (Figs. 8, 9) showed the same result. Snakes from Duval County look like *L. g. getula*, but have an ontogenetically lightened interband dorsal pattern like *L. g. floridana* (Krysko 1995, 2001), and this sample was placed either in the *L. g. getula* subclade or in an Atlantic/Peninsula subgroup made up entirely of intermediate phenotypes (Fig. 8). Blaney (1977, 1979) hypothesized that kingsnakes from the Outer Banks, Dare County, North Carolina, were relict intergrades between *L. g. floridana* and *L. g. getula* because these individuals possessed an intermediate phenotype between these two geographic races. Our morphological data always place Dare County samples with the Duval County sample (Figs. 8, 9), but our molecular analyses group the Duval County sample within the peninsula and Dare County (i.e., Outer Banks) haplotypes were identical to those from the adjacent North Carolina mainland and as far away as southwestern Georgia (Table 2; also see Krysko 2001, Krysko & Franz 2003). Barbour and Engels (1942) described the Outer Banks populations as *L. g. sticticeps*, however, this name was rejected by Blaney (1977, 1979) and our results support his conclusion. Means & Krysko (2001) showed that some morphological characters are unique to the Eastern Apalachicola Lowlands populations and intermediate phenotypes between these snakes and *L. g. getula* are found in the surrounding region suggesting gene flow. These intermediate phenotypes are found as far away as Bay County to the west, Calhoun County to the north, and Jefferson County to the east. Although the frequency of these intermediate phenotypes tapers off considerably as one moves further away from the Eastern Apalachicola Lowlands, these data suggest genes have been exchanged over a substantial distance. Means & Krysko's (2001) gene flow hypothesis is supported by our morphological data as intermediate phenotypes from southern Gulf County to the west and Wakulla County to the east are found within the Eastern Apalachicola Lowlands subclade (Figs. 8, 9). Additionally, molecular data support Means & Krysko's (2001) hypothesis illustrating exchange of genes between Eastern Apalachicola Lowlands snakes with mostly morphological intermediates from the surrounding region (Figs. 10, 11; also see Krysko 2001; Krysko & Franz 2003). It is quite interesting that the unnamed polymorphic and morphologically distinctive Eastern Apalachicola Lowlands populations are identified by more synapomorphies than the other currently recognized *L. getula* subspecies in the eastern

United States (Figs. 8–11). Because the Eastern Apalachicola Lowlands populations overlap in distribution with a number of other endemic plants and animals (Clewell 1977; Coile 1996; Gilbert 1987; James 1961; Judd 1982; Ward 1979; Yerger 1977; for a list of species *see* Table 1 in Means & Krysko 2001), these snakes likely evolved locally along with other endemics, and we believe deserve taxonomic recognition.

Taxonomy. Many current systematic studies are carried out with an underlying assumption that phylogenetic patterns exist not only between, but also within recognized species (i.e., phylogeographic investigations; *see* Avise 1994). One question that arises is how (if at all) to treat subclades or infraspecific entities in a formal nomenclatural system (taxonomy), although many researchers are currently simply elevating these taxa to species status without much thought. However, species are not necessarily the least inclusive monophyletic group discerned in phylogenetic studies, although they have sometimes been treated as such. As noted by Mishler & Brandon (1987:408), “It is not sufficient to say that a species is the smallest diagnosable cluster...or even monophyletic group, because such groups occur at all levels...”. Mishler & Brandon (1987:397) advocated a Phylogenetic Species Concept (PSC) that “uses a (monistic) grouping criterion of monophyly in a cladistic sense, and a (pluralistic) ranking criterion based on those causal processes that are *most important* in producing and maintaining lineages in a particular case. Such causal processes can include actual interbreeding, selective constraints, and developmental canalization.” Around the same time, a similar viewpoint was proposed by Donoghue (1985). Mishler & Theriot (2000:46) slightly redefined the PSC, which is also called the Apomorphic Species Concept (ASC; *see* Judd *et al.* 2002), stating that “a species is the least inclusive taxon recognized in a formal phylogenetic classification.” But notably, Mishler & Theriot (2000:46) go on to state that, “As with all hierarchical levels of taxa in such a classification, organisms are grouped into species because of evidence of monophyly. Taxa are ranked as species rather than at some higher level because they are the smallest monophyletic groups *deemed worthy* of formal recognition, because of the amount of support for their monophyly and/or because of their importance in biological processes operating on the lineage in question.” Note the words “*most important*” and “*deemed worthy*” in these definitions. It is clear that these definitions leave it up to the researcher to decide which clades are considered to be evolutionary significant and/or worthy of taxonomic naming. Such decisions are often subjective, as previously noted by O’Hara (1993). Thus, there may not be a clear demarcation between populations, connected by tokogenetic relationships and species-lineages, showing a phylogenetic pattern. The decision as to which putative clades should be recognized as species and which should be more appropriately considered as infraspecific entities (whether formally or informally named) is often problematic, and we suggest that researchers be more cautious about how to recognize these types of natural groups and not simply elevate infraspecific clades to species status without thought.

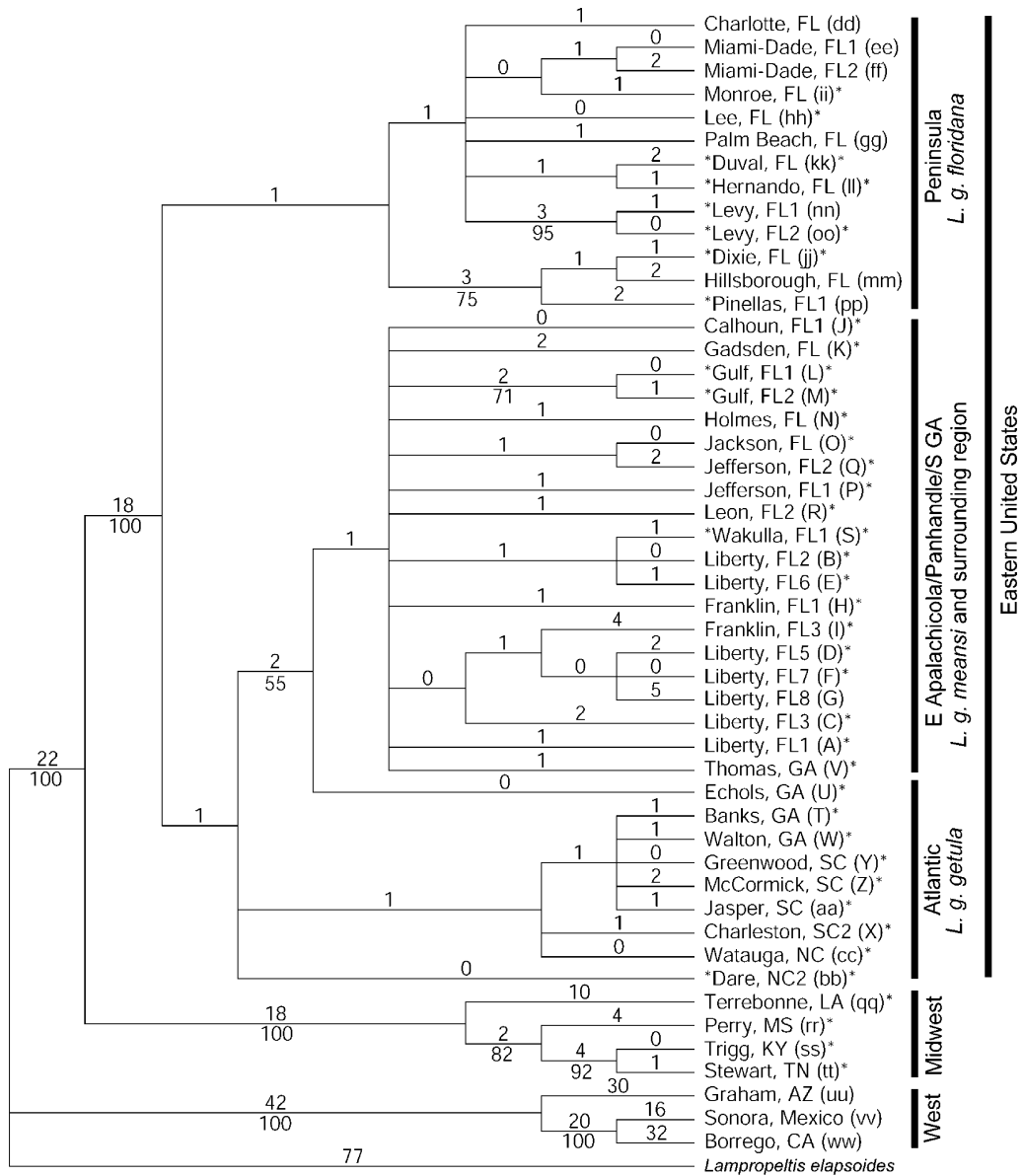


FIGURE 10. Randomly selected representative of 1169 most parsimonious trees with number of base differences (above) and bootstrap values (> 50%, below) from unweighted maximum parsimony analysis with 1886 base pairs of combined cytochrome-*b* and ND4 region mtDNA genes in kingsnakes of the *Lampropeltis getula* complex. Letter(s) in parentheses indicate distinct haplotype. Putative morphological intermediates are indicated with an asterisk before sample locality. An asterisk after haplotype indicates that the same individual was used for morphological analyses in this study.

Wilson & Brown (1953) were the first to remark that the subspecies concept had been misapplied (in the past). Subspecies were commonly identified by too few and arbitrary

delimited characters, and in many cases where several characters were used, each character varied independently because of differing locally adaptive pressures resulting in different subspecies distributions (depending upon the character chosen by the systematist) and arbitrary division of clines (Wilson & Brown 1953; also *see* Frost & Hillis 1990). Although we certainly concur with their views especially as applied to taxonomic work occurring in the early and middle twentieth century, we do not believe that there has been an absolute justification in the literature to completely disregard the use of infraspecific names, especially when they refer to natural clades (also *see* Smith *et al.* 1997) given that we now have the ability through phylogenetic analysis of molecular and morphological data to develop reasonably well supported relationships of biological entities within many species (especially when geographically widely distributed). Thus, the idea of completely abandoning the subspecies concept, although reasonable at the time of Wilson & Brown (1952), requires reconsideration. We take a pragmatic standpoint, noting that phylogenetically meaningful groups (= clades or natural groups) are often discernable within currently recognized species, and that these clades often correlate with biogeography. We believe it is often useful to refer to such infraspecific clades formally as other researchers have done using cladistic analyses below the species level (*see* Hammond 1990[1991]; Wilken & Hartman 1991; Campbell 1983, 1986). Herein, we expand the ASC (= Phylogenetic Species Concept *sensu* Donoghue 1985; Mishler 1985; Mishler & Brandon 1987; Mishler & Theriot 2000), following the example of Wilken & Hartman (1991). We see no reason why phylogenetically meaningful groups (i.e., natural monophyletic groupings of populations) within a species cannot be formally named as subspecies. In this study, after much deliberation such monophyletic groups are ranked as subspecies (and not as species) because the support for their monophyly is less, either because of interbreeding with individuals of adjacent populations or because of a more recent evolutionary divergence. We have tried to consistently apply our view of species and subspecies to the *Lampropeltis getula* complex, but we are aware that other researchers might have a different philosophical view regarding our taxonomic arrangement and consider our groupings of populations in the eastern United States at the specific (instead of the subspecific) level and view morphological intermediates as mere hybrids.

Individuals of *Lampropeltis getula* from the western side of the Apalachicola River were once described as a distinct subspecies, *L. g. goini* (Neill & Allen 1949). Neill & Allen (1949) named this taxon based on nine specimens restricted to Calhoun and northern Gulf counties (*see* Fig. 1 map in Means & Krysko 2001), but they did not examine any snakes elsewhere and appear to have been completely unaware of the extreme morphologically divergent populations found on the adjacent (eastern) side of the Apalachicola River, Florida's largest river. Blaney (1977, 1979) rejected *L. g. goini* by speculating that it represents a relict Pleistocene morphological intermediate (or hybrid) between Florida panhandle *L. g. getula* and now disjunct peninsular *L. g. floridana*. Means

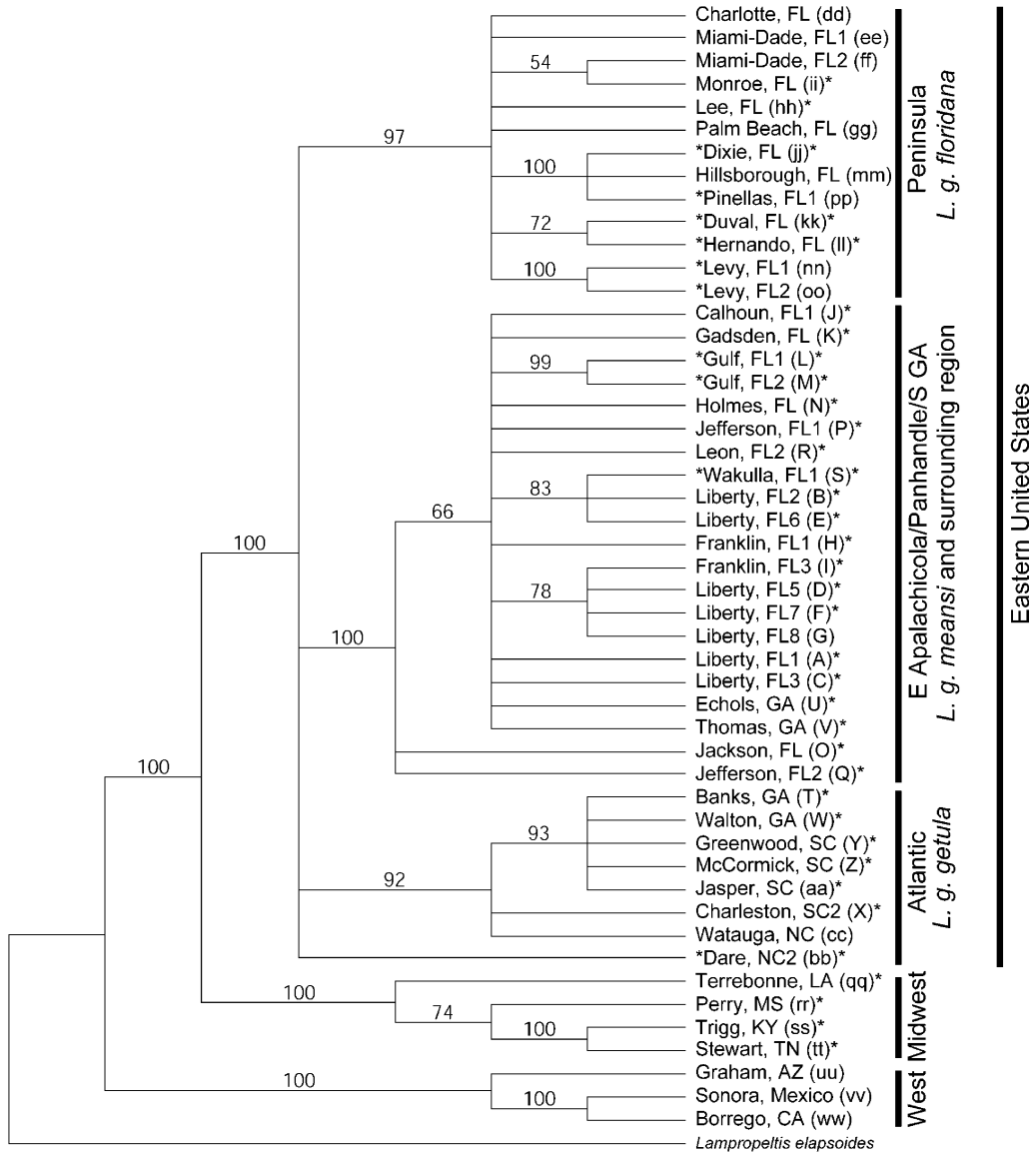


FIGURE 11. Majority rule consensus phylogeny inferred from Bayesian analysis with posterior probabilities (above branches) using 1886 base pairs of combined cytochrome-*b* and ND4 region mtDNA genes in kingsnakes of the *Lampropeltis getula* complex. Letter(s) in parentheses indicate distinct haplotype. Putative morphological intermediates are indicated with an asterisk before sample locality. An asterisk after haplotype indicates that the same individual was used for morphological analyses in this study.

(1977) and Means & Krysko (2001) also rejected *L. g. goini* and agreed with Blaney that the holotype (and paratypes) is a snake showing intermediate morphological features, but they believed it is intermediate between unnamed populations in the Eastern Apalachicola Lowlands (between the Apalachicola and Ochlockonee rivers) and *L. g. getula* that

surrounds the region (also *see* Means 1978, 1992). Therefore, we must follow the ICZN (International Code of Zoological Nomenclature 1999) for naming the Eastern Apalachicola Lowlands populations, as names found to denote more than one taxon (i.e., morphological intermediate or hybrid) are not available, as they are individuals, not populations, and hence not taxa (*see* Articles 1.3.3, 17, and 23.8). Additionally, because the name “*goini*” is attached to the holotype, which has been relegated to intermediate (or hybrid) status by numerous researchers it is irrelevant that this name has been applied to *L. getula* populations in other areas and a new name is warranted for populations in the Eastern Apalachicola Lowlands (L. Wilson, and A. Polaszek & S. Morris [ICZN] pers. comm.).

Although our discussion is not meant to be exhaustive, we feel it is important here to briefly discuss some other commonly utilized species concepts and how, using our data, these might effect the taxonomic treatment of *Lampropeltis getula* populations in the eastern United States. Under the Biological Species Concept (BSC; *sensu* Mayr 1969), species are reproductively incompatible, and subspecies are more or less allopatric populations that can be distinguished morphologically but are reproductively compatible (Mayr 1969; Smith *et al.* 1997). Our species delineation, which is based on the ASC, would be in agreement with the BSC. Under the Diagnostic Species Concept (DSC; Phylogenetic Species Concept *sensu* Wheeler & Platnick 2000), species are considered to be the minimal diagnosable group and taxonomic subgroups are discouraged (Davis & Nixon 1992; Wheeler & Platnick 2000). Under the Evolutionary Species Concept (ESC; *sensu* Wiley & Mayden 2000), species are considered to be lineages with their own tendencies and historical fates held together by tokogenetic relationships/descent and infraspecific taxa are not recognized. Our suggestion that the eastern United States populations of *L. getula* constitute a distinct species (as based on the ASC) is in agreement with the DSC and ESC, because these populations form a well-supported (100%) monophyletic group. However, no subspecies would be recognized under these approaches. The Genealogical Species Concept (GSC; *sensu* Baum 1992; Baum & Donoghue 1995; Baum & Shaw 1995) is an extension of the ASC that stresses the assessment of historical relationships via gene coalescence. Thus, species are an exclusive group of organisms that are more closely related to each other than they are to any organism outside of that group (de Queiroz & Donoghue 1990; Baum 1992; Baum & Donoghue 1995; Baum & Shaw 1995). This approach, in our case, would probably yield similar results to the ASC, although the suggested methodology involves the use of more DNA regions.

Obviously, these various approaches can lead to different conclusions regarding the recognition of biological entities, and some may also lead to an underestimate of biological diversity. We believe that it is useful to combine phylogenetic analyses with biogeography (= phylogeography; *see* Avise 1994), and this paper compliments other studies on the biogeography of *Lampropeltis getula* (Means & Krysko 2001; Krysko &

Franz 2003). With reassessment of species concepts and use of updated laboratory techniques, researchers will benefit in scrutinizing gray zones (i.e., the taxonomic “line of death”, Wheeler & Platnick 2000:57) between tokogenetic and phylogenetic realms (also see O’Hara 1993). We prefer to use the ASC over other currently utilized species concepts because it allows us to recognize natural groups (i.e., clades recognized at the level of species) as well as natural groups (i.e., subclades within species), and thus usefully represent phylogenetic patterns near such gray zones. Furthermore, in this case the ASC leads to a more accurate estimate of biological diversity.

Systematic Account

Lampropeltis getula meansi ssp. nov.

Common name. English: Apalachicola Lowlands Kingsnake; Spanish: Serpiente rey de las tierras bajas de Apalachicola.

Holotype. UF 73433 (field tag DBM 1360), an adult male collected 9 June 1970 in the Apalachicola National Forest on FH-13 ca. 3.2 km W SR 67, Liberty County, Florida, United States, by D. Bruce Means (Fig. 12).

Paratypes. All specimens from the Eastern Apalachicola Lowlands: UF 55449, male, Liberty County, FL; UF 55365, male, Apalachicola National Forest, NFR 126, 0.1 km S NFR 111, Liberty County, FL; UF 55362, female, Apalachicola National Forest, NFR 107, 1.2 km E NFR 122, Liberty County, FL; UF 55421, male, Apalachicola National Forest, NFR 111, 1.6 km E NFR 120, Liberty County, FL; UF 55385, male, Apalachicola National Forest, SR 65, 4.8 km S Clio, Liberty County, FL; UF 73638, female, Apalachicola National Forest, SR 67, 12.8 km S Telogia, Liberty County, FL; UF 128273, male, Tate’s Hell Swamp, US 98, 0.8 km W Carrabelle, Franklin County, FL; UF 73639, male, Tate’s Hell State Forest, SR 65, 1.6 km S Whiskey George Creek, Franklin County, FL.

Diagnosis. A large-sized, polymorphic population of *Lampropeltis getula* distinguished from all others by its overall light dorsal coloration, having either narrow or wide crossbands with considerably lightened interbands, or being non-banded (striped or patternless). Combinations of these basic phenotypes also occur regularly in the wild. The ventral pattern is also variable, being either bicolored, loose checkerboard with interspersed bicolored scales, or mostly dark.

Description of holotype. 1040 mm SVL; 155 mm tail; on both sides of head: 1 + 2 oculars, 2 + 3 + 4 temporals, 7 + 7 supralabials, 9 + 9 infralabials; 52 subcaudals; 21 DSR at midbody; 211 ventrals; dorsal pattern non-banded (patternless); ventral pattern bicolored cephalad with dark pigment suffused with bicolored scales caudally (Fig. 12).

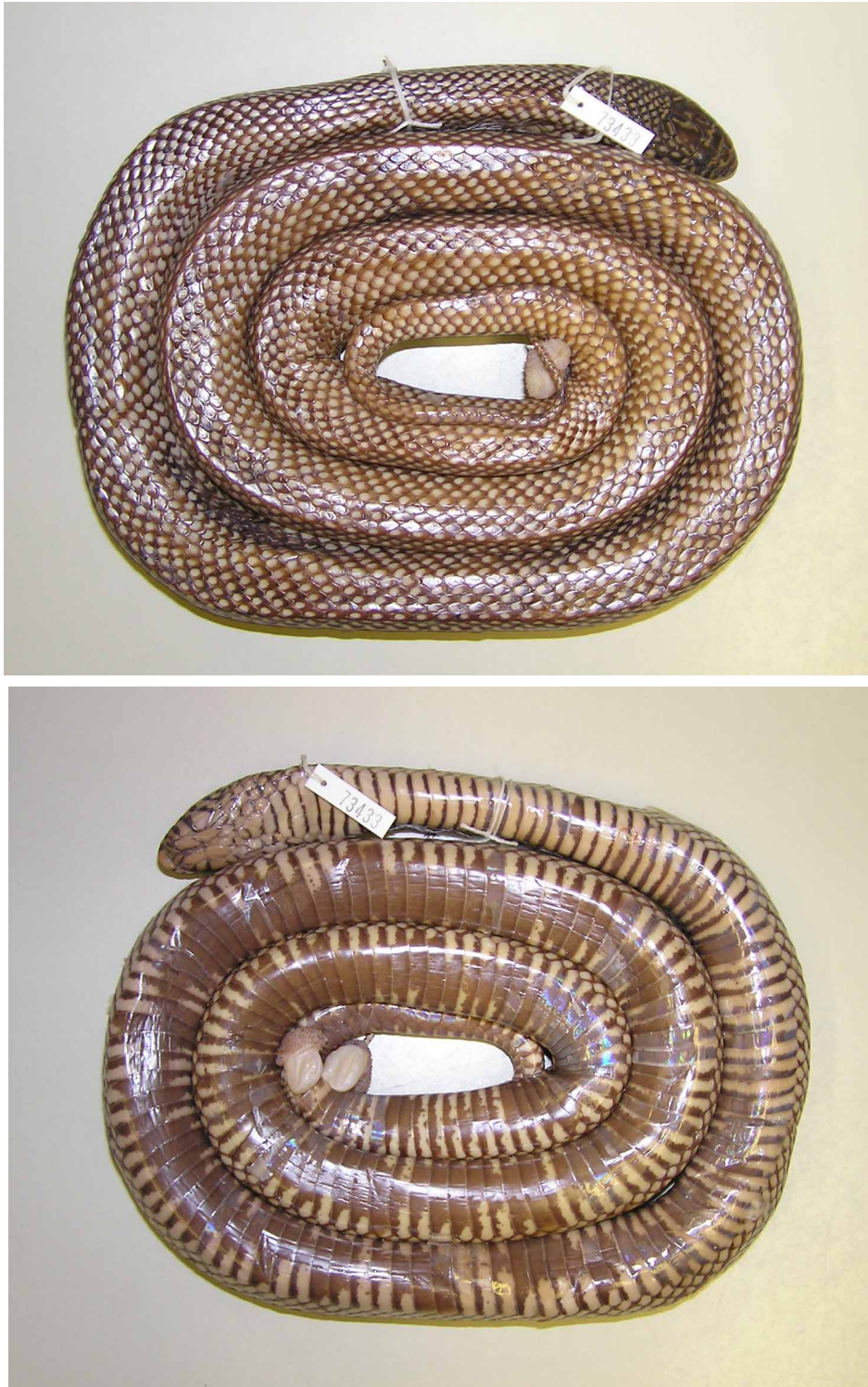


FIGURE 12. Holotype of the Eastern Apalachicola Lowlands kingsnake (*Lampropeltis getula mansi*): dorsal (above) and ventral (below) views.

Variation. 250 mm to 1425 mm SVL; 1 + 2 oculars; arrangement of temporals ($n = 95$ head sides) 2 + 3 + 4 (74.7%), 3 + 3 + 4 (2.10%), 2 + 3 + 3 (9.47%), 2 + 2 + 4 (4.21%), 2 + 3 + 5 (3.15%), 2 + 2 + 3 (4.21%), 1 + 3 + 4 (1.05%), 1 + 3 + 3 (1.05%); 7 + 7 supralabials; infralabials ($n = 91$ head sides) 9 (54.9%), 10 (43.9%), 11 (1.0%); subcaudals in males 47 to 53 (mean = $50.6 \pm \text{S.E.} = 0.43$, $n = 31$), females 42 to 53 (mean = 45.5 ± 0.59 , $n = 17$); 21 DSR at midbody; ventrals 206 to 222 (mean = 212.7 ± 0.47 , $n = 60$). Dorsal pattern variable: crossbands wide (up to the entire body length) and numbering from 1 to 25 (mean = 16.0 ± 1.31 , $n = 26$) in females and 1 to 23 (mean = 9.6 ± 0.82 , $n = 65$) in males. Gradual ontogenetic interband lightening occurs on newborns' normally black interbands on the anterior 1/2 to 3/4 of each scale, which varies from 25–100% of the intensity of the light crossbands in the adult stage (*see* Figs. 7, 8 in Means & Krysko 2001). Adults that possess interbands the same intensity of the light crossbands appear to be non-banded (striped or patternless), but they can be distinguished from truly non-banded morphs due to the difference in morphology between the light colored crossband and interband scale types (Means & Krysko 2001).

Distribution. Found in the Eastern Apalachicola Lowlands in the Florida panhandle between the Apalachicola and Ochlockonee rivers and south of Telogia Creek, Franklin and Liberty counties. Individuals of this taxon are also occasionally found in the southwestern Apalachicola Lowlands on the western side of the Apalachicola River. Morphological intermediates (i.e., *L. g. goini*) between *L. g. meansi* and *L. g. getula* are found mostly in the surrounding region from southern Gulf and Franklin counties to the west, north to Calhoun County, and east into northern Liberty (north of Telogia Creek), Gadsden, Leon, Wakulla, and Jefferson counties.

Etymology. The taxon name is a noun, named for Dr. D. Bruce Means in recognition of his discovery of the first known Eastern Apalachicola Lowlands kingsnake, as well as his contributions to our knowledge of the flora and fauna of the Coastal Plains.

Remarks. In the early to mid 1970s, it was not uncommon to encounter up to five *Lampropeltis g. meansi* crossing roads during the spring mating season (Krysko & Smith 2005). However, after travelling thousands of kilometers and hours on these same roads during the 1990s, KLK found only one individual, which had just been killed by a vehicle (Krysko & Smith 2005). Due to the rarity and severely declining populations of nearly all *Lampropeltis getula* in Florida (Krysko 2001, 2002; Krysko & Smith 2005), *L. g. meansi* should be listed at the state and/or federal level.

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Appendix 1. Sample (OTU); Voucher number, and locality of kingsnakes in the *Lampropeltis getula* complex used for morphological analyses. Asterisk next to sample number in parentheses indicates that identical individual was used in DNA analyses (Appendix 3; paper in progress; but see Krysko 2001; Krysko & Franz 2003).

No.	Sample	Voucher No., Locality
1	San Diego, CA ¹	UF 116020; United States: California, San Diego County
2	San Diego, CA ²	UF 116023; United States: California, San Diego County
3	Sonora, Mexico	UF 123994, Mexico: Sonora
4	Duval, TX	UF 116062; United States: Texas, Duval County, Hwy 59, 9.5 km N of Freer
5	Terrebonne, LA	KLK-519 (*Lg 59); United States: Louisiana, Terrebonne Parish, Houma
6	Perry, MS	UF 128260 (*Lg 90); United States: Mississippi, Perry County
7	Calloway, KY	UF 128259 (*Lg 56); United States: Kentucky, Calloway County
8	Trigg, KY	UF 128264 (*Lg 93); United States: Kentucky, Trigg County
9	Dare, NC ¹	UF 128283 (*Lg 121); United States: North Carolina, Dare County, Hatteras Island
10	Dare, NC ²	UF 128281 (*Lg 111); United States: North Carolina, Dare County, Hatteras Island
11	Watauga, NC	UF 128275 (*Lg 29); United States: North Carolina, Watauga County, Triplett
12	Charleston, SC ¹	UF 128278 (*Lg 37); United States: South Carolina, Charleston County, Adams Run
13	Charleston, SC ²	UF 128282 (*Lg 120); United States: South Carolina, Charleston County, Edisto Island
14	Greenwood, SC	UF 128284 (*Lg 129); United States: South Carolina, Greenwood County, US 221, 2.6 km W of Hwy 10
15	Jasper, SC	UF 128280 (*Lg 99); United States: South Carolina, Jasper County
16	Banks, GA	UF 128267 (*Lg 117); United States: Georgia, Banks County, Yonah Church Rd, 9.8 km W of Homer
17	Echols, GA	KME-m10 (*Lg 18); United States: Georgia, Echols County, Statenville
18	Randolph, GA	UF 128285 (*Lg 133); United States: Georgia, Randolph County
19	Thomas, GA	UF 128286 (*Lg 134); United States: Georgia, Thomas County, Ochlockonee River
20	Walton, GA	UF 121162 (*Lg 119); United States: Georgia, Walton County, Loganville
21	Bay, FL	UF 128279 (*Lg 52); United States: Florida, Bay County, SR 22, 32.1 km W of Wewahitchka

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Appendix 1 (continued)

No.	Sample	Voucher No., Locality
22	Miami-Dade, FL	UF 19675; United States: Miami-Dade County, Krome Ave ca. 16 km S of Tamiami Trail
23	Calhoun, FL	UF 114321 (*Lg 5); United States: Florida, Calhoun County, Blountstown
24	Dixie, FL	UF 128269 (*Lg 115); United States: Florida, Dixie County, CR 361, 5.7 km S of Rocky Creek
25	Duval, FL	KME-f11 (*Lg 16); United States: Florida, Duval County, Jacksonville
26	Franklin, FL ¹	UF 128263 (*Lg 45); United States: Florida, Franklin County, US 98, 7 km E of C30
27	Franklin, FL ²	UF 128273 (*Lg 46); United States: Florida, Franklin County, US 98, 9.5 km W of Carrabelle
28	Franklin, FL ³	UF 128262 (*Lg 55); United States: Florida, Franklin County, Tates Hell Swamp near New River
29	Gadsden, FL	UF 128271 (*Lg 47); United States: Florida, Gadsden County, US 90 5.7 km W of Quincy
30	Gulf, FL ¹	KME-m25 (*Lg 11); United States: Florida, Gulf County, Port St. Joe
31	Gulf, FL ²	KME-m26 (*Lg 15); United States: Florida, Gulf County, Port St. Joe
32	Hernando, FL	UF 111101 (*Lg 23); United States: Florida, Hernando County, Hernando Beach
33	Hillsborough, FL	UF 128258; United States: Florida, Hillsborough County, Brandon
34	Holmes, FL	UF 128261 (*Lg 65); United States: Florida, Holmes County, Rt 179A, 3.8 km SW of SR 2
35	Jackson, FL	KLK-491 (*Lg 107); United States: Florida, Jackson County, CR 271
36	Jefferson, FL	UF 128270 (*Lg 50); United States: Florida, Jefferson County, Goosepasture Rd, 1.9 km S of Tram Rd
37	Lee, FL	UF 128277 (*Lg 35); United States: Florida, Lee County, Gasparilla Island, Boca Grande
38	Leon, FL ¹	KME-m3 (*Lg 13); United States: Florida, Leon County, Bloxham cutoff
39	Leon, FL ²	UF 128272 (*Lg 51); United States: Florida, Leon County, Meridian Rd, 0.2 km S of Meridian Hills Rd
40	Levy, FL	UF 95556; United States: Florida, Levy County, 9.6 km E of Cedar Key
41	Liberty, FL ¹	UF 105383 (*Lg 10); United States: Florida, Liberty County, SR 67, 3.7 km S of SR 20

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Appendix 1 (continued)

No.	Sample	Voucher No., Locality
42	Liberty, FL ²	UF 128266 (*Lg 26); United States: Florida, Liberty County, SR 67, just N of Liberty-Franklin County line
43	Liberty, FL ³	UF 114323 (*Lg 27); United States: Florida, Liberty County, near junction of NFR 103 and 116
44	Liberty, FL ⁴	UF 114322 (*Lg 28); United States: Florida, Liberty County, near junction of NFR 103 and 116
45	Liberty, FL ⁵	DBM-104 (*Lg 30); United States: Florida, Liberty County
46	Liberty, FL ⁶	DBM-50 (*Lg 32); United States: Florida, Liberty County, NFR 110, 2.9 km S of jct 111
47	Liberty, FL ⁷	KLK-247 (*Lg 58); United States: Florida, Liberty County, NFR 139
48	Monroe, FL	UF 123777 (*Lg 36, KLK-490); United States: Florida, Monroe County, Key Largo
49	Palm Beach, FL	UF 99739 (KLK-94005); United States: Florida, Palm Beach County, Pahokee
50	Pinellas, FL	UF 128265; United States: Florida, Pinellas County
51	Wakulla, FL ¹	KME-m13 (*Lg 12); United States: Florida, Wakulla County, junction of NFR 313 and 312
52	Wakulla, FL ²	KME-f3 (*Lg 14); United States: Florida, Wakulla County, Arren

Appendix 2. Data matrix for kingsnakes in the *Lampropeltis getula* complex used for cladistic analyses. See Table 1 for character descriptions.

No.	Sample (OTU)	Character State															
		1	2	3a	3b	3c	3d	3e	4	5	6	7	8	9	10	11	12
1	San Diego, CA ¹	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	San Diego, CA ²	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2
3	Sonora, Mexico	1	1	1	1	0	0	0	1	2	1	1	0	0	3	2	1
4	Duval, TX	1	1	1	0	0	0	0	1	3	1	1	1	0	3	2	1
5	Terrebonne, LA	2	1	1	0	0	0	0	1	4	1	1	1	0	3	2	1
6	Perry, MS	2	1	1	0	0	0	0	1	4	1	1	1	0	3	2	1
7	Calloway, KY	2	1	1	0	0	0	0	1	2	1	1	0	0	3	2	1
8	Trigg, KY	2	1	1	0	0	0	0	1	2	1	1	0	0	3	2	1
9	Dare, NC ¹	2	2	1	0	1	0	0	1	4	1	2	2	1	2	1	2
10	Dare, NC ²	2	2	1	0	1	0	0	1	4	1	2	2	1	2	1	2
11	Watauga, NC	2	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
12	Charleston, SC ¹	2	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
13	Charleston, SC ²	2	2	1	0	1	0	0	2	1	1	2	0	1	2	1	3
14	Greenwood, SC	2	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
15	Jasper, SC	2	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
16	Banks, GA	2	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
17	Echols, GA	2	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
18	Randolph, GA	2	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
19	Thomas, GA	2	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
20	Walton, GA	2	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
21	Bay, FL	2	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
22	Miami-Dade, FL	1	1	1	0	0	0	0	1	4	1	2	2	1	3	1	2
23	Calhoun, FL	2	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
24	Dixie, FL	2	1	1	0	0	0	0	1	4	1	2	2	1	2	1	2
25	Duval, FL	2	2	1	0	1	0	0	1	4	1	2	2	1	2	1	2
26	Franklin, FL ¹	2	3	1	0	1	1	0	2	4	2	2	2	1	2	1	3
27	Franklin, FL ²	2	4	1	0	1	1	1	3	4	2	2	2	1	1	1	4
28	Franklin, FL ³	2	3	1	0	1	1	0	2	4	2	2	2	1	2	1	3
29	Gadsden, FL	1	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
30	Gulf, FL ¹	2	3	1	0	1	1	0	2	4	2	2	2	1	2	1	3
31	Gulf, FL ²	2	3	1	0	1	1	0	2	4	2	2	2	1	2	1	3
32	Hernando, FL	1	1	1	0	0	0	0	1	4	1	2	2	1	2	1	2
33	Hillsborough, FL	1	1	1	0	0	0	0	1	4	1	2	2	1	3	1	2

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Appendix 2 (continued)

No.	Sample (OTU)	Character State															
34	Holmes, FL	1	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
35	Jackson, FL	2	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
36	Jefferson, FL	2	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
37	Lee, FL	1	1	1	0	0	0	0	1	4	1	2	2	1	3	1	2
38	Leon, FL ¹	2	3	1	0	1	0	0	1	1	1	2	0	1	2	1	3
39	Leon, FL ²	1	2	1	0	1	0	0	1	1	1	2	0	1	2	1	2
40	Levy, FL	1	1	1	0	0	0	0	1	4	1	2	2	1	2	1	2
41	Liberty, FL ¹	2	3	1	0	1	1	0	2	4	2	2	2	1	2	1	3
42	Liberty, FL ²	2	3	1	0	1	1	0	2	4	2	2	2	1	2	1	3
43	Liberty, FL ³	2	3	1	0	1	1	0	2	4	2	2	2	1	2	1	3
44	Liberty, FL ⁴	2	3	1	0	1	1	0	2	4	2	2	2	1	2	1	3
45	Liberty, FL ⁵	2	4	1	0	1	1	1	4	4	2	2	2	1	1	1	4
46	Liberty, FL ⁶	2	4	1	0	1	1	1	3	4	2	2	2	1	1	1	4
47	Liberty, FL ⁷	2	3	1	0	1	1	0	2	4	2	2	2	1	2	1	3
48	Monroe, FL	1	1	1	0	0	0	0	1	4	1	2	2	1	3	1	2
49	Palm Beach, FL	1	1	1	0	0	0	0	1	4	1	2	2	1	3	1	2
50	Pinellas, FL	1	1	1	0	0	0	0	1	4	1	2	2	1	2	1	2
51	Wakulla, FL ¹	1	3	1	0	1	1	0	2	4	2	2	2	1	2	1	3
52	Wakulla, FL ²	2	3	1	0	1	1	0	2	4	1	2	2	1	2	1	3

Appendix 3. Haplotype; Sample (OTU); Genbank numbers; voucher number (Lab [Lg] and Field Nos.), and locality of kingsnakes, *Lampropeltis getula* complex, used for DNA analyses. Asterisk next to lab number in parentheses indicates that identical sample was used in morphological analyses (see Appendix 1). Shared haplotypes are also listed in Table 2.

Haplotype	Sample	Genbank Nos.; Voucher No. (Lab [Lg] and Field Nos.); Locality
A	Liberty, FL ¹	DQ360325, DQ360458; UF 105383 (*Lg 10, KLK 211); U.S.: Florida, Liberty County, SR 67, 3.7 km S SR 20
B	Liberty, FL ²	DQ360326, DQ360459; (*Lg 26, KLK 213); U.S.: Florida, Liberty County, SR 67, just N Liberty-Franklin County line
C	Liberty, FL ³	DQ360327, DQ360460; (*Lg 27, KLK 239); U.S.: Florida, Liberty County, near junction NFR 103 and 116
C	Liberty, FL ⁴	DQ360328, DQ360461; (*Lg 28, KLK 240); U.S.: Florida, Liberty County, near junction NFR 103 and 116
D	Liberty, FL ⁵	DQ360329, DQ360462; (*Lg 30, DBM 104); U.S.: Florida, Liberty County
E	Liberty, FL ⁶	DQ360330, DQ360463; (*Lg 32, DBM 50); U.S.: Florida, Liberty County, NFR 110, 2.9 km S NFR 111
F	Liberty, FL ⁷	DQ360331, DQ360464; (*Lg 58, KLK 247); U.S.: Florida, Liberty County, NFR 139
G	Liberty, FL ⁸	DQ360332, DQ360465; (Lg 31, KLK 537); U.S.: Florida, Liberty County
H	Franklin, FL ¹	DQ360323, DQ360456; (*Lg 45, KLK 220); U.S.: Florida, Franklin County, US 98, 7 km E C30
I	Franklin, FL ³	DQ360324, DQ360457; (*Lg 55, KLK 231); U.S.: Florida, Franklin County, Tates Hell Swamp, near New River
J	Bay, FL	DQ360310, DQ360443; (*Lg 52, KLK 227); U.S.: Florida, Bay County, SR 22, 32.1 km W Wewahitchka
J	Calhoun, FL ¹	DQ360308, DQ360441; UF 114321 (*Lg 5, KLK 31); U.S.: Florida, Calhoun County, Blountstown
J	Calhoun, FL ²	DQ360309, DQ360442; (Lg 6, KLK 32); U.S.: Florida, Calhoun County, Blountstown
J	Franklin, FL ²	DQ360312, DQ360445; (*Lg 46, KLK 221); U.S.: Florida, Franklin County, US 98, 9.5 km W Carrabelle
J	Leon, FL ¹	DQ360311, DQ360444; (*Lg 13, KME m3); U.S.: Florida, Leon County, Bloxham cutoff
K	Gadsden, FL	DQ360313, DQ360446; (*Lg 47, KLK 222); U.S.: Florida, Gadsden County, US 90 5.7 km W Quincy
L	Gulf, FL ¹	DQ360314, DQ360447; (*Lg 11, KME m25); U.S.: Florida, Gulf County, Port St. Joe

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Appendix 3 (continued)

Haplotype	Sample	Genbank Nos.; Voucher No. (Lab [Lg] and Field Nos.); Locality
M	Gulf, FL ²	DQ360315, DQ360448; (*Lg 15, KME m26); U.S.: Florida, Gulf County, Port St. Joe
N	Holmes, FL	DQ360316, DQ360449; (*Lg 65, KLK 257); U.S.: Florida, Holmes County, Rt 179A, 3.8 km SW SR 2
O	Jackson, FL	DQ360317, DQ360450; (*Lg 107, KLK 491); U.S.: Florida, Jackson County, CR 271
P	Jefferson, FL ¹	DQ360318, DQ360451; (*Lg 50, KLK 225); U.S.: Florida, Jefferson County, Goosepasture Rd, 1.9 km S Tram Rd
Q	Jefferson, FL ²	DQ360319, DQ360452; (Lg 105, KLK 538); U.S.: Florida, Jefferson County
R	Leon, FL ²	DQ360320, DQ360453; (*Lg 51, KLK 226); U.S.: Florida, Leon County, Meridian Rd, 0.2 km S Meridian Hills Rd
S	Wakulla, FL ¹	DQ360321, DQ360454; (*Lg 12, KME m13); U.S.: Florida, Wakulla County, junction of NFR 313 and 312
S	Wakulla, FL ²	DQ360322, DQ360455; (*Lg 14, KME f3); U.S.: Florida, Wakulla County, Arren
T	Banks, GA	DQ360333, DQ360466; (*Lg 117, KLK 350); U.S.: Georgia, Banks County, Yonah Church Rd, 9.8 km W Homer
U	Echols, GA	DQ360334, DQ360467; (*Lg 18, KME m10); U.S.: Georgia, Echols County, Statenville
V	Thomas, GA	DQ360335, DQ360468; (*Lg 134, KLK 524); U.S.: Georgia, Thomas County, Ochlockonee River
W	Walton, GA	DQ360336, DQ360469; UF 121162 (*Lg 119); U.S.: Georgia, Walton County, Loganville
X	Charleston, SC ²	DQ360337, DQ360470; (*Lg 120, KLK 528); U.S.: South Carolina, Charleston County, Edisto Island
Y	Greenwood, SC	DQ360338, DQ360471; (*Lg 129, KLK 522); U.S.: South Carolina, Greenwood County, US 221, 2.6 km W Hwy 10
Z	McCormick, SC	DQ360339, DQ360472; (Lg 24, KLK 531); U.S.: Florida, South Carolina, McCormick County, Long Cane Creek, junction SR 81 and SR 28
aa	Jasper, SC	DQ360340, DQ360473; (*Lg 99, KLK 526); U.S.: South Carolina, Jasper County
bb	Mitchell, GA	DQ360342, DQ360475; (*Lg 17, KME m27); U.S.: Georgia, Mitchell County, Cotton
bb	Randolph, GA	DQ360343, DQ360476; (*Lg 133, KLK 523); U.S.: Georgia, Randolph County

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Appendix 3 (continued)

Haplotype	Sample	Genbank Nos.; Voucher No. (Lab [Lg] and Field Nos.); Locality
bb	Dare, NC ²	DQ360341, DQ360474; (*Lg 111, KLK 525); U.S.: North Carolina, Dare County, Hatteras Island
cc	Dare, NC ¹	DQ360346, DQ360479; (*Lg 121, KLK 530); U.S.: North Carolina, Dare County, Hatteras Island
cc	Dare, NC ³	DQ360347, DQ360480; (Lg 104, KLK 532); U.S.: North Carolina, Dare County, Hatteras Island
cc	Watauga, NC	DQ360344, DQ360477; (*Lg 29, KLK 520); U.S.: North Carolina, Watauga County, Triplett
cc	Charleston, SC ¹	DQ360345, DQ360478; (*Lg 37, KLK 521); U.S.: South Carolina, Charleston County, Adams Run
dd	Charlotte, FL	DQ360293, DQ360426; (Lg 34, KLK 533); U.S.: Florida, Charlotte County, SR 776, S Englewood
ee	Miami-Dade, FL ¹	DQ360294, DQ360427; (Lg 3, KLK 94021); U.S.: Florida, Miami-Dade County, C-111 basin
ff	Miami-Dade, FL ²	DQ360295, DQ360428; (Lg 7, KLK 161); U.S.: Florida, Miami-Dade County, Krome Ave, 2 km S Tamiami Trail
gg	Hendry, FL	DQ360300, DQ360433; (Lg 19, KLK 534); U.S.: Florida, Hendry County, Hwy 80A, 18 km SE Clewiston
hh	Lee, FL	DQ360296, DQ360429; (*Lg 35, KLK 527); U.S.: Florida, Lee County, Gasparilla Island, Boca Grande
hh	Pinellas, FL ²	DQ360297, DQ360430; UF 121121 (*Lg 4, KLK 30); U.S.: Florida, Pinellas County, Pinellas Park
ii	Monroe, FL	DQ360298, DQ360431; UF 123777 (*Lg 36, KLK 490); U.S.: Florida, Monroe County, Key Largo
gg	Palm Beach, FL	DQ360299, DQ360432; (Lg 20, KLK 535); U.S.: Florida, Palm Beach County, King Ranch S South Bay
jj	Dixie, FL	DQ360301, DQ360434; (*Lg 115, KLK 316); U.S.: Florida, Dixie County, CR 361, 5.7 km S Rocky Creek
kk	Duval, FL	DQ360302, DQ360435; (*Lg 16, KME f11); U.S.: Florida, Duval County, Jacksonville
ll	Hernando, FL	DQ360303, DQ360436; UF 111101 (*Lg 23, KLK 230); U.S.: Florida, Hernando County, Hernando Beach
mm	Hillsborough, FL	DQ360304, DQ360437; (Lg 8, KLK 195); U.S.: Florida, Hillsborough County, Gibsonton
nn	Levy, FL ¹	DQ360305, DQ360438; (Lg 22, KLK 536); U.S.: Florida, Levy County, just N Cedar Key

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Appendix 3 (continued)

Haplotype	Sample	Genbank Nos.; Voucher No. (Lab [Lg] and Field Nos.); Locality
oo	Levy, FL ²	DQ360336, DQ360469; (Lg 116, KLK 539); U.S.: Florida, Levy County, Cedar Key
pp	Pinellas, FL ¹	DQ360337, DQ360470; (Lg 2, KLK 160); U.S.: Florida, Pinellas County, Gandy Blvd
qq	Terrebonne, LA	DQ360348, DQ360481; (*Lg 59, KLK 519); U.S.: Louisiana, Terrebonne Parish, Houma
rr	Perry, MS	DQ360349, DQ360482; (*Lg 90, KLK 259); U.S.: Mississippi, Perry County
ss	Trigg, KY	DQ360350, DQ360483; (*Lg 93, KLK 262); U.S.: Kentucky, Trigg County
tt	Stewart, TN	DQ360351, DQ360484; (Lg 96 KLK 529); U.S.: Tennessee, Stewart County
tt	Calloway, KY	DQ360352, DQ360485; (*Lg 56, KLK 232); U.S.: Kentucky, Calloway County
uu	Graham, AZ	AF337081; (JF25sY); U.S.: Graham County, AZ
vv	Sonora, Mexico	AF337070; (JF14sY); Mexico: Sonora
ww	Borrego Desert, CA	AF337079; (JF23sY); U.S.: Borrego Desert, CA
	L. elapsoides	AF337091; (JF35s); U.S.: Georgia, Dawson County, Amicalola Falls State Park