New Species of Lampropeltis (Serpentes: Colubridae) from the Sierra Madre Occidental, México

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ABSTRACT.—We describe a new species of Lampropeltis from the Pacific versant of the Sierra Madre Occidental in western México based on external morphology, scutellation, and molecular data. This species appears to be closely related to Lampropeltis pyromelana and Lampropeltis mexicana. Divergence may have resulted from geographic isolation on the west side of the Continental Divide in the Sierra Madre Occidental and subsequent adaptation to a unique ecological region.

RESUMEN.—Describimos una nueva especie de Lampropeltis para la vertiente de Pacífico de la Sierra Madre Occidental en el oeste de México basado en morfología, escamación y datos moleculares. Esta especie aparentemente está muy cercanamente relacionada a Lampropeltis pyromelana y Lampropeltis mexicana. Aislamiento geográfico del lado oeste de la división continental de la Sierra Madre Occidental y adaptaciones consecuentes, pudieron haber dado resultado a la divergencia de otra taxa en esta región única.

While conducting field research on montane Crotalus in the Mexican state of Durango, a specimen representing an undescribed species of Lampropeltis was found dead on the road on Mexican Hwy. 40 near the Durango/Sinaloa border. An additional specimen from this area was subsequently located in a small herpetological museum collection. Despite the amount of traffic on this highway since its paving in 1960 and the research conducted in this region using Mexican Hwy. 40 as a transect (Webb, 1984), only two specimens have been found. Herein we describe this new species and compare it with other taxa in the genus Lampropeltis.

MATERIALS AND METHODS
The sex of the specimens was determined by making a small subcaudal incision posterior to
the anal plate to determine the presence or absence of a hemipenis. All measurements were made to the nearest 0.1 mm with a vernier caliper. Scale counts were made with the aid of a dissecting microscope. Color descriptions were made from alcohol-preserved specimens, field notes, and color photos of the holotype before preservation. The coordinates and elevation of the type-locality were determined using a handheld Garmin GPS II Plus. Abbreviations used for museum specimens are as follows: UANL (Universidad Autónoma de Nuevo León) and FWMSH (Forth Worth Museum of Science and History).

Because of the extreme variability in color and pattern in the genus Lampropeltis, a molecular phylogenetic analysis was performed to assist in the diagnosis of the new species. A 868 bp fragment of the mitochondrial ND4-subunit 4 (ND4) and the tRNA\(^{Tyr}\), tRNA\(^{Ser}\), and partial tRNA\(^{Leu}\) genes of the holotype was sequenced and compared to one outgroup species and six other species of tricolored Lampropeltis from central México (for a listing of specimens, see Appendix 1). This fragment of mitochondrial DNA was amplified using the primers ND4 and Leu specified in Arévalo et al. (1994), and the forward primers CornF3 (CTCAYATTTGATATCAACA) and LND4#2 (CTCAACAAACAGACCTAAATCCCT) and reverse primer CornR2 (GTTAATTAGTARTCAAYATATTYCTATCAACACA) and LND4#2 (CTCAACAAACAGACCTAAATCCCT) and reverse primer CornR2 (GTTAATTAGTARTCAAYATATTYCTATCAACACA) and LND4#2 (CTCAACAAACAGACCTAAATCCCT) and reverse primer CornR2 (GTTAATTAGTARTCAAYATATTYCTATCAACACA) and LND4#2 (CTCAACAAACAGACCTAAATCCCT) and reverse primer CornR2 (GTTAATTAGTARTCAAYATATTYCTATCAACACA) and LND4#2 (CTCAACAAACAGACCTAAATCCCT) and reverse primer CornR2 (GTTAATTAGTARTCAAYATATTYCTATCAACACA) and LND4#2 (CTCAACAAACAGACCTAAATCCCT) and reverse primer CornR2 (GTTAATTAGTARTCAAYATATTYCTATCAACACA) and LND4#2 (CTCAACAAACAGACCTAAATCCCT) and reverse primer CornR2 (GTTAATTAGTARTCAAYATATTYCTATCAACACA) and LND4#2 (CTCAACAAACAGACCTAAATCCCT) and reverse primer CornR2 (GTTAATTAGTARTCAAYATATTYCTATCAACACA) and LND4#2 (CTCAACAAACAGACCTAAATCCCT). The template DNA was amplified in 100 ll reactions using 0.06 M Tris, 0.015 M (NH\(_4\))\(_2\)SO\(_4\), 0.0015 M MgCl\(_2\), 0.78 M dimethyl sulfoxide, 0.025 mM each dNTP, 1 mM each primer, and 2.5 U Taq polymerase in a GeneAmp PCR System 9700 thermal cycler. Amplification conditions consisted of 35 cycles of denaturing at 95\(^\circ\)C for 30 sec, primer annealing at 50\(^\circ\)C for 60 sec, and an extension at 72\(^\circ\)C for 60 sec, followed by a final extension at 72\(^\circ\)C for 5 min. PCR products were verified in agarose minigels and then prepared for sequencing using QIAquick PCR Purification Kit (QIAGEN, Valencia, CA) or Concert\(^{TM}\) Rapid PCR Purification System (Life Technologies, Carlsbad, CA). The cleaned products were electrophoresed alongside pGEM-3Z(f)(p) sequencing standard (Applied Biosystems, Norwalk, CT) in an agarose minigel to estimate final template concentration. The sequencing reactions were performed with the original primers using BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Norwalk, CT). Cycling parameters were 25 cycles of 96\(^\circ\)C for 30 sec, 50\(^\circ\)C for 60 sec, and 60\(^\circ\)C for 4 min. The completed sequencing reactions were cleaned of excess dyes by Sephadex G-50 in CENTRI-SEP Columns (Princeton Separations, Inc., Adelphia, NJ). The reactions were electro-

phoresed and analyzed on an ABI Prism 377 DNA Sequencer (Applied Biosystems, Norwalk, CT). The aligned sequences were entered into PAUP* 4.0b10 (Vers. 4, D. L. Swofford, Sinauer Associates, Sunderland, MA 1999) and analyzed using maximum parsimony (MP; Swofford et al., 1996) and maximum likelihood (ML; Felsenstein, 1981). All characters were weighted equally, and gaps were treated as a fifth character state. Maximum parsimony analyses were conducted using exhaustive searches with starting trees obtained via stepwise addition with 2500 random addition sequences, accelerated character transformation (ACCTRAN), and tree-bisection-reconnection (TBR) branch swapping. All characters were treated as unordered. Nonparametric bootstrap (BP) analyses (Felsenstein, 1985) of 2500 pseudoreplicates were performed on MP analyses to examine the relative support of each relationship in the resultant topologies. Maximum-likelihood trees were calculated via heuristic search. For the substitution model, transition: transversion ratios were estimated and a discrete approximation to gamma distribution was estimated for among-site rate variation. For all other options, default settings were maintained, thus yielding the equivalent of the HKY model (Hasegawa et al., 1985). The number of diagnostic nucleotide states was determined in MacClade 4.0 (Vers. 3, W. P. Maddison and D. R. Maddison, Sinauer Associates, Sunderland, MA, 2000).

**Species Description**

Lampropeltis webbi sp. nov.

Lampropeltis mexicana—Bryson et al., 2001 [Mis application].

Holotype.—UANL 5684, a female (Figs. 1, 2) collected by Robert Bryson, Deron Hartman, and Javier Banda on 30 June 2000 from 4.0 km west of El Palmito on Hwy. 40, Municipio Concordia, Sinaloa, México (23\(^\circ\)33 39.14 20 N, 105\(^\circ\)59 47.20 W), 2000 m elevation.

Paratype.—FWMSH 6716, a female collected on 28 August 1968 by ‘‘W. J. Voss, Pratt et al.’’ from 29.1 km southwest of Buenos Aires, or 80.1 km west of El Salto via Hwy. 40, Municipio El Salto, Durango, México.

Diagnosis.—A species of tricolored kingsnake most closely resembling Lampropeltis pyromelana and Lampropeltis mexicana but differing from those species in a number of characters. Lampropeltis webbi can be distinguished from L. pyromelana based on a primarily black snout, the smaller number of white annuli, and a different head pattern. It differs from L. mexicana in having a higher number of ventral and subcaudal scales and in having a different body pattern. In addition, L. webbi is distin-
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Fig. 1. Lampropeltis webbi (holotype, UANL 5684), a juvenile female. Dorsal, lateral, and ventral aspects of the head.

Fig. 2. Lampropeltis webbi (holotype, UANL 5684), a juvenile female, SVL 5221 mm. Photo taken minutes after death.

guished from L. pyromelana and L. mexicana and all other members of this genus, by mitochondrial DNA markers. The following combination of nucleotide states within the ND4-Leu gene region is diagnostic for L. webbi in our analysis. The numbers refer to the positions within the aligned sequences referenced in Appendix 1; position 1 corresponds to position 11703 of the complete Dinodon semicarinatus mitochondrial genome (GenBank accession number NC 001945). These character states are fixed within and unique to L. webbi in our analysis: 218: T, 220: G, 358: G, 361: T, 562: G, 566: T, 585: T, 590: A, 646: T, 679: G, and 685: G.

Description of the Holotype.—Juvenile female (Figs. 1, 2); snout-vent length (SVL) 221 mm; tail length (TL) 45 mm; tail 16.9% of total length; eye large, diameter 2.3 mm; pupil round; head distinct from neck; maximal width of head 7.3 mm; head 12.9 mm long from snout to posterior margin of jaw; neck 4.1 mm wide; 16 maxillary teeth on right side.

Dorsal head scales symmetrical, internasals, prefrontals, supraoculars, and parietals paired; parietals large, longer than wide; single frontal, longer than wide; prefrontals contact loreal; rostral about twice as wide as high, visible from above; nasals divided; loreal wider than high; one large preocular, higher than wide, not touching frontal, and in contact with second and third supralabials; two postoculars, lower in contact with fourth and fifth supralabial; temporals 1/1p2p3, left; 1/1p3, right; 7-7 supralabials, third and fourth enter orbit, sixth largest; 9-9 infralabials, first pair in contact behind mental; mental triangular; two pairs of chinshields, anterior longer and larger than the posterior; posterior chinshields joined medially, separated posteriorly by gulars; infralabials 1-4 in contact with anterior chinshields; fifth infralabial in contact with posterior chinshields. Dorsal scales smooth. Standard dorsal scale reductions (Dowling, 1951) as follows: 23(10) 3p4/3p4 (126/120) 21 4p5/4p5 (147/145) 19 (218). Anal entire; 216 ventrals; 66 divided subcaudals, including the cornified tail tip.

Color in Life.—There are 36 black-edged, orange-red body blotches, and 36 pale body annuli. An additional 13 blotches and 15 (four are incomplete) annuli are found on the tail. The ground color is grayish-white. The first eight
intercalary marks are also black-edged orange-red spots that become progressively smaller posteriorly. The intercalary spots following the first eight are invaded by black pigment until they are small and black near the tail. Many of the intercalary spots are united ventrally with black marks on the belly.

The head has a grayish-white W-shaped mark (open end toward the snout) across the frontal and prefrontals, surrounded by a black head cap that extends from the rostral to the posterior edge of the parietals. The head cap contains three gray-tan spots, one in each of the parietals, the third on the posterior suture between the parietals. A dark brown area occurs on the suture between the internasals and the posterior edge of the rostral. Each nasal scale is black. Black spots are present on the first six supralabials of each side, six on the right infralabial, five on the left, and on each of the lower second temporal scales.

The nuchal orange-red blotch is slightly larger than all other body blotches, with a slightly wider black edge on its anterior margin. The grayish-white ground color between the black head cap and the nuchal blotch extends forward along each side of the head to the posterior edge of the eye. The 13 orange-red tail bands become narrow and invaded with black pigment toward the tail tip.

Color in Preservative.—The dorsal surface of head has three pale spots within the parietal shield, one each almost in the center of the parietal, and the third situated on the medial margins of each parietal; posterior third of frontal scale with a pale broad “W”; wings of the “W” extending forward along the outer edge of the supraoculars; remainder of frontal black; posterior third of prefrontals black; anterior part of the prefrontal pale, anterior medial edge of nasal scales with black spot, with lateral part of nasals pale; black ring around each nostril; rostral reticulated with brown; the first black band behind head angling posteriorly towards venter; the band medially touches the parietal, passing ventroposteriorly to third scale row, and encircling the pale nuchal spot. Each pale dorsal blotch is edged with black; the length of each pale blotch varies from three to 10 scales; each pale blotch extends laterally to third scale row. Each black border covers one to two scales; black borders somewhat variable as to placement laterally, extending to upper half of scale row one to lower one half of scale row 3, at least anteriorly. Venter densely reticulated with black, less so anteriorly. First 11 ventrals with more cream than black; remainder become progressively more black, so that black predominates posteriorly (except for the vent area). The vent is cream; subcaudal area with equal amounts of black and red. Tail with uncountable banded pattern.

Description of the Paratype (where Different).—Female, SVL 306 mm; TL 65 mm (17.5% of total length); eye diameter 2.3 mm. The head is crushed and in bad condition. Dorsal head scales on left side are in general agreement with holotype, head scales on right side are damaged; temporals 1/1p2 right; 1/1p2p3 left; 7-7 supralabials; 9-9 infralabials; two pairs of chinshields, posterior as long as anterior; posterior chinshields totally separated by gulars. Dorsal scale reductions: 23(10) 3p4/3p4 (156/166) 21 4p5/4p5 (159/169) 19 (221). Ventrals 221; subcaudals 67.

The color pattern of the paratype is quite different from the holotype. The parietals are mostly black with pale cream on the posterior and somewhat lateral edges, and some pale cream reticulation in the medial part. Frontal mostly black, with the interior, posterior edge of the supraoculars cream; this diverging cream edge extends anteriorly on the right side of the head, touching the prefrontal and nasal scales, which are dark brown; posterior edge of prefrontals black; outer edge of left prefrontal and nasal scales with dark brown reticulation; rostral lightly reticulated with brown. Body with 32 tan blotches and 32 pale body annuli (plus one incomplete). First nuchal blotch nine scales in length, followed by blotches with scale lengths varying from 4-6.5 scales in length. Length of red spaces vary from one to two and one-half scales; tan blotches extend laterally to third scale row. Red space extends laterally to first scale row. The black edging of red bands begins laterally along the upper one half of the first scale row, occasionally beginning only on the second scale row, or upper half of first and lower half of second scale row. Tail with 14 gray tail bands, about 11 red bands, and 13 pale annuli. Red bands incomplete ventrally. Venter heavily reticulated with black posteriorly. First 80 ventrals with black spots scattered across ventrals, tending to be sparse near the chin to about the 60th ventral.

Comparisons.—Lampropeltis webbi is distinguished from L. pyromelana and L. mexicana by a number of characters. Lampropeltis pyromelana has 46-85 white annuli and a white snout (Tanner, 1983), whereas L. webbi has 36 and 32 annuli and a primarily black snout. Of these characters, snout color is particularly useful in distinguishing L. webbi from L. pyromelana as all of the subspecies of L. pyromelana have white snouts (Tanner, 1983). Lampropeltis mexicana has between 190-211 ventrals and 51-65 subcaudals (Garstka, 1982). Lampropeltis webbi has a much higher number of ventrals (216 and 221) and 66 and 67 subcaudals. Although L. webbi resembles
L. mexicana in having a primarily black head, it differs in dorsal color and pattern by lacking a light-edged red center blotch and any alternating reduced markings (Garstka, 1982). However, as noted by Garstka (1982), the extreme variability in color and pattern demonstrated within L. mexicana and similar species makes using only these characters untenable.

Both Tanner (1953) and Garstka (1982) suggest patterns of clinal variation within L. pyromelana and the L. mexicana group, respectively. Tanner (1953) stated that number of ventrals in L. pyromelana are highest in the south (Lampropeltis pyromelana knoblochi, 235) and lowest in the north (Lampropeltis pyromelana pyromelana, 213). Garstka (1982) suggested a similar relationship in the clinal variation in the number of ventrals in the L. mexicana group but with counts highest in the north (Lampropeltis alterna, 230) and lowest in the south (Lampropeltis ruthveni, 182). Lampropeltis webbi has 216 and 221 ventrals. Both specimens of L. webbi were collected south of the documented range of L. pyromelana and to the west of the middle of the range of the L. mexicana group and do not fall into the clinal patterns of either group.

Phylogenetic Analyses.—The new mtDNA sequences generated for the taxa examined have been deposited in GenBank (Appendix 1). The nucleotide sequences span a total of 868 bp. Of these, 122 are parsimony informative. Corrected sequence divergences (K2p; Kimura, 1980) ranged from 5.2 to 5.6% between L. webbi and L. pyromelana and from 7.8 to 9.7% between L. webbi and other species in the ingroup (Table 1). The MP exhaustive search with all characters weighted equally resulted in two trees of 359 steps in length with a consistency index of 0.68 and a retention index of 0.58 (Fig. 3A). The MP tree demonstrates a L. webbi   L. pyromelana (pyromelana  knoblochi) clade, which is strongly supported (95%) by bootstrap analyses using maximum parsimony branch-and-bound searches (Fig. 3A). The final ML tree (ln likelihood = -2181.28) was obtained with previously estimated transition: transversion ratio of 7.95 (kappa = 5.1747) and gamma shape parameter of 0.16. The ML phylogram presented in Figure 3B maintains branch lengths proportional to the number of changes. Topologies resulting from the MP and ML analyses were similar and recovered the same nodes. Both trees suggest that L. webbi is a distinct species, separate from L. pyromelana. This view is consistent with the phylogenetic species concept (e.g., Cracraft, 1989) which requires species to be diagnosable monophyletic units.

The discordance between the phylogenetic relationships of the mexicana and triangulum groups seen in this study and those based on morphology, as proposed by Garstka (1982) and others (Smith, 1942, 1944; Webb, 1962; Williams, 1988), may be caused by lineage sorting and/or introgressive hybridization during a northeastern radiation from a common ancestor (Bryson, 2002). As such, the mtDNA topologies (i.e., gene trees) presented in this study may not be congruent with the species tree and true evolutionary history of these groups. Evidence for such incongruence has been addressed elsewhere (Ballard et al., 2002; Taggart et al., 2001).

Habitat and Distribution.—Both specimens were collected on the Pacific versant of the Sierra Madre Occidental in Mixed Boreal-Tropical habitat (Webb, 1984) near the Durango/Sinaloa state border. This area is characterized by steep mountainsides covered with dense pine (Pinus) and oak (Quercus) forest, a thick understory, and epiphytic plants. An abrupt climate change occurs between the moist Mixed Boreal-Tropical habitat and the drier, cooler Pine-Oak habitat to the east (Webb, 1984).

The holotype was collected dead on the road (DOR) at 1628 h on 30 June 2000. The ambient air
temperature was approximately 248°C, and the area had received rain recently. The paratype was collected DOR on a rainy night on 28 August 1968. The habitat where the holotype was found is heavily vegetated and steep (Fig. 4). Rock cuts are present along the north side of the road. The south side is characterized by steep hillsides and rocky drainages.

Etymology.—The specific epithet is a patronym honoring Robert G. Webb for his countless hours of research on the herpetofauna of northwestern México. His contributions have greatly enhanced our understanding of this region, and with his help, the paratype was located.

DISCUSSION
Tanner et al. (1972:16) suggested that the rugged terrain and diverse climate of the Sierra Madre Occidental to the west of El Salto, Durango, was responsible for the development through adaptation of a number of subspecies. Indeed, Webb (1984) recognized five ecological regions in 296 km along Hwy. 40 in southwestern Durango and southern Sinaloa. Deep barrancas traverse the high mountains in the area along the Durango/Sinaloa border. Geographic isolation on the west side of the Continental Divide in the Sierra Madre Occidental and subsequent adaptation to a unique ecological region may have resulted in divergence of L. webbi from L. pyromelana to the north and L. mexicana to the east.

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HASEGAWA, M., H. KISHINO, AND T.-A YANO. 1985. Dating of the human-ape splitting by a molecular clock of


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APPENDIX I

Localities for Specimens Used in Molecular Analysis.—Voucher numbers and GenBank accession numbers are in parentheses following species name. Museum and private collection abbreviations are as follows: SRSU, Sul Ross State University; UAA, Universidad Autónoma de Aguascalientes; UANL, Universidad Autónoma de Nuevo León; GRQ, George Raymond Queen; SD, Stan Draper; SH, Stephen Hammack. The outgroup species (Pituophis catenifer, GenBank AF138763) and two other tricolored species (Lampropeltis zonata multicincta, GenBank AF136195; L. pyromelana pyromelana, GenBank AF138761) used in this study were previously published sequences retrieved from GenBank.