Zootaxa 4729 (4): 451–481
https://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4729.4.1
http://zoobank.org/urn:lsid:zoobank.org:pub:C111AD4F-6740-4695-9C79-C598EE5AFDE1

Accessing cryptic diversity in Neotropical rattlesnakes (Serpentes: Viperidae: *Crotalus*) with the description of two new species

RUBÉN ALONSO CARBAJAL-MÁRQUEZ[1,4,5], JOSÉ ROGELIO CEDEÑO-VÁZQUEZ[1], ARELY MARTÍNEZ-ARCE[1], EDGAR NERI-CASTRO[2] & SALIMA C. MACHKOUR- M'RABET[3]

[1]*Departamento de Sistemática y Ecología Acuática. El Colegio de la Frontera Sur, Unidad Chetumal, Av. Centenario Km 5.5, Chetumal, 77014, Quintana Roo, México*
[2]*Instituto de Biotecnología de la Universidad Nacional Autónoma de México, Av. Universidad #2001, Col. Chamilpa C.P. 62210 Cuernavaca, Morelos, México*
[3]*Departamento de Conservación de la Biodiversidad. El Colegio de la Frontera Sur, Unidad Chetumal, Av. Centenario Km 5.5, Chetumal, 77014, Quintana Roo, México*
[4]*Conservación de la Biodiversidad del Centro de México, A.C. Andador Torre de Marfil No. 100, 20229, Aguascalientes, Aguascalientes, México.*
[5]*Corresponding author. E-mail: redman031@hotmail.com*

Abstract

Members of the *Crotalus durissus* species complex are widely distributed from Mexico to Argentina in areas with mainly seasonally dry tropical deciduous forest. Although four species (*C. culminatus*, *C. durissus*, *C. simus* and *C. tzabcan*) are currently recognized, species limits remain to be tested. Previous genetic studies suggest that *C. durissus* and *C. simus* may be paraphyletic and that at least one cryptic species may be present. We analyzed 2596 bp of DNA sequence data from three mitochondrial and one nuclear gene to infer phylogenetic relationships in the Neotropical rattlesnakes. We also examined museum and wild specimens as well as captive animals to analyze morphological characters. Our results suggest that current taxonomy of the *Crotalus durissus* species complex does not reflect evolutionary history. We found strong support for five independent lineages within *Crotalus simus* (*sensu lato*), with genetic and morphological evidence for three previously recognized taxa and two new species, as well as three major lineages within *C. durissus* that each represent species hypothesis to be tested with additional evidence. We also found support to retain *C. totonacus* in the *Crotalus molossus* species complex. We suggest conservative taxonomic changes to the complex and related species, but more evidence is needed (e.g., morphology, ecology and venom composition) to clarify relationships among species.

Key words: *Crotalus durissus*, *Crotalus simus*, tropical deciduous forest, molecular phylogeny

Resumen

Los miembros del complejo de especies *Crotalus durissus* tienen una amplia distribución desde México hasta Argentina, principalmente en zonas con bosque tropical deciduo. Aunque se reconocen actualmente cuatro especies (*C. culminatus*, *C. durissus*, *C. simus* y *C. tzabcan*), los límites entre estas no son claros. Estudios genéticos previos sugieren que *C. durissus* y *C. simus* pueden ser parafiléticos y que al menos una especie críptica puede estar presente. Analizamos una secuencia de 2596 pares de bases de ADN provenientes de tres genes mitocondriales y uno nuclear, para inferir las relaciones filogenéticas en las especies de víboras de cascabel neotropicales. Por otro lado, se examinaron especímenes de museo, silvestres y ejemplares en cautiverio para analizar caracteres morfológicos. Nuestros resultados sugieren que la taxonomía actual del grupo de especies *Crotalus durissus* no refleja su historia evolutiva. Los resultados obtenidos muestran un fuerte apoyo para cinco linajes independientes en *Crotalus simus* (*sensu lato*) con evidencia genética y morfológica para tres taxones previamente reconocidos y dos especies nuevas, y tres linajes en *C. durissus*, que representan hipótesis de especies para ser probadas con evidencia adicional. También encontramos apoyo para mantener a *C. totonacus* en el complejo de especies *Crotalus molossus*. Sugerimos cambios taxonómicos conservadores para el complejo y especies relacionadas, pero se necesita más evidencia (e.g., morfología, ecología y composición bioquímica del veneno), para aclarar las relaciones entre especies.

Palabras clave: *Crotalus durissus*, *Crotalus simus*, bosque tropical deciduo, filogenia molecular

Accepted by P. Passos: 18 Dec. 2019; published: 31 Jan. 2020

Introduction

The members of the *Crotalus durissus* species complex known as Neotropical rattlesnakes, are widely distributed from central-south Mexico to central Argentina across lowlands, mainly in seasonally dry tropical deciduous forest, and together with the *Crotalus molossus* species complex belongs to the *Crotalus durissus* species group (Murphy *et al*. 2002; Reyes-Velasco *et al.* 2013). Currently, the complex contains four recognized species: *C. culminatus* Klauber 1952, *C. durissus* Linnaeus 1758, *C. simus* Latreille in Sonnini & Latreille 1801, and *C. tzabcan* Klauber 1952 (Wüster *et al*. 2005; Alencar *et al*. 2016). The nominal species, *C. durissus*, is the widest ranging species in the group, distributed in lowlands across South America. *Crotalus durissus* currently contains six subspecies: *C. d. cumanensis* Humboldt 1813, *C. d. durissus* Linnaeus 1758, *C. d. marajoensis* Hoge 1966, *C. d. ruruima* Hoge 1966, *C. d. terrificus* (Laurenti 1768), and *C. d. trigonicus* Harris and Simmons 1978 (Uetz *et al.* 2019). *Crotalus unicolor* Van Lidth de Jeude 1887, from Aruba Island and *C. vegrandis* Klauber 1941, from the savannas of southern Venezuela, are considered different species from *C. durissus* by some authors (McCranie 1984; Rivas *et al*. 2012; Uetz *et al.* 2019), while others regard it as a subspecies of *C. durissus* (Klauber 1956; Campbell & Lamar 2004; Wüster *et al.* 2005). *Crotalus culminatus* was previously considered as a subspecies of *C. simus* (Klauber, 1952; Campbell & Lamar 2004), with distribution in the Río Balsas Basin and along the Pacific coastal plains and foothills from Southwestern Michoacán southward to the Isthmus of Tehuantepec in Mexico. *Crotalus simus* (*sensu lato*) ranges from the Atlantic versant in central Veracruz to Chiapas and to western Honduras and crosses over the Pacific versant in the Isthmus of Tehuantepec, Oaxaca, along the Pacific coastal plains and foothills of Chiapas to western Costa Rica (Campbell & Lamar 2004; Wüster *et al*. 2005). *Crotalus tzabcan* also was previously considered as subspecies of *C. simus*, and occurs in the Yucatán Peninsula, including Campeche, northeast Chiapas, Quintana Roo, Tabasco and Yucatán, Mexico, northern Belize and El Petén, Guatemala (Lee 1996; Campbell 1998; Campbell & Lamar 2004). *Crotalus totonacus* Gloyd and Kauffeld 1940, previously was considered within the *Crotalus durissus* species complex, but recent studies transferred it to the *Crotalus molossus* species complex (Campbell & Lamar 2004; Wüster *et al*. 2005; Anderson & Greenbaum 2012; Alencar *et al*. 2016).

Since the description of *Crotalus durissus*, the taxonomic composition of the group has changed several times (see Klauber, 1952; Campbell & Lamar 2004; Murphy *et al.* 2002; Savage *et al*. 2005; Reyes-Velasco *et al.* 2013). The most recent molecular studies that include members of the *Crotalus durissus* species complex (Wüster *et al*. 2005; Blair & Sanchez-Ramírez 2016; Alencar *et al*. 2016) found strong support for a monophyletic assemblage that includes *C. culminatus*, *C. durissus*, *C. simus* and *C. tzabcan.* Wüster *et al*. (2005), found evidence that *C. durissus* and *C. simus* are paraphyletic, and that at least one cryptic species was present in the complex. Although this study extensively sampled the geographic range of the *Crotalus durissus* species complex, some geographical regions were missing, mainly in Mexico and Central America. Here, we combine all available molecular sampling with new tissues samples from Mexico, and include a nuclear gene and morphological data (complementary scutellation and color pattern dataset) to examine the phylogenetic relationships and show congruence to cryptic species along the distribution of Neotropical rattlesnakes.

Material and methods

Species criteria. We follow the approach of Meik *et al.* (2018), considering all multicellular organisms as constituting a multidimensional frequency distribution of peaks and troughs of relative cohesion in development, genomic architecture and other emergent properties that result from the collective history of shared reproductive opportunity and compatibility (i.e. processes operating through interacting replicators and meta-populations; see de Queiroz 2007; Hart 2010). Therefore, we consider species as proxy labels that circumscribe patterns of biological variation among individual organism. Thus, species delimitation is the testing whether collections of organisms correspond to these peaks of relative biological cohesion or similarity. Our view of species does not require absolute reproductive isolation or discrete diagnostic differences among groups, as we consider speciation a process with no distinct beginning or ending, and any temporal partitioning of organism into species as they grade from ancestor to descendant lineages will be artificial. We consider three primary criteria for evaluating hypotheses of species boundaries: (i) evolutionary history of putative lineages; (ii) relative cohesion in multivariate dimensions across different biological attributes or axes; and (iii) comparisons with congeneric taxa that have been broadly accepted as species on the basis of criteria i and ii above.

Taxon sampling and molecular data assembly. Tissue samples were obtained from 39 specimens from 31 localities throughout the distribution of *C. culminatus, Crotalus simus* (*sensu lato*), *C. tzabcan*, *C. basiliscus* (Cope 1864)*, C. m. nigrescens* Gloyd 1936*, C. ornatus* Hallowell 1845, and *C. totonacus* (Appendix 1). Genomic DNA was extracted mostly from ventral scale clippings, shed skin, and blood samples, following the procedures described in Fetzner (1999). The DNA concentration in each sample was then estimated using a Qubit® 2.0 fluorometer (Invitrogen). We generated sequence data for tissue samples from museum vouchers, along with individuals not deposited in museum collections (highlighted in bold in Appendix 1). All the specimens from which we obtained samples and included in the genetic analyses were morphologically identified following Campbell & Lamar (2004), and Anderson & Greenbaum (2012). We used sequences from species of *Crotalus durissus* species group available in Genbank up to January 2018 (Appendix 1), previously used by Wüster *et al.* (2005) and Anderson & Greenbaum (2012), and following their previous identifications.

We amplified the mitochondrial gene fragments cytochrome *b* (cyt *b*), NADH dehydrogenase subunit 4 (*ND4*), NADH dehydrogenase subunit 2 (*ND2*), and nuclear gene oocyte maturation factor (*c-mos*) using previously published primers (Table 1). We set up PCR reactions to a final volume of 50 µl, using 2.0 µl genomic DNA, 1.0 µl of each primer (10 µM; Integrate DNA Technologies), 10 µl of PCR buffer (5X; Promega), 1.0 µl total dNTPs (10 mM; Promega), 8.0 µl of $MgCl_2$ (25 mM; Promega), 0.2 µl of GoTaq Flexi DNA polymerase (1 U; Promega), and 26.8 µl of ultrapure water for all the genetic fragments. We followed Pook *et al*. (2000) and Ashton & de Queiroz (2001) to set up amplification conditions, which involved an initial denaturation step of 4 min at 94°C, 35 cycles of denaturation for 1 min at 94°C, primer annealing for 1 min at 50°C (55°C for *ND2*), extension for 2 min at 72°C, ending with a final extension step for 3 min at 72°C. We visualized the amplified products (PhotoDoc-It, UVP) on 1% agarose gels with GelRed (Biotum) as the post-gel staining method, and sequenced by Macrogen (Seoul, S. Korea-http://dna.macrogen.com) using the same primers as for amplification.

TABLE 1. Primers used for the amplification and sequencing of gene fragments in this study.

Locus	Primer	Reference	Sequence (5'-3')	**BP**
cyt *b*	*703Bot-L*(modificado)	Pook *et al*. 2000	TCAAACATCTCAACCTGATGAAA	758
cyt *b*	*MVZ16-H* (modificado)	Pook *et al*. 2000	GGCAAATAGGAAGTATCATTCTG	758
ND4	*ND4*	Arévalo *et al*. 1994	CACCTATGACTACCAAAAGCTCATGTAGAAGC	900
ND4	*Leu*	Arévalo *et al*. 1994	CATTACTTTTACTTGGATTTGCACCA	900
ND2	*L4437b*	Kumazawa *et al*. 1996;1998	CAGCTAAAAAAGCTATCGGGCCCATACCCATACC	1067
ND2	*Trna-trpR*	Ashton & de Queiroz 2001	GGC TTT GAA GGC TMC TAG TTT	1067
c-mos	S77	Lawson *et al*. 2005	CATGGACTGGGATCAGTTATG	570
c-mos	S78	Lawson *et al*. 2005	CCTTGGGTGTGATTTTCTCACCT	570

We assembled and edited the sequences using Sequencher v4.1.4 (Gene Codes Corporation, Ann Arbor, MI). We validated the amplified fragments with BLAST (http://www.ncbi.nlm.nih.gov). We performed the sequence alignment using Muscle (Edgar 2004) using the default settings in Mega v7 (Kumar *et al*. 2016) and adjusted visually with Mesquite v3.31 (Maddison & Maddison 2017). We verified that stop codons were not present in the data. We phased the nuclear gene (*c-mos*) using the default settings in the program PHASE in DnaSP (Rozas *et al*. 2003) and retained the most probable haplotype before concatenation.

Phylogenetic inference. We treated gaps in the alignment as missing data. We concatenated the different gene sequences for each specimen and analyzed the data jointly. To determine the appropriate model of sequence evolution for each gene fragment, as well as for the concatenated dataset of mitochondrial and nuclear genes fragments, the computer program jModelTest v2.1.6 (Darriba *et al*. 2012) was employed, for each gene, under the Akaike Information Criterion (AIC; Akaike 1973). We also incorporated the following species as outgroups: *Agkistrodon contortrix*, *A. piscivorus*, *Crotalus tigris*, *C. viridis*, *Sistrurus catenatus* and *S. miliarius* following the phylogeny generated by Alencar *et al.* (2016; Appendix 1).

We utilized two datasets to infer the phylogenetic reconstruction of the *Crotalus durissus* species group: the first dataset was composed only of mitochondrial sequences (cyt *b*+*ND4*+*ND2*) and included all terminal taxa, the second dataset combined mitochondrial and nuclear sequences (cyt *b*+*ND4*+*ND2*+*c-mos*) and also included

all terminal taxa. We performed phylogenetic analyses using maximum likelihood (ML), with RAxML-HPC 2 (Stamatakis 2014) in CIPRES Science Gateway platform (Miller *et al*. 2010); nodal support was assessed using the rapid-bootstrapping algorithm with 1000 non-parametric bootstraps (Felsenstein 1985). All ML estimates and tests were run under the GTRCAT model. Nodes were considered well-supported if bootstrap support (BS) was over 70. For the Bayesian inference (BI) analysis, we used the same two datasets as in ML analyses, but partitioned by gene and tested in MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003), with 15 million generations and four parallel Markov chains with default heating settings, under the GTR+G+I model for each gene. Trees were sampled every 1000 generations and 25% of them were discarded as ‘‘burn-in”. The resulting saved trees were used to calculate posterior probabilities (PP) for each partition through a 50% majority-rule consensus tree. We used Tracer 1.6 (Rambaut *et al*. 2018) to assess convergence and effective sample sizes (ESS) for all parameters. Nodes were considered well-supported if PP > 0.95. The resulting tree was divided into two figures (Fig. 1A–B), one for each of the species complexes within the *Crotalus durissus* group for better resolution. Sequence divergence within and among populations was estimated by using uncorrected pairwise (*p*) distances calculated in Mega v. 7 (Kumar *et al*. 2016).

Haplotype network. Pattern of haplotype sharing in the *c-mos* dataset was visualized in NETWORK 5.0.0.3 (Bandelt *et al*. 1999), under the median-joining (MJ) algorithm, with the next priors an epsilon value=10, and as a post-processing to purge superfluous links and median vectors from the network, a Maximum Parsimony calculation (MP) was executed (Polzin & Daneshmand 2003).

Morphological data. We examined 142 specimens available to us from museum vouchers, captive collections and wild specimens with known locality, from across the distribution of the *Crotalus durissus* species complex (Appendix 2). Institutional abbreviations follow Sabaj Pérez (2016) except for: Instituto de Biotecnología, UNAM, Cuernavaca, Morelos, Mexico (IBt); Unidad de Manejo Ambiental Barranca Honda, Tlaltizapán, Morelos, Mexico (UMABH); Unidad de Manejo Ambiental OCTOLAB, Emiliano Zapata, Veracruz, Mexico (UMA-OCTOLAB); Colección Herpetológica, Facultad de Biología, Universidad Juárez del Estado de Durango, Gómez Palacio, Durango, Mexico (UJED-CHFCB); Unidad de Manejo Ambiental Palancoatl, Veracruz, Mexico (UMA-Palancoatl); Colección de Anfibios y Reptiles de Tabasco, Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco, Mexico (UJAT-CART). We classified snakes as juveniles (≤ 800 mm total length) and adults (> 800 mm total length), based on the minimum reproductive size of *C. durissus* (Almeida-Santos *et al*. 2004a, b; Barros *et al*. 2012). We examined 35 characters (meristic, qualitative and color pattern) traditionally used for distinguishing species in the *Crotalus durissus* species complex (see Klauber 1952; Campbell & Lamar 2004) for morphological congruence of cryptic species. We first noted the number or arrangement of meristic characters, including number of ventrals (VEN), subcaudals (SBC), rattle-fringe scales (RFS), middorsal scaler rows (MDR), midtail scale rows (MTR), number of dorsal body blotches (DBB), scale width of the paravertebral stripes (WPS), scale length of the paravertebral stripes (LPS), internasals (INS), canthals (CAS), supralabials (SLS), infralabials (ILS), prefoveals (PFO), anterior intersupraoculars (AIS), supraloreal (SLO), infraloreal (ILO), presupraloreal (PSL), postloreal (PLO), and postsupraloreal (TSL). We then noted the qualitative characters, including the presence vs. absence of intercanthals (ICS), presence vs. absence of first infralabial divided (DFI), presence vs. absence of postrostral (PRT), contact or not contact between prenasal and first supralabial (PRE-SLS), presence vs. absence of interpreocular (IPO), presence vs. absence of superciliar (SCI), and lacunal-supralabial contact vs. not contact (LAC-SLS). For bilateral characters, we considered the left side, and the right side when damage to the specimen prevented us from recording the former. For scale counts of the width and length of paravertebral stripes, we considered that of the widest and longest stripe. For DFI, PRT, PRE-SLS, IPO, SCI, and LAC-SLS, we considered when at least in one side was present. We then measured tail length in millimeter, and standardized this measurement by dividing by total length (tail length ratio). Finally, we examined aspects of color pattern, including number of scales wide and coloration of postocular stripe, presence vs. absence of secondary and tertiary blotches, presence vs. absence of paravertebral stripe bordered by buff scale, presence vs. absence of paravertebral stripes with light center, contrast between dorsal blotches and ground color (approximated with a binary scale, high vs. low), contact or not contact between paravertebral stripes with supraoculars (PRV-SPO), and presence vs. absence of prefrontal bar interrupted (PFB). We express qualitative characters as percentages for descriptive purposes. Snout-vent length (SVL) and tail length (TL) were measured using a meter stick to the nearest millimeter. Scale counts were made with the aid of a stereoscope microscope when necessary. Sex was determined by evaluating the presence/absence of hemipenes.

Morphological data were subjected to principal component analysis (PCA) on 16 meristic scale and color pattern characters displaying variation: VEN, SBC, MDR, INS, DBB, WPS, LPS, SLS, ILS, PFO, AIS, SLO, ILO,

PSL, PLO, and TSL. Specimens for which data were missing or not applicable were omitted from the analysis. We standardized all variables to a mean of zero and unit variance prior to analysis in order to ensure equal weights. We then performed a Linear Discriminant Analysis (LDA), using the mtDNA clades to evaluate the quantitative discrimination in meristic characters between these groups.

We analyzed qualitative characters using a Pearson chi-squared test of independence which evaluated whether frequencies of individuals assigned to each category were independent across putative genetic lineages. Statistical tests were conducted in PAST 3.25 (Hammer *et al*. 2001).

The lineages identified in this species complex show no single exclusive diagnostic morphological character. However, unique combinations of morphological characters diagnosed most species. The initial separation of taxa was based on the analysis of meristic data, and complemented with qualitative characters and color pattern.

Results

Sequence data. We generated new sequences for the *Crotalus durissus* species group: *C. culminatus* (cyt *b*: 2; *ND4*: 2; *ND2*: 1; *c-mos*: 2 individuals), *Crotalus simus* (*sensu lato*) (cyt *b*: 16; *ND4*: 16; *ND2*: 5; *c-mos*: 16 individuals), *C. tzabcan* (cyt *b*: 8; *ND4*: 8; *ND2*: 3; *c-mos*: 6 individuals), *C. basiliscus* (cyt *b*: 3; *ND4*: 3; *ND2*: 1; *c-mos*: 3 individuals), *C. ornatus* (cyt *b*: 1; *ND4*: 1; *ND2*: 0; *c-mos*: 1 individual) and *C. totonacus* (cyt *b*: 9; *ND4*: 9; *ND2*: 1; *c-mos*: 9 individuals). Adding the sequences retrieved from GenBank, we obtained a dataset of 127 specimens, including 87 terminals of the ingroup.

Phylogenetic analyses. For the three fragments of mtDNA, we obtained a 2002 bp alignment (675 bp of cyt *b*, 657 bp of *ND4*, and 670 bp of *ND2*). For the four fragments concatenated of mtDNA + nDNA, we added an additional fragment of 594 bp of *c-mos* gene sequences, resulting in a matrix composed of 2596 bp aligned. Both datasets yielded 97 singletons haplotypes. Sample, haplotype, and GenBank accession numbers are in Appendix 1. Some of our samples failed to amplify for *ND2*, and *c-mos* markers. Also, only sequences of cyt *b*, *ND4*, and *ND2*, for some specimens of *Crotalus durissus* (*sensu lato*), *C. basiliscus*, *Crotalus molossus* (*sensu lato*), *C. ornatus*, *C. culminatus*, *C. simus, C. tzabcan*, *C. totonacus* and outgroups were available from GenBank, as well as only sequences of *c-mos* for *Crotalus molossus* (*sensu lato*) and *C. ornatus* were available. In spite of these missing data, these taxa were included in analyses of the three-gene fragments (cyt *b+ND4+ND2*) and four-gene data set (cyt *b+ND4+ND2+c-mos*), because the placement of taxa that are missing a significant portion of sequence data can be accurately inferred in a phylogeny, given an adequate number of informative characters (Wiens 2003; Pyron *et al*. 2011; Anderson & Greenbaum 2012).

Results from mtDNA and mtDNA + nDNA datasets were highly congruent. This may be the result of bias caused by the more rapid rate of mutation from the mitochondrial genes (Wiens *et al*. 2010), while the *c-mos* gene is more conservative. The tree topologies recovered for the mtDNA and mtDNA + nDNA datasets are the best scoring trees found by RAxML (-ln *L*=-15,771.28 and -ln *L*=-17,270.83, respectively), under the GTRCAT approximation. The topologies recovered through ML and BI analyses were concordant, and the nodes robustly supported, the differences being restricted to minor rearrangements within the major clades (Fig. 1 A–B, only four dataset depicted). We found the *Crotalus durissus* species complex is represented by two major clades, the norhten clade (*C. culminatus*) and the southern clade (*C. durissus*), and sisters of the *Crotalus molossus* species complex. The northen clade includes populations from the Pacific versant in Michoacán to surroundings of Tonalá, Chiapas, across the Tehuantepec Isthmus, and including the Balsas River Basin and Grijalva River Basin, almost reaching the Central Valleys of Oaxaca, and the states of México, Morelos and Puebla, and from the Atlantic versant in central-south Veracruz, and was represented by three well supported clades (*C. culminatus* in yellow, *C. simus*-Isthmus-Chiapas in purple, and *C. simus*-Veracruz in light blue; Figs. 1A, 4). The southern clade including populations from southeast of Tonalá in the Pacific coast of Chiapas, and Yucatán Peninsula, Mexico to Costa Rica (Central America), and from Colombia to Argentina (South America), was represented by three well supported clades (*C. simus*-Central America in orange, *C. tzabcan* in green, and *C. durissus* in dark blue, grey and red; Figs. 1A, 4). Three clades were recovered within *C. durissus* (South America), two of them with high support values (*C. d. cumanensis* + *C. d. unicolor* in dark blue, and *C. d. durissus* + *C. d. ruruima* + *C. d. terrificus* in red; bootstrap > 70; PP> 95; Figs. 1A). The clade including specimens from areas south of the Amazon River (*C. d. cascavella, C. d. collilineatus,* and *C. d. terrificus*) presented weak support values (bootstrap=55; PP=74) and poor resolution. The populations north of the Amazon are paraphyletic and present strong support, except the node that links *C. d. cumanensis*-La Guaira + *C. d. vegrandis* with

the more southerly clades (bootstrap=48; Pp=52). Based on the mean pairwise divergences (*p*-distance) of the eight clades which ranges from 0.006 to 0.074 in the mtDNA dataset, we consider them all as candidate species (Table 2). Also, we obtained seven major clades within the *Crotalus molossus* species complex from North America (*C. m. nigrescens*, *C. m. oaxacus*, *C. totonacus*, *C. ornatus*, *C. m. molossus*, *C. basiliscus*-north and *C. basiliscus*-south), with strong support, with the exception of the single sequence of *C. m. oaxacus* (bootstrap=42; Pp=72; Fig. 1B).

TABLE 2. Uncorrected *p*-distances within and among clades of *Crotalus durissus* species complex, which are calculated from the combined mtDNA (cyt *b*, *ND4*, *ND2*, 2002 bp).

	Clades	WGMD	1	2	3	4	5	6	7	8
1	*C. d. durissus*	0.006	-							
2	*C. d. vegrandis*	0.004	0.013	-						
3	*C. d. cumanensis*	0.005	0.024	0.026	-					
4	*C. simus*-Central America	0.008	0.017	0.016	0.027	-				
5	*C. tzabcan*	0.003	0.065	0.063	0.077	0.062	-			
6	*C. culminatus*	0.004	0.061	0.059	0.071	0.059	0.074	-		
7	*C. simus*-Isthmus-Chiapas	0.018	0.064	0.062	0.070	0.063	0.072	0.031	-	
8	*C. simus*-Veracruz	0.008	0.066	0.066	0.076	0.067	0.074	0.056	0.050	-

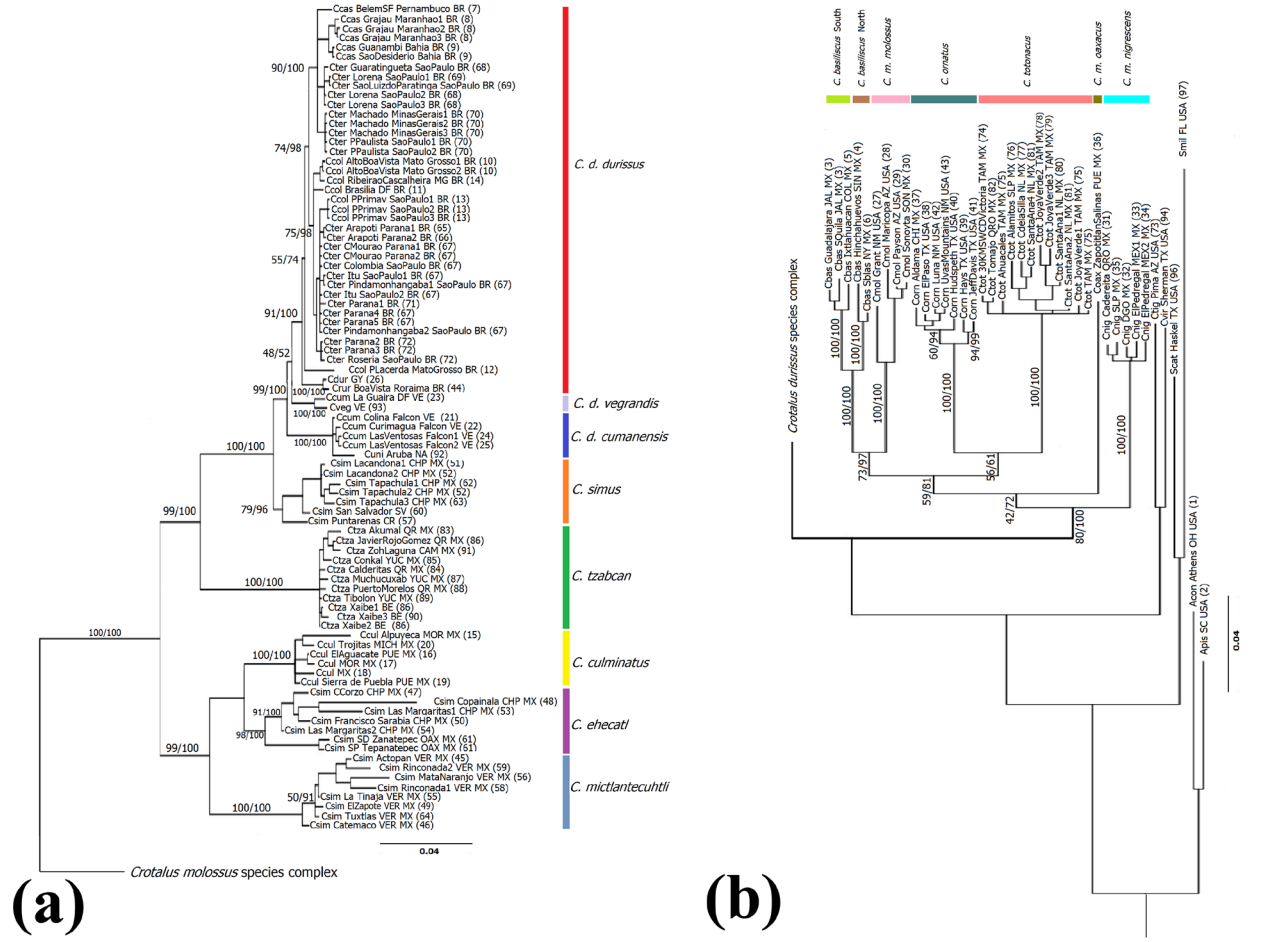

FIGURE 1. (**A**) Maximum-likelihood phylogram of the four genes (cyt *b, ND4*, *ND2*, *c-mos*, 2596 bp) analysis (-ln *L*=-17,270.83). Tip labels are as follows: 3-letter subspecies code for the *Crotalus durissus* species complex, following Campbell & Lamar (2004), locality and haplotypes in parentheses, see Appendix 1. Numbers along branches indicate bootstrap support-ML and Bayesian posterior probability. For clarity, support is only shown for important nodes. (**B**) Maximum-likelihood phylogram of the four genes (cyt *b, ND4*, *ND2*, *c-mos*, 2596 bp) analysis (-ln *L*=-17,270.83). Tip labels are as follows: 3-letter subspecies code for the *Crotalus molossus* species complex and outgroups, following Campbell & Lamar (2004) and Anderson & Greenbaum (2012), locality and haplotypes in parentheses, see Appendix 1. Numbers along branches indicate bootstrap support-ML and Bayesian posterior probability. For clarity, support is only shown for important nodes.

Haplotype network. Network of the *c-mos* haplotype generated from sequences in the *Crotalus durissus* species group showed low genetic divergence within members, with considerable shared haplotypes. However, members of the *Crotalus durissus* species complex: *C. simus*-Veracruz (Actopan-MN072556), *C. culminatus* (Alpuyeca-MN072553; Trojitas-MN072554), *C. tzabcan* (Akumal-MN072581; Calderitas-MN072582; Conkal-MN072583; JavierRojoGómez-MN072584; PuertoMorelos-MN072585), *C. simus*-Central America (Tapachula1-MN072569; Tapachula2- MN072570; Tapachula3-MN072571) contained exclusive sets of haplotypes, none of which were shared with other forms in this gene. Also *C. ornatus* (Hays-JN620925; Luna-JN620917), and *C. totonacus* (JoyaVerde2-MN072576) of the *Crotalus molossus* species complex, contained exclusive sets of haplotypes (Fig. 2).

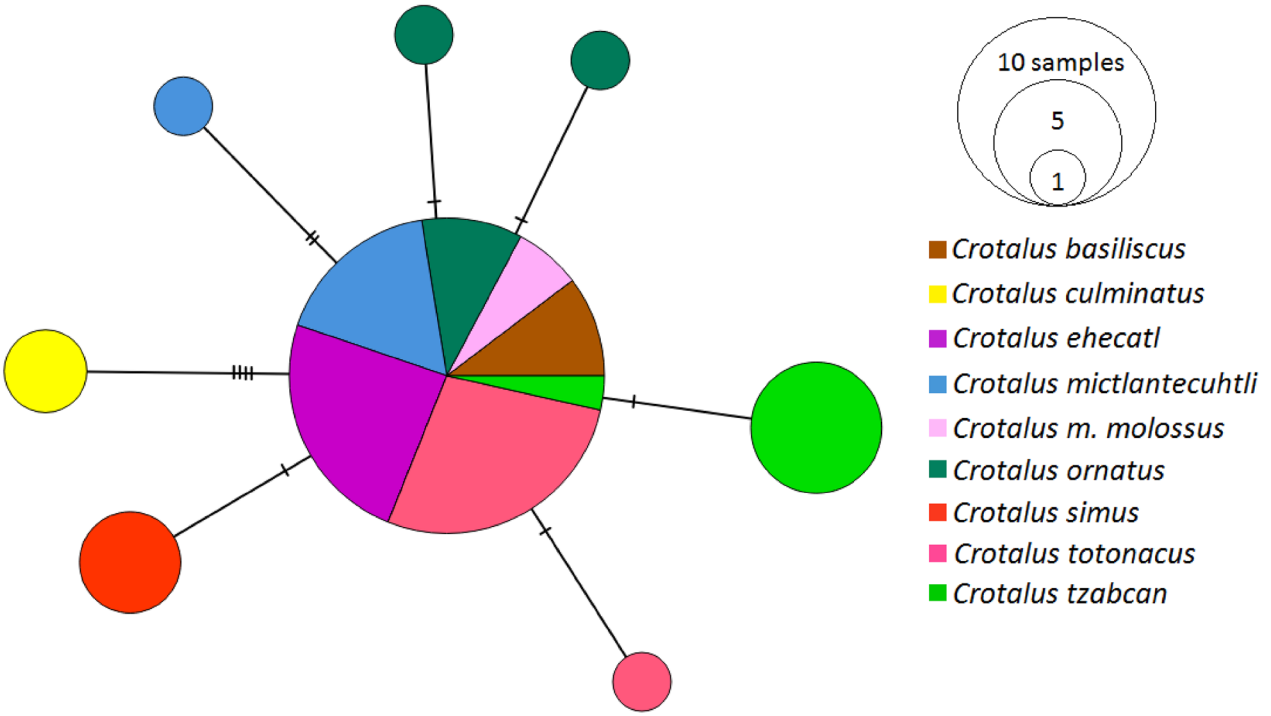

FIGURE 2. Haplotype network from specimens of *Crotalus durissus* species group based on the *c-mos* nuclear gene.

Morphology. No single exclusive morphological character distinguished any one of the five clades of the *Crotalus durissus* species complex with morphological data available. However, unique combinations of morphological characters diagnosed most species, particularly the scales width of paravertebral stripe, presence of intercanthal scales, presence of an interpreocular scale, presence of a divided first infralabial scale, lacunal and supralabial scales contact, contact between paravertebral stripes and supraoculars, and number of dorsal body blotches (Table 3).

The PCA analysis demonstrated that species within our delimitation scheme and based only in meristic data, formed relative cohesive groups, mostly for *C. simus*-Veracruz (light blue), *C. culminatus* (yellow), and *C. tzabcan* (green), but with some individuals that fell outside the clusters (Fig. 3A). The first two axes of the PCA accounted for 37% of the total variation in morphological characters. The PC1 explained 26.2% of the total variation and loaded strongly and with positive values with TSL, PFO and negatively with WPS. PC2 explained 10.8% and loaded strongly and positively with VEN, SBC, SLS and ILS. Plotting PC1 vs. PC2 revealed relative distinct groupings of the five lineages, with morphological overlap between highly differentiated mtDNA + nDNA clades like *C. simus*-Veracruz (light blue) with *C. simus*-Central America (orange), and between *C. simus*-Isthmus-Chiapas (purple) and *C. tzabcan* (green) (Fig. 3A). Reanalysis grouping by two major clades with morphological meristic data, the northern clade (*C. culminatus*-yellow, *C. simus*-Isthmus-Chiapas-purple and *C. simus*-Veracruz-light blue; Fig. 3B) and the southern clade (*C. simus*- Central America-orange and *C. tzbcan*-green; Fig. 3C), reveals relative discrimination, but there is still overlap in meristic characters. In the northern clade, the first two axes accounted for 44.4% (PC1=35%; PC2=9.4%) of the total variation. PC1 loaded strongly and positively with TSL, PFO, and DBB, and negatively with WPS. PC2 loaded strongly and positively INS, ILO, and negatively with SBC. *Crotalus simus*-Veracruz revealed clustering, while many individuals of *C. simus*-Isthmus-Chiapas were grouped within the morphospace of *C. culminatus* (Fig. 3B). While, in the southern clade, the first two axes accounted for 27.5% (PC1=14.7%; PC2=12.8%) of the total variation. PC1 loaded strongly and positively with PFO, AIS, and PLO, and

negatively with VEN. PC2 loaded strongly and positively with MDR, SLS, ILS, and WPS. *Crotalus simus*-Central America is not so tight clustered, with some individuals that fell inside of a more clustered morphospace of *C. tzabcan* (Fig. 3C).

TABLE 3. Summary of morphometric, meristic, qualitative, and color pattern characters for members of the *Crotalus durissus* species complex. Range followed by mode in parentheses for all characters except for tail length ratio where range is followed by mean in parentheses. Ventrals, subcaudals, tail length ratio, and dorsal body blotches counts are shown for males (above) and females (below). Characters for *C. durissus* from Campbell & Lamar (2004). Names of species reflect the taxonomy proposed here. (*n*=number of specimens).

Character	***C. mictlantecuhtli***	***C. ehecatl***	***C. culminatus***	***C. tzabcan***	***C. simus***	***C. durissus***
Ventrals	168–176 (170) *n*=17	168–186 (181) *n*=6	170–182 (175) *n*=14	175–187 (181) *n*=37	170–177 (170) *n*=4	155–179
	169–178 (174) *n*=18	177–187 (187) *n*=8	178–185 (178) *n*=10	181–194 (186) *n*=19	172–184 *n*=5	163–190
Subcaudals	27–32 (28) *n*=17	27–32 (31) *n*=6	25–32 (28) *n*=14	28–32 (31) *n*=37	27–30 (30) *n*=4	25–32
	20–26 (23) *n*=18	21–26 (24) *n*=9	20–25 (22) *n*=10	21–25 (23) *n*=19	22–23 (23) *n*=5	18–26
Rattle fringe scales	10–12 (10) *n*=32	10–12 (10) *n*=13	10–12 (10) *n*=18	10–12 (10) *n*=55	10–12 (12) *n*=5	—
Mid-dorsal scales rows	27–29 (27) *n*=35	27–31 (27) *n*=15	27–33 (29) *n*=25	26–29 (27) *n*=56	27–29 (27) *n*=8	25–33 (27)
Mid-tail scales rows	10–11 (11) *n*=32	10–13 (11) *n*=13	10–13 (11) *n*=25	11–14 (11) *n*=56	10–12 (11) *n*=6	—
Scales width of paravertebral stripe	2–3 (3) *n*=34	1–3 (2) *n*=15	1–2 (1) *n*=25	1–3 (2) *n*=56	2 *n*=9	2–4
Scales length of paravertebral stripe	18–35 (26) *n*=34	9–33 (22) *n*=15	15–38 (33) *n*=25	11–42 (28) *n*=56	20–39 (27) *n*=8	1–4 head length
Tail length ratio	8.7–9.8 (9.3) *n*=3	9.3–10.3 (9.7) *n*=3	6.7–10.9 (9) *n*=11	7.6–10.5 (9.2) *n*=35	13.2 n=1	—
	9.3 *n*=1	6.5–8.8 (7.4) *n*=7	6.3–7.4 (7) *n*=6	6.4–11.5 (7.4) *n*=19	5.8–11.7 (8.6) *n*=4	—
Internasals	2 *n*=35	2 *n*=16	2–4 (2) *n*=25	2 *n*=56	2 *n*=10	2
Canthals	2 *n*=35	2 *n*=16	2–4 (2) *n*=25	2 *n*=56	2 *n*=10	2
Intercanthals presence %	0 *n*=35	18.7 *n*=16	60 *n*=25	0 *n*=56	10 *n*=10	0–2
Supralabials	13–16 (15) *n*=34	14–16 (15) *n*=16	14–17 (15) *n*=25	13–18 (16) *n*=56	13–15 (14) *n*=10	11–18 (13-16)
Infralabials	15–17 (15) *n*=34	13–18 (16) *n*=16	14–18 (17) *n*=25	14–20 (16) *n*=56	15–17 (15) *n*=10	12–20 (14-17)
First infralabial (% divided)	0 *n*=35	18.7 *n*=16	56 *n*=25	75 *n*=56	0 *n*=10	50
Postrostral (% present)	0	12.5 *n*=16	44 *n*=25	8.9 *n*=56	0	—
Prenasal-Supralabial contact (% present)	100 *n*=34	93.7 *n*=16	80 *n*=25	96.4 *n*=56	100 *n*=10	100
Interpreocular %	0 *n*=34	50 *n*=16	20 *n*=25	0 *n*=55	0 *n*=10	—
Prefoveals	3–7 (5) *n*=34	5–10 (5) *n*=16	4–9 (6) *n*=24	2–7 (5) *n*=55	5–9 (6) *n*=10	3–10 (6-8)
Anterior intersupraoculars	2–5 (2) *n*=34	2–5 (3) *n*=16	2–5 (3) *n*=25	2–4 (2) *n*=55	2–4 (2) *n*=10	2–5 (2)
Supraloreal	0–1 (1) *n*=34	1 *n*=16	1 *n*=24	1 *n*=55	1 *n*=10	1
Infraloreal	0–1 (1) *n*=34	1 *n*=16	1–2 (1) *n* =24	1 *n*=55	1 *n*=10	1

.....continued on the next page

TABLE 3. (Continued)

Character	*C. mictlantecuhtli*	*C. ehecatl*	*C. culminatus*	*C. tzabcan*	*C. simus*	*C. durissus*
Presupraloreal	0 *n*=34	0–1 (0) *n*=16	0–1 (0) *n*=24	0–1 (0) *n*=55	0–1 (0) *n*=10	0
Postloreal	0 *n*=34	0–2 (0) *n*=16	0–2 (0) *n*=24	0–1 (0) *n*=55	0–1 (0) *n*=10	0
Postsupraloreal	0–1 (0) *n*=34	1 *n*=16	1–2 (2) *n*=24	0–1 (1) *n*=55	0–1 (1) *n*=10	1
Superciliar %	0 *n*=34	0 *n*=16	20.8 *n*=24	0 *n*=55	10 *n*=10	—
Lacunal-supralabial contact %	68 *n*=34	56.2 *n*=16	16.6 *n*=24	59.2 *n*=55	0 *n*=10	0
Postocular stripe	3–3.5-light center	3 light center	2–3 faded	3 faded	3 faded	2
Dorsal body blotches	23–27 (24) *n*=17	25-31(31) *n*=6	22–30 (26) *n*=14	20–29 (24) *n*=37	24–27 (24) *n*=4	18–32 (26)
	22–26 (23) *n*=18	25–30 (30) *n*=9	26–31 (27) *n*=11	21–28 (25) *n*=19	21–29 *n*=4	—
Secondary blotches	present	present	present	present	present	present
Tertiary blotches	not conspicuos	not conspicuos	not conspicuos	present	present	present
Paravertebral stripe bordered below by buff scale	yes	yes	yes	yes	yes	—
Paravertebral stripe with light center	some-present	only nape	no	no	no	—
Contrast blotches vs ground color	low	low	high	high	high	varialbe
Contact paravertebral stripes with supraoculars %	97 *n*=35	68.7 *n*=16	24 *n*=25	85.5 *n*=55	80 *n*=10	—
Prefrontal bar interrupted %	100 *n*=35	93.7 *n*=16	96 *n*=25	40 *n*=55	90 *n*=10	—

Discriminant functions were unable to entirely separate the five clades with meristic data available of the *Crotalus durissus* species complex. First two discriminant functions correspond to 92% of the entire variation (DF1=60%; DF2=32%), and were strongly correlated with WPS, VEN, and SBC. Despite overlapping in the bivariate space between them, the clades attained values above 57% of individuals correct allocated through Jackknife classification (Table 4; Fig. 3D), with mostly cohesive clusters consisting of *C. simus*-Veracruz, *C. culminatus* and *C. tzabcan*. By separating between the two major clades in the *Crotalus durissus* species complex, we obtained a better discrimination, with values above the 92% of individuals correct allocated in the northern clade, and 60% for *C. simus*-Central America and 92.8% for *C. tzabcan* from the southern clade (Table 4).

Frequencies of qualitative characters of the five clades varied significantly among lineages in condition of ICS (X^2=61.4, *df*=4, p < 0.0001); DFI (X^2=63.3, *df*=4, p < 0.0001); PRT (X^2=29.4, *df*=4, p < 0.0001); PRE-SLS (X^2=12.8, *df*=4, p < 0.05); IPO (X^2=45.8, *df*=4, p < 0.0001); SCI (X^2=21.5, *df*=4, p < 0.0001); LAC-SLS (X^2=26.5, *df*=4, p < 0.0001); PRV-SPO (X^2=47.7, *df*=4, p < 0.0001); and PFB (X^2=56.8, *df*=4, p < 0.0001).

Taxonomic decision. The mtDNA + nDNA analyses revealed eight deep haplotype clades with geographically cohesive distributions in the *Crotalus durissus* species complex: *C. simus*-Veracruz, *C. simus*-Isthmus-Chiapas, *C. culminatus*, *C. tzabcan*, *C. simus*-Central-America, *C. d. cumanensis*, *C. d. durissus*, and *C. d. vegrandis* (only specimens with tissue samples, sequences or phenotypic data herein analyzed were plotted in the map; Fig. 4). The level of mtDNA sequence divergence between these clades equal or exceed those found on other rattlesnake species (Anderson & Greenbaum 2012; Meik *et al.* 2018; Table 2). Although in the PCA and DFA not all group could be fully discriminated against one other (Fig. 3), the diagnosis from them may be complemented by the qualitative characters of scaling and color pattern (Table 3).

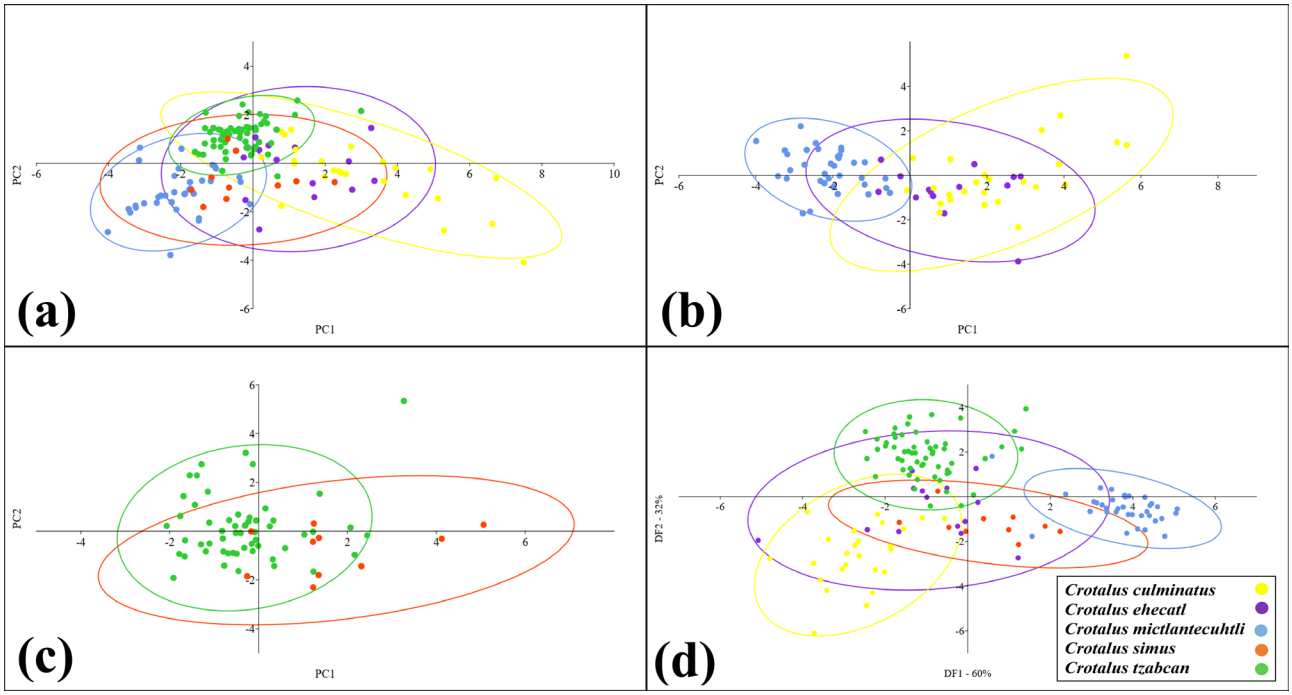

FIGURE 3. (A), Results of the principal component analyses between the members of the *Crotalus durissus* species complex with 95% confidence regions. PC1 and PC2 together explain 33.4% of the total variance. (B), Reanalysis including only members of norhten clade (*Crotalus culminatus*); (C), members of southern clade (*Crotalus durissus*) with morphological data available. (D), Bivariate plots with 95% confidence regions for the first two axes derived from scores of discriminant analyses for members of *Crotalus durissus* species complex.

TABLE 4. Classificatory matrix from discriminant analysis for five defined clades, and the northern and southern clades of the *Crotalus durissus* species complex, showing individuals correctly allocated based on the classification by Jackknife.

Clades	1	2	3	4	5	Total	%
C. simus-Veracruz-1	32	1	0	1	1	35	91.4
C. simus-Isthmus-Chiapas-2	1	8	2	2	1	14	57.1
C. culminatus-3	0	4	18	0	3	25	72
C. tzabcan-4	0	2	0	51	3	56	91
C. simus-Central America-5	1	3	0	0	6	10	60
Total	34	18	20	54	14	140	
Northern clade							
C. simus-Veracruz-1	35	0	0			35	100
C. simus-Isthmus-Chiapas-2	0	13	1			14	92.8
C. culminatus-3	0	0	25			25	100
Total	35	13	26			74	
Southern Clade							
C. tzabcan-4				52	4	56	92.8
C. simus-Central America-5				0	6	6	60
Total				52	10	66	

Based on our three criteria for species recognition, we find evidence that support the recognition of five species in the *Crotalus durissus* species complex (*C. simus*-Veracruz, *C. simus*-Isthmus-Chiapas, *C. culminatus*, *C. tzabcan*, and *C. simus*-Central-America), two of them as new species. Pairwise comparison show one or several lines of evidence for the taxa involved. The pairwise species-level distinctness of the mitochondrial defined clades is summarized in Table 5. Due the lack of nDNA sequences and comprehensive morphological review, the remaining

three clades from South America (*C. d. cumanensis*, *C. d. durissus*, and *C. d. vegrandis*), need to be proved with additional evidence, to support (or not) them as different species (Padial *et al*. 2010).

Crotalus simus-Veracruz is supported as new species. With respect to evolutionary history, this taxon appears most closely related to *C. culminatus* and *C. simus*-Isthmus-Chiapas. Also, is morphologically diagnosable from the rest of clades, but less differentiated from *C. simus*-Central America. It also has a set of unique *c-mos* haplotypes, and other set shared with *C. simus*-Isthmus-Chiapas, *C. tzabcan* and other species in the *Crotalus molossus* species complex. Such sharing of haplotypes can be the result of the retention of ancestral polymorphism due there is no documented sympatry between them.

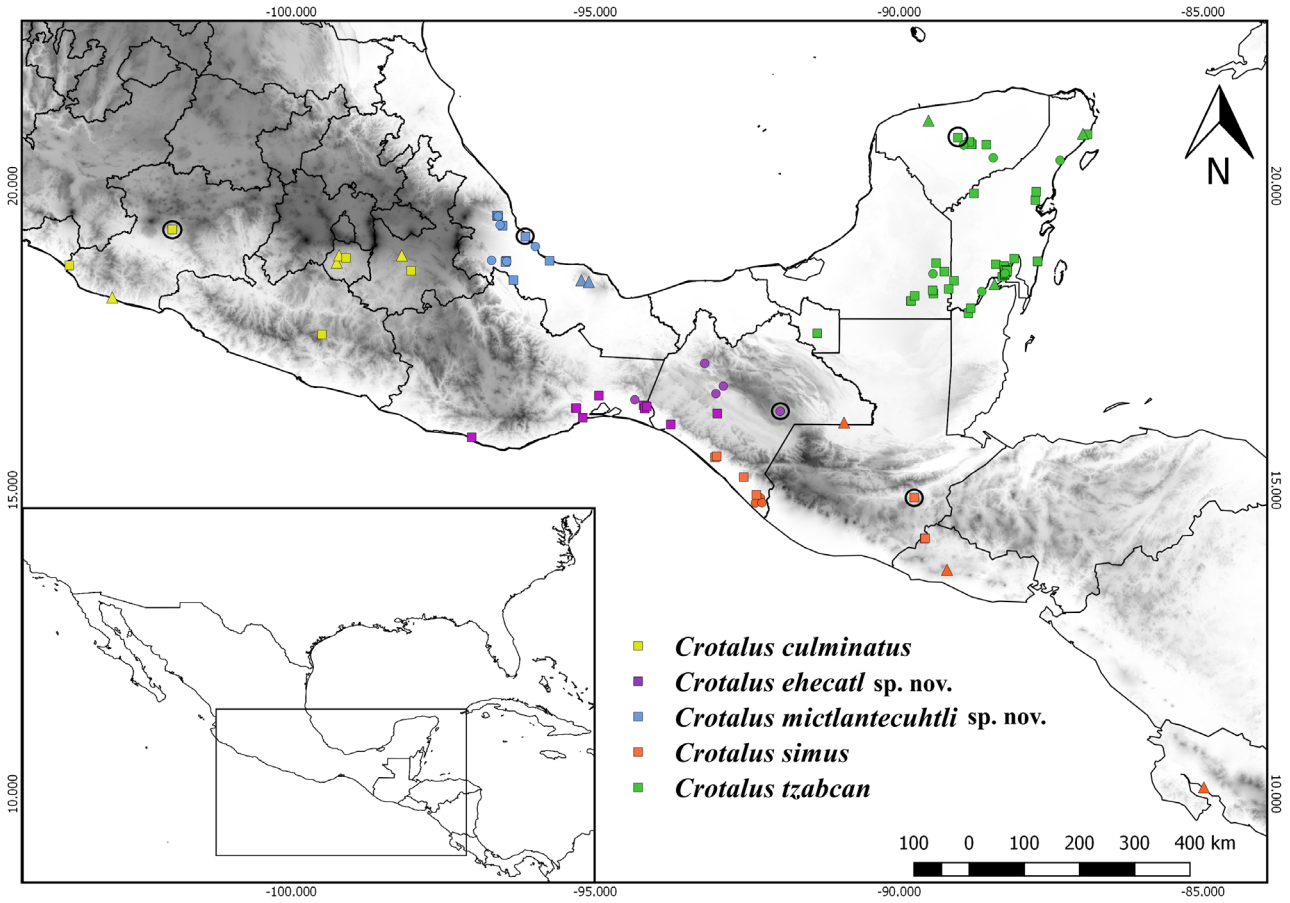

FIGURE 4. Geographic distribution of species in the *Crotalus durissus* species complex distributed across Mexico and Central America. Circled dots indicate type localities. The circles indicate specimens where sequence and morphology were obtained; squares only morphology; triangles only sequences. Names of species reflect our proposed taxonomy.

Crotalus simus-Isthmus-Chiapas is supported as new species. This taxon appears most closely related to *C. culminatus* and *C. simus*-Veracruz, and being morphologically diagnosable from the rest of clades, but more similar to *C. culminatus*. Additionally, does not share *c-mos* haplotypes with *C. culminatus* and *C. simus*-Central America and with some sets of *C. simus*-Veracruz and *C. tzabcan*. However, shares some other *c-mos* sets whit these last two clades mentioned above, and species in the *Crotalus molossus* species complex, but without sympatry, thus, can be result of retention of ancestral haplotypes.

Crotalus culminatus is supported as a valid species, being more closely related to *C. simus*-Isthmus-Chiapas and *C. simus*-Veracruz, and morphologically diagnosable from the rest of clades, but more similar to *C. simus*-Isthmus-Chiapas. Additionally, lacks of *c-mos* haplotype sharing and it is not sympatric with the rest of clades.

Crotalus tzabcan is supported as a valid species. This taxon is more closely related to *C. simus*-Central America and clades from South America (*C. d. cumanensis*, *C. d. durissus*, and *C. d. vegrandis*). Additionally, it can be morphologically diagnosed from the rest of the clades, being more similar to *C. simus*-Central America, and probably sympatric in northern Belize and Guatemala. It also possesses a unique set of *c-mos* haplotypes, but share some sets with *C. simus*-Veracruz, *C. simus*-Isthmus-Chiapas, and species within the *Crotalus molossus* species complex, without sympatry, so can be result of retention of ancestral haplotypes.

Crotalus simus-Central America is supported as a valid species. This taxon is more closely related to *C. tzabcan* and clades from South America, being morphologically diagnosable from the rest of clades, but less differentiated of *C. tzabcan*, and probably sympatric in northern Belize and Guatemala. Additionally, possess a set of unique *c-mos* haplotypes.

In terms of nomenclature, three of the five clades have available names: *C. culminatus*, *Crotalus simus* (*sensu stricto*) and *C. tzabcan*. While the remaining two clades (*C. simus*-Veracruz and *C. simus*-Isthmus-Chiapas), previously were considered *Crotalus simus* (*sensu lato*; Klauber 1952; Campbell & Lamar 2004; Savage *et al.* 2005; Wüster *et al*. 2005), and do not appear to have available names, we therefore describe them as new species.

***Crotalus mictlantecuhtli* sp. nov.**

Figs. 5–6, Table 3

Crotalus simus—Savage *et al*. (2005): 370; Wüster *et al*. (2005): 1097 (Fig.1), 1103; Heimes (2016): 470, 518 (Map 186).
Crotalus simus simus—Campbell & Lamar (2004): 584 (Map 109), 586.
Crotalus durissus durissus—Klauber (1941): 64, 65; Smith & Taylor (1950): 348; Armstrong & Murphy (1979): 10; McCranie (1993): 577.2 (Map 1), 577.5.

Holotype. Adult male (SDNHM-22416) collected on 27 May of 1934 by W. A. King in Veracruz city (19.18833°, -96.14000°; 13 m above sea level; asl hereafter), state of Veracruz, Mexico.

Paratype. Adult male (SDNHM-24026) collected 14 July of 1935 by W. A. King in Tierra Blanca (18.44981°, -96.35995°; 68 m asl), municipality of Tierra Blanca, state of Veracruz, Mexico.

Diagnosis. A rattlesnake belonging to the *Crotalus durissus* species complex, characterized as other species by a prominent vertebral process and conspicuous scale tuberculations. *Crotalus mictlantecuhtli* is not sympatric with any congeners, but its range closely approaches those of *C. simus*-Isthmus-Chiapas and *C. tzabcan. Crotalus mictlantecuhtli* can be distinguished from all members of the *Crotalus durissus* species complex by exclusive combination of the following characters: paravertebral stripes with three scale rows, commonly paravertebral stripes with light center, presupraloreal scale absent, postloreal scale absent, usually postsupraloreal scale absent, contact between lacunal and supralabial scales in 68% (*n*=34) of specimens, postocular stripes of 3.0–3.5 scales width, with light center, contact between paravertebral stripes and supraocular scales in 97% (*n*=34) of specimens, and dark prefrontal bar interrupted.

Comparisons. *Crotalus mictlantecuhtli* is closely related to species of the northern clade (*C. culminatus*) of the *Crotalus durissus* species complex, and differ from them by having 168–176 (mode=170) ventral scales in males, 169–178 (mode=174) females (vs. 170–182 [175] males, 178–185 [178] females in *C. culminatus*); mid-dorsal scale rows 27–29 (mode=27) (vs. 27–33 [29] in *C. culminatus*); width of paravertebral stripe of three scales, commonly with light center (vs. usually one in *C. culminatus*); intercanthal scales absent (vs. 60% present in *C. culminatus*); first infralabial scales not divided (vs. 56% divided in *C. culminatus*); postrostral scale absent (vs. 44% present in *C. culminatus*); prenasal-supralabial scales contact in 100% (vs. 80% present in *C. culminatus*); interpreocular scale absent (vs. 20% present in *C. culminatus*); usually two anterior intersupraocular scales (vs. usually three in *C. culminatus*); presupraloreal absent (vs. often absent in *C. culminatus*); postloreal absent (vs. often absent in *C. culminatus*); postsupraloreal scale usually absent (vs. usually two in *C. culminatus*); superciliar scale absent (vs. 20.8% present in *C. culminatus*); lacunal-supralabial scales contact in 68% (vs. 16.6% in *C. culminatus*); postocular stripe 3.0–3.5 scales width and light center (vs. three scales and faded coloration in *C. culminatus*); dorsal body blotches in males usually 24 (23–27) (vs. usually 26 [22–30] in *C. culminatus*); 23 (22–26) in females (vs. usually 27 [26–31] in *C. culminatus*); contact between paravertebral stripes and supraoculars in 97% (vs. 24% in *C. culminatus*); prefrontal bar interrupted in 100% (vs. 96% in *C. culminatus*). *Crotalus mictlantecuhtli* is distinguished from *C. simus* by the absence of intercanthal scales (vs. presence of 10%), absence of presupraloreal scale (vs. rarely present), absence of postloreal scale (vs. rarely present), postsupraloreal scale rarely present (vs. usally present), superciliar scale absent (vs. rarely present), contac between lacunal and supralabial scales of 68% (vs. not contact), postocular stripe of 3.0–3.5 scales with light center (vs. three scales and faded), tertiary blotches not conspicuous (vs. conspicuous), paravertebral stripes often with light center (vs. usually without light center), contact of paravertebral stripes with supraoculars of 97% (vs. contact of 80%), prefrontal bar interrupted in 100% (vs. interrupted in 90%).

Description of the holotype. SVL 1283 mm, TL 132 mm, head length 59 mm, head width 43 mm; middorsal

scale rows 29; midtail scale rows 11; rattle fringe scales 10; ventrals 170; subcaudals 28; supralabials 15; infralabials 16; rostral slightly higher than wide; internasals two, equally wider than long; canthal one on either side; intercanthals absent, with four scales in the internasal-prefrontal area; anterior intersupraoculars two; posterior intersupraoculars three; suture between intersupraoculars and canthals not evident; supraoculars the largest scales of the head scales; pre- and postnasals approximaltely equal in size; nostril mostly contained in postnasal; prenasals contact first supralabials; prefoveals five; lacunals contact the third supralabials; infraloreal and supraloreal present on both sides; postsupraloreal only on left side; upper preocular larger; lower preocular contacts loreal; interpreocular absent; five scales below center of the eye, between orbit and edge of the lip; mental triangular; first infralabials undivided; genials long, intergenials and submentals absent.

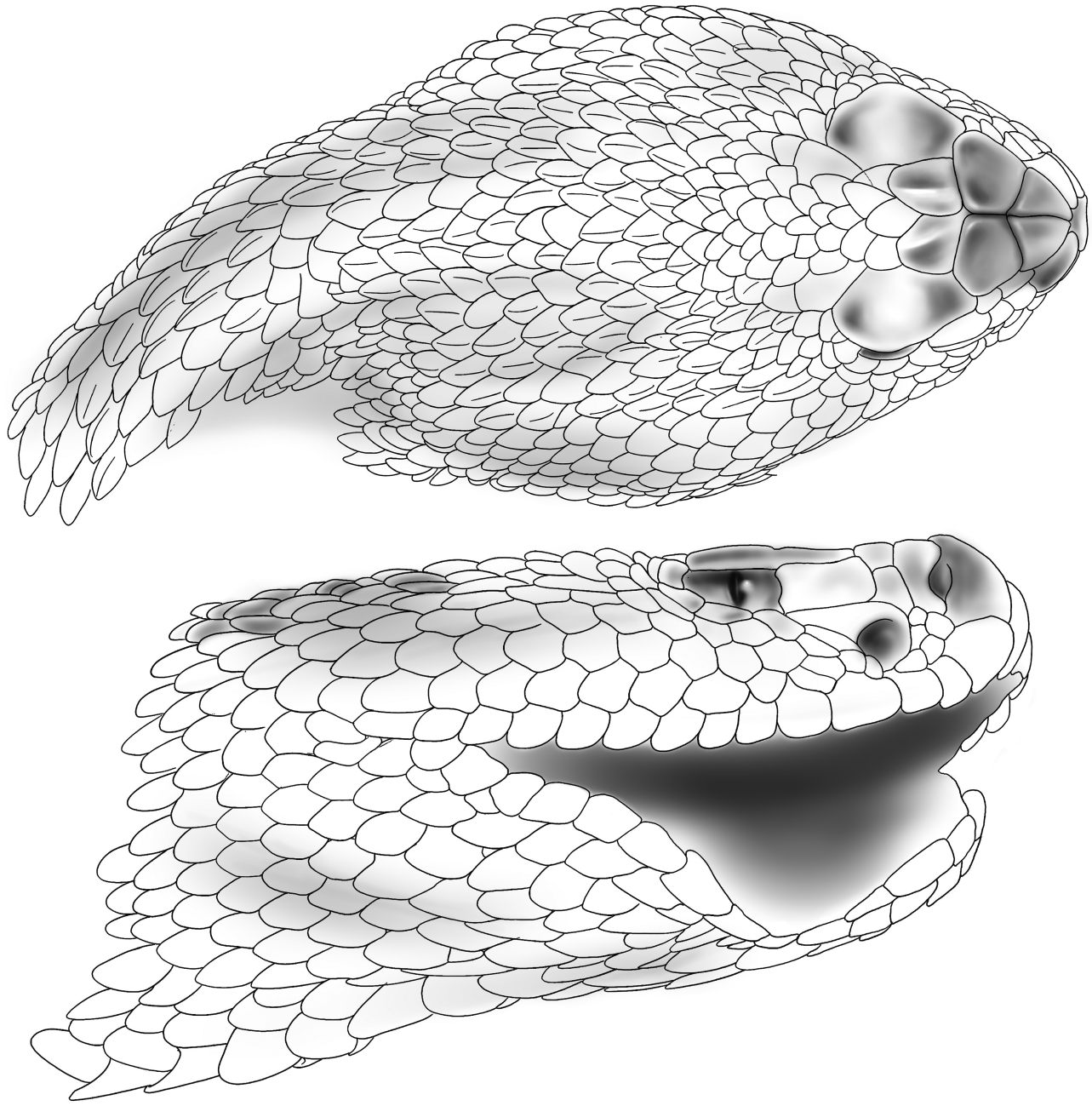

FIGURE 5. Dorsal and lateral view of the head of the holotype of *Crotalus mictlantecuhtli* (SDNHM 22416).

Crotalus mictlantecuhtli is not brightly patterned, usually having less contrast between the dark brown blotches and brown interspaces. Because is a preserved specimen, the coloration may have been faded. Head yellow cream above, dark brown prefrontal bar interrupted encompassing the canthals and anterior edges of intersupraoculars and supraoculars; dark brown paravertebral stripes reach the posterior edge of supraoculars with three to five scales

width in the nape and light center; diagonal dark brown postocular bars 3.0–3.5 scales wide, not conspicuous as the paravertebrals, with light center; sides of head cream, with a brown spot beneath and posterior of loreal pit; underside of the head immaculate cream. Pattern of body consists of a pair dark brown paravertebral stripes 22 scales length, three scales wide, with light central scale, separated by light stripe three scales wide; first row of scales below each paravertebral buff, somewhat lighter than the lateral scales; 25 dark brown dorsal diamonds, sharper posteriorly, somewhat truncated anteriorly, each one bordered by a white scale, with light brown center, a secondary blotch associated in each side, below the lateral angle of the primary, of four to five scales and same color, tertiary blotches between secondary blotches not conspicuous; lateral areas between diamonds punctuated with light brown; toward the tail, the pattern becomes undifferentiated from ground color; tail brown above, buff below, without crossbands evident.

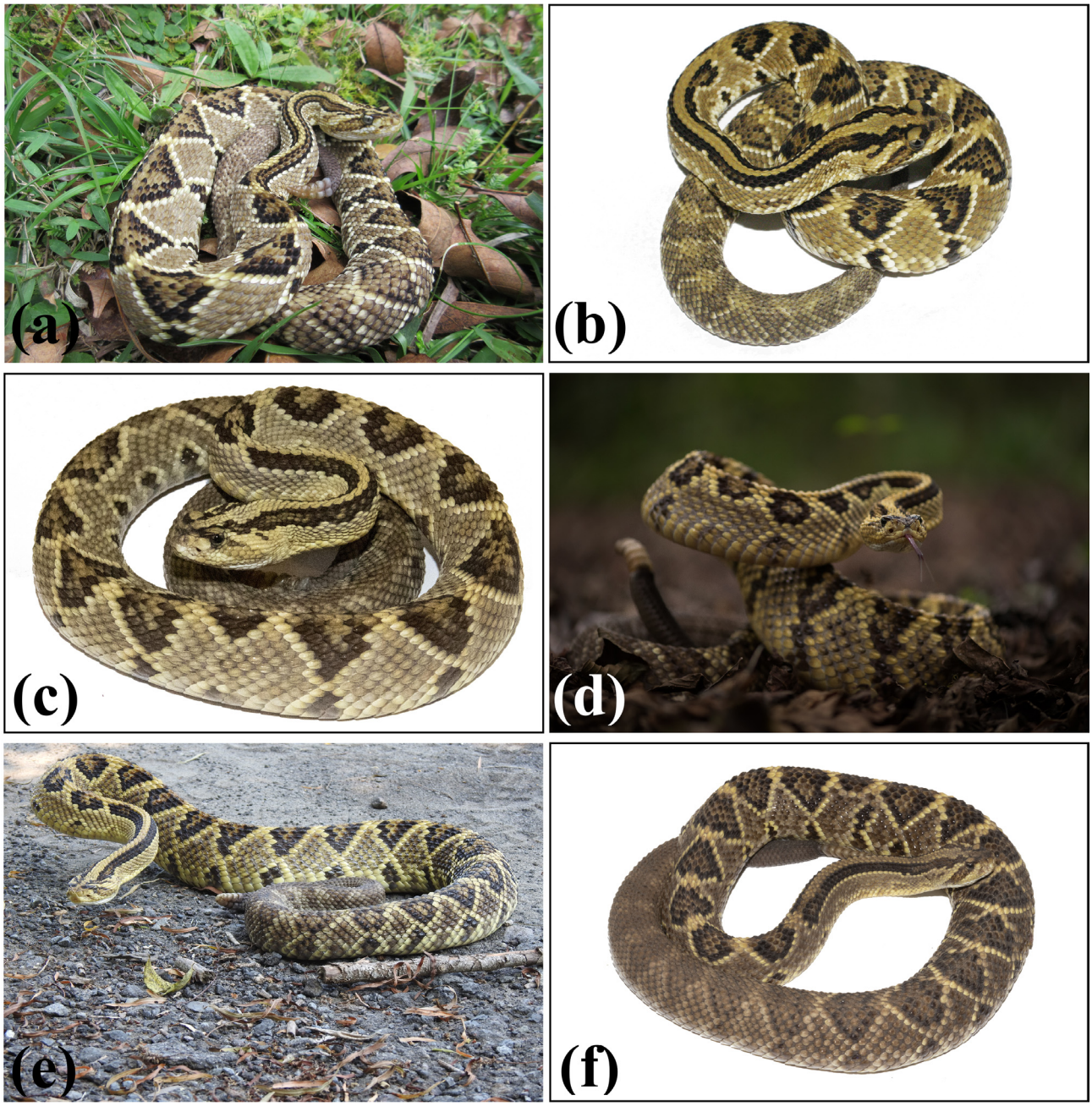

FIGURE 6. *Crotalus mictlantecuhtli* in life, (A) juvenile specimen from El Zapote, Alvarado, Veracruz; (B) neonate specimen from Puerto de Veracruz, Veracruz; (C) adult specimen from Rinconada, Emiliano Zapata, Veracruz; (D) adult specimen from La Tinaja, Cotaxtla, Veracruz; (E) adult specimen from El Colibrí, La Antigua, Veracruz; (F) adult specimen from Rinconada, Emiliano Zapata, Veracruz. Photos by M.A. de la Torre Loranca (A), E. Centenero Alcalá (B, C, F), J.M. González Villa (D), and I. Ajactle Tequiliquihua (E).

Color in life. Color in life varies with adult specimens presenting low contrast between blotches and interspaces, although some are more contrasting (Fig. 6).

Variation. Largest male 1283 mm SVL, 132 mm TL (SDNHM 22416), and female 1360 mm SVL, 140 mm TL (UMA Palancoatl 50); ventrals 168–176 (mode=170; *n*=17) in males, 169–178 (mode=174; *n*=18) in females; subcaudals 27–32 (mode=28; *n*=17) in males, 20–26 (mode=23; *n*=18) in females; midbody scale rows 27 (*n*=27) or 29 (*n*=8); width of paravertebral stripe two (*n*=1) or three (*n*=33) scales; length of the paravertebral stripe 18–35 (mode=26; n=34); supraloreal scales 0 (*n*=8) or 1 (*n*=26); infraloreal scales 0 (*n*=2) or 1 (*n*=32); postsupraloreal scales 0 (*n*=20) or 1 (*n*=14); contact between lacunal and supralabial scales (*n*=23) or no contact (*n*=11); dorsal body blotches 23–27 (mode=24; *n*=17) in males, 22–26 (mode=23; *n*=18) in females; contact between paravertebral stripes and posterior edge of supraoculars (*n*=34) or no contact (*n*=1). We refer to Table 3 for variation in additional quantitative and qualitative features.

Etymology. The specific epithet "*mictlantecuhtli*", derives from the Nahuatl word "*Mictlantecuhtli*" and means "Lord of Mictlán" or "Lord of the place of the dead". In Mexican mythology (Aztec), Zapotec and Mixtec, *Mictlantecuhtli* is the god of the underworld and the dead. The species name is used as an invariable noun in apposition to the generic name. We suggested the vernacular name: Veracruz Neotropical rattlesnake.

Habitat and distribution. *Crotalus mictlantecuhtli* inhabits mostly open dry areas with rocky outcrops in tropical deciduous forest and seasonal rain forest along the Atlantic versant of Mexico from central-south Veracruz, from 0–1200 m asl. This species is known from the state of Veracruz, and may range to western Tabasco, and northeastern Oaxaca in the Atlantic slope on the border with Veracruz (Fig. 4).

Crotalus ehecatl **sp. nov.**

Figs. 7–8, Table 3

Crotalus culminatus—Heimes (2016): 439 (in part);
Crotalus simus—Savage *et al*. (2005): 370; Wüster *et al*. (2005): 1097 (Fig.1), 1103; Heimes (2016): 470 (in part), 492 (Fig. 619), 518 (Map 186).
Crotalus simus simus—Campbell & Lamar (2004): 584 (Fig. 212; Map 109), 586, Plate 956.
Crotalus durissus durissus—Klauber (1941): 61, 64, 65, 67, 71 ; Smith & Taylor (1950): 348 ; Armstrong & Murphy (1979): 10; McCranie (1993): 577.2 (Map 1), 577.5.

Holotype. Adult male (ECO-CH-H-3778) collected on 22 October of 2016 by Jorge Arturo Hidalgo García, in San José Tintonishac (16.29366°, 91.961840°; 1504 m asl), Las Margaritas, state of Chiapas, Mexico.

Paratypes. Six specimens, all from Mexico. Chiapas: Juvenile female (ECO-CH-H-3777) collected 08 October of 2016 by J.A. Hidalgo García, in San José Tintonishac (16.2941°, -91.95931°; 1497 m asl), municipality of Las Margaritas; juvenile female (ECO-CH-H-3776) collected on 24 September of 2015 by T. Ramírez Valverde and R.A. Carbajal Márquez, at 12.2 km west of Chiapa de Corzo (16.706618°, -92.896292°; 1071 m asl), municipality of Chiapa de Corzo. Oaxaca: adult female (SDSNH-24383) and adult male (MCZ-R-27819) collected on 1929 by W. W. Brown Jr., at San Pedro Tepanatepec (16.368660°, - 94.193445°; 63 m asl), municipality of San Pedro Tepanatepec; collected on July 1927 by W.W. Brown Jr., at San Pedro Tepanatepec (16.368660°, - 94.193445°; 63 m asl), municipality of San Pedro Tepanatepec. Adult female (MCZ-R-27821) collected July 1927 by Wilmot W. Brown Jr., at San Pedro Tepanatepec (16.368660°, - 94.193445°; 63 m asl), municipality of San Pedro Tepanatepec. Adult male (MCZ-R-46485) collected on January 1942 by W. Barker near Santo Domingo Tehuantepec (16.324765°, -95.238529°; 52 m asl), municipality of Santo Domingo Tehuantepec.

Diagnosis. A rattlesnake belonging to the *Crotalus durissus* species complex, characterized as other species by a prominent vertebral process and conspicuous scale tuberculations. The distribution range of *C. ehecatl* closely approaches to those of *C. culminatus* and *C. simus*, and to a lesser extent to *C. mictlantecuhtli*. *Crotalus ehecatl* can be distinguished from all members of the *Crotalus durissus* species complex by exclusive combination of the following characters: paravertebral stripes of two scale rows, usually paravertebral stripes with light center on the nape, length of paravertebral stripes of 22 scales, 31 dorsal body blotches, intercanthal scales in 18.7% (*n*=16) of specimens, interpreocular scale in 50% (*n*=16) of specimens, first infralabial scale divided in 18.7% (*n*=16), postrostral scale in 12.5% (*n*=16), usually 1 postsupraloreal scale, contact between lacunal and supralabial scales in 56.2% (*n* =16) of specimens, postocular stripe of three scales, usually with light center, contact between paravertebral stripes and

supraocular scales in 68.7% (*n*=16) of specimens, and a dark prefrontal bar interrupted in 93.7% (*n*=16) of specimens.

Comparisons. *Crotalus ehecatl* is most closely related to species of the northern clade (*C. culminatus*) of the *Crotalus durissus* species complex, and is distinguished from these species by having 168–186 (mode=181) ventral scales in males, 177–187 (mode=187) in females; number of subcaudal scales 27–32 (mode=31) in males, 21–26 (mode=24) in females (vs. 25–32 [28] males, 20–25 [22] females in *C. culminatus,* and 27–32 [28] males, 20–26 [23] females in *C. mictlantecuhtli*); mid-dorsal scale rows 27–31 (mode=27) (vs. 27–33 [29] in *C. culminatus*); width of paravertebral stripe of two scales, usually with light center in the nape (vs. three commonly with light center in *C. mictlantecuhtli*, and usually one in *C. culminatus*); intercanthal scales present in 18.7% (vs. absent in *C. mictlantecuhtli*, and 60% in *C. culminatus*); first infralabial scales divided in 18.7% (vs. no divided in *C. mictlantecuhtli*, and 56% in *C. culminatus*); postrostral scale present in 12.5% (vs. absent in *C. mictlantecuhtli*, and 44% in *C. culminatus*); prenasal-supralabial scales contact in 93.7% (vs. present in 100% in *C. mictlantecuhtli*, and 80% in *C. culminatus*); interpreocular scale present in 50% (vs. absent in *C. mictlantecuhtli*, and 20% in *C. culminatus*); usually three anterior intersupraocular scales (vs. usually two in *C. mictlantecuhtli*); presupraloreal sometimes present (vs. absent in *C. mictlantecuhtli*); one or two postloreal scales sometimes present (vs. absent in *C. mictlantecuhtli*); usually one postsupraloreal scale (vs. usually absent in *C. mictlantecuhtli*, and two in *C. culminatus*); superciliar scale absent (vs. present in 20.8% in *C. culminatus*); lacunal-supralabial scales contact in 56.2% (vs. 68% in *C. mictlantecuhtli* and 16.6% in *C. culminatus*); post-ocular stripe of three scales in width and usually with a light center (vs. 3.0–3.5 scales width and usually light center in *C. mictlantecuhtli*, and three scales width and faded coloration in *C. culminatus*); usually 31 (25–31) dorsal body blotches in males (vs. usually 24 [23–27] in *C. mictlantecuhtli*, and 26 [22–30] in *C. culminatus*); in females 30 (25–30) (vs. usually 23 [22–26] in *C. mictlantecuhtli*, and 27 [26–31] in *C. culminatus*); contact between paravertebral stripes and supraoculars in 68.7% (vs. 97% in *C. mictlantecuhtli*, and 24% in *C. culminatus*); and the prefrontal bar interrupted in 93.7% (vs. 100% in *C. mictlantecuhtli*, and 96% in *C. culminatus*). *Crotalus ehecatl* is distinguished from *C. simus* by higher number of ventral scales 168–186 (181) vs. 170–177 (170), less number of scales length of paravertebral stripe 9–33 (22) vs. 20–39 (27), intercanthal scales present in 18.7% (vs. presence of 10%), first infralabial scale divided in 18.7% (vs. not divided), postrostral scale present in 12.5% (vs. absent), contact between prenasal and first supralabial scale of 93.7% (vs. contact of 100%), interpreocular scale present in 50% (vs. absent), usually three (2–5) anterior intersupraoculars (vs. usally two [2–4]), superciliar scale absent (vs. rarely present), contac between lacunal and supralabial sales of 56.2% (vs. not contact), postocular stripe of three scales with light center (vs. three scales and faded), tertiary blotches not conspicuous (vs. conspicuous), paravertebral stripes often with light center only in the nape (vs. without light center), contact of paravertebral stripes with supraoculars of 68.7% (vs. contact of 80%), prefrontal bar interrupted in 93.7% (vs. interrupted in 90%).

Description of the holotype. SVL 1204 mm, TL105 mm, head length 51 mm, head width 32.6 mm; middorsal scale rows 27, mid-tail scale rows 10, rattle fringe scales 10; ventrals 168, subcaudals 27; supralabials 14–15, infralabials 13–16; rostral slightly higher than wide; internasals two equally wider than long; canthal one on either side; intercanthals three, with seven scales in the internasal-prefrontal area; anterior intersupraoculars five + six; suture between intersupraoculars and canthals not evident; supraoculars the largest scales of the head scales; pre- and postnasals approximately equal in size; nostril mostly contained in the postnasal; prenasals contact first supralabials; prefoveals seven; lacunals contact the third supralabials; infraloreal, supraloreal and postsupraloreal present on both sides; upper preocular larger; lower preocular contacts loreal; interpreocular absent; five scales below center of the eye, between the orbit and the edge of the lip; mental triangular; first infralabial of the left side divided; genials long, intergenials and submentals absent.

Crotalus ehecatl is not brightly patterned, usually having less contrast between the dark brown-black blotches and yellow-brown interspaces. Head yellow cream above, dark brown internasals and prefrontal bar interrupted encompassing the canthals and anterior edges of intersupraoculars and supraoculars; dark brown, near black paravertebral stripes do not reach the posterior edge of supraoculars, with three to five scale width in the nape, and light center; diagonal dark brown postocular bars of three scales wide, not conspicuous as the paravertebrals, with light center; sides of the head yellow cream, with a brown spot beneath and posterior of the loreal pit; underside of the head immaculate cream. Pattern of the body consist of a pair of dark brown, near black paravertebral stripes 16 scales length, two scales wide, separated by a dark yellow buff stripe of three scales wide, somewhat lighter than the lateral scales; first row of scales below each paravertebral buff, lighter than the lateral scales; 29 black dorsal dia-

monds, sharper posteriorly and somewhat truncated anteriorly, each one bordered by a cream white scale and light brown center, a secondary blotch associated in each side, below the lateral angle of the primary, comprised of four to five scales and same color, tertiary blotches between secondary blotches not conspicuous; lateral areas between diamonds punctated with light brown; toward the tail, the pattern becomes obscure; tail dark gray above and buff below, with crossbands slightly evident.

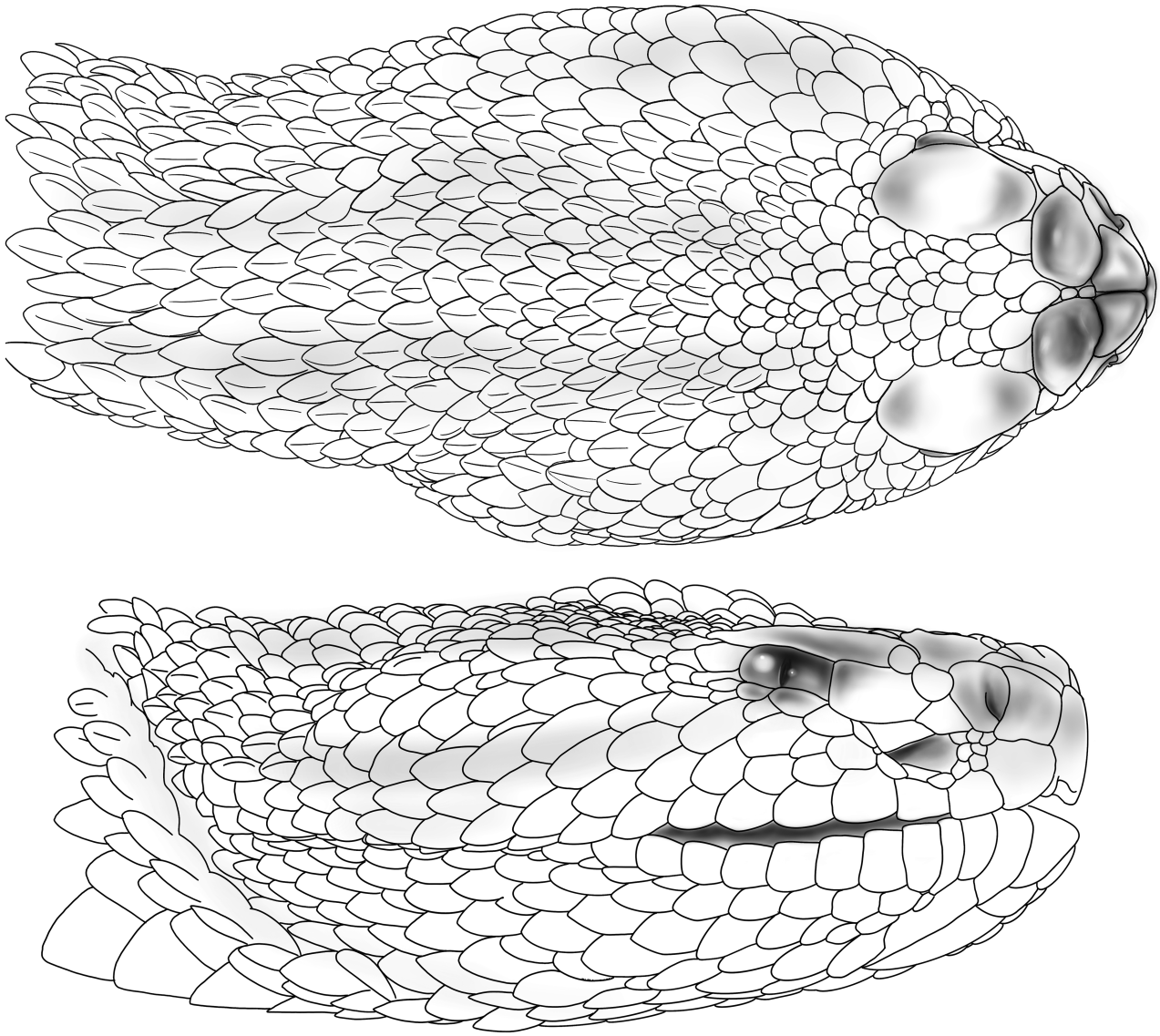

FIGURE 7. Dorsal and lateral view of the head of the holotype of *Crotalus ehecatl* (ECO-CH-H 3778).

Color in life. Color in life varies with adult specimens presenting low contrast between blotches and interspaces, although some are more contrasting (Fig. 8).

Variation. Largest male 1653 mm in total length (UTA-R 51456; see Campbell & Lamar 2004), and female 1180 mm SVL, 85 mm TL (MCZ-R 27821); ventrals 168–186 (mode=181; *n*=6) in males, 177–187 (mode=187; *n*=8) in females; subcaudals 27–32 (mean=31; *n*=6) in males, 21–26 (mean=24; *n*=9) in females; midbody scale rows 27–31 (mode=27; *n*=15); width of paravertebral stripe 1–3 (mode=2; *n*=15); length of the paravertebral stripe 9–33 (mode=22; *n*=15); one supraloreal, one infraloreal and one postsupraloreal in all examined specimens; contact between lacunal and supralabial scales (*n*=9), or no contact (*n*=6); dorsal body blotches 25–31 (mode=31; *n*=6) in males, 25–30 (mode=30; *n*=9) in females; contact between paravertebral stripes and posterior edge of supraoculars (*n*=11), or no contact (*n*=5). We refer to Table 3 for variation in additional quantitative and qualitative features.

Etymology. The specific epithet "*ehecatl*", derives from the Nahuatl word "*Ehēcatl*" and means "The wind" or "Lord of the wind". In Mexican mythology (Aztec), *Ehécatl* is the god of the wind. It is usually interpreted as one of the manifestations of *Quetzalcóatl*, the feathered serpent, taking the name of *Ehécatl-Quetzalcóatl*, appearing in

the breath of living beings and in the breezes that bring the clouds with rain for the sowings. His breath starts the movement of the Sun, and brings life to what is inert. Also, he clears the way for *Tláloc*. The species name is used as an invariable noun in apposition to the generic name. We suggested the vernacular name: Tehuantepec Isthmus Neotropical rattlesnake.

Habitat and distribution. *Crotalus ehecatl* inhabits mostly open dry areas with rocky outcrops in tropical deciduous forest and seasonal rain forest along the Pacific versant of Mexico from central-south Oaxaca, southward across the Tehuantepec Isthmus to west of Tonalá, Chiapas, almost reaching the Central Valleys in Oaxaca, and in the Grijalva River basin reaching Comitán, Chiapas, from 0–1585 m asl. This species is known from the state of Oaxaca, and Chiapas, and may range to southeastern Veracruz, and eastern Huehuetenango, Guatemala (Fig. 4).

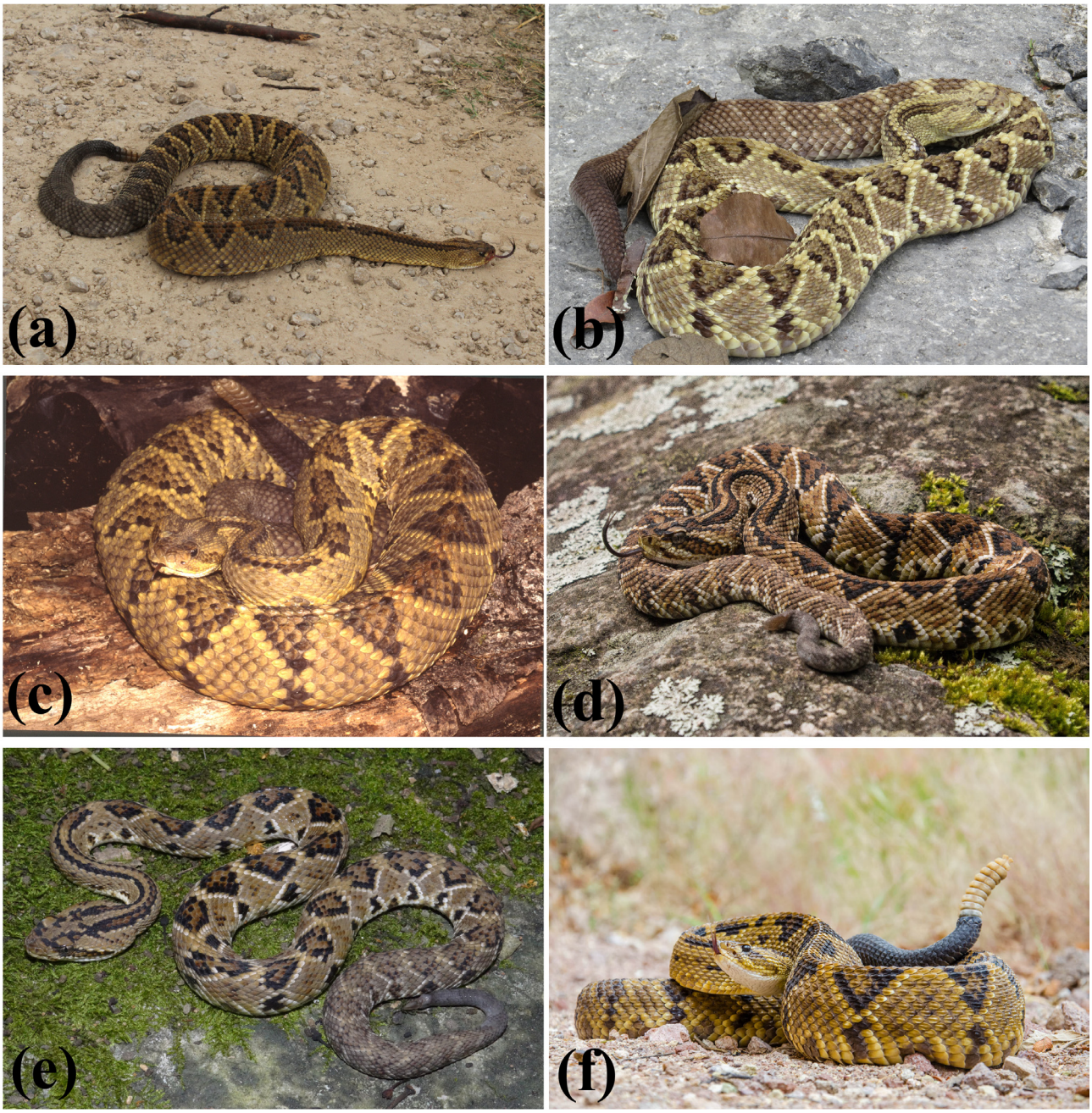

FIGURE 8. *Crotalus ehecatl* in life, (A) ECO-CH-H 3778, holotype from San José Tintonishac, Las Margaritas, Chiapas; (B) adult specimen from Tuxtla Gutiérrez, Chiapas; (C) UTA-R 51456, adult male from Santa Inés, Santa María Chimalapa, Oaxaca; (D) neonate specimen from Santa María Mixtequilla, Oaxaca; (E) neonate specimen from San Pedro Tapanatepec, Oaxaca; (F) adult specimen from San Pedro Totolápam, Oaxaca. Photos by J.A. Hidalgo García (A), E.B. Jiménez Díaz (B), E.N. Smith courtesy of J.A. Campbell (C), I.T. Ahumada Carrillo (D), HERP.MX (E), and F. Martínez Belmar (F).

TABLE 5. Distinguishing datasets and analyses supporting species status for the five mtDNA defined clades of the *Crotalus durissus* species complex considered in this study. Above the diagonal: evidence from nDNA; below the diagonal: evidence from morphology.

	C. simus-Veracruz	*C. simus*-Isthmus-Chiapas	*C. culminatus*	*C. tzabcan*	*C. simus*-Central America
***C. simus*-Veracruz**	–	some haplotype sharing, not sympatry	not haplotype sharing, not sympatry	some haplotype sharing, not sympatry	not haplotype sharing, not sympatry
***C. simus*-Isthmus-Chiapas**	phenotypically distinct	–	not haplotype sharing, not sympatry	some haplotype sharing, not sympatry	not haplotype sharing, probably sympatry
C. culminatus	phenotypically distinct	phenotypically distinct	–	not haplotype sharing, not sympatry	not haplotype sharing, not sympatry
C. tzabcan	phenotypically distinct	phenotypically distinct	phenotypically distinct	–	not haplotype sharing, probably sympatry
***C. simus*-Central America**	moderately diagnosable	phenotypically distinct	phenotypically distinct	moderately diagnosable	–

Discussion

This study represents the most comprehensive phylogenetic analysis of any member of the *Crotalus durissus* species group to date, including lineages that had not been previously sampled. Our results illustrate that species diversity is currently underestimated in this group which belongs to one of the most studied snake genera, in part by subtle patterns of morphological variation. Apparently, the *Crotalus durissus* species complex is comprised by two major clades, the northern (*C. culminatus*) restricted to Mexico, and the southern (*C. durissus*) from southern Mexico to South America, meanwhile *C. molossus* represents the sister complex of species distributed in North America.

Our phylogenetic inferences support three lineages within the northern clade (*C. ehecatl*, *C. culminatus*, and *C. mictlantecuhtli*) and three within the southern clade (*C. durissus, C. simus*, and *C. tzabcan*). The phylogenetic pattern recovered was well supported and agrees mostly with Wüster *et al*. (2005) and confirms that the apparent allopatric populations from central-south Veracruz, previously referred as *C. simus*, represent an independent sister lineage of *C. culminatus* and *C. ehecatl*. Previously, Klauber (1952) had suggested that with additional evidence the Veracruz populations could be recognized as a distinct taxon. We find that specimens from the central-south coast, Isthmus of Tehuantepec, Oaxaca and Grijalva River Basin in Chiapas, previously assigned to *C. simus* based on morphology (see Klauber 1952; McCranie 1993; Campbell & Lamar 2004), represent a lineage more closely related to *C. culminatus*, named here as *C. ehecatl*, while the populations from the Yucatán Peninsula (*C. tzabcan*) are sisters of the coastal Chiapas to Costa Rica populations (*C. simus*) and *C. durissus* represents the populations from South America. This distribution pattern of the different lineages in the northern and southern clades is very similar to that of other reptiles that inhabit the tropical deciduous forest in Mexico and Central America, including pitvipers from the same region, such as species of the genus *Agkistrodon* and *Porthidium* (Bryson *et al*. 2008; Porras *et al*. 2013; Zarza *et al*. 2019). *Crotalus mictlantecuhtli* is allopatric, but distributes near to *C. ehecatl* and *C. tzabcan* ranges, but without confirmed sympatry. There is a distribution gap between the range of *C. ehecatl* and *C. culminatus*, located approximately between the surroundings of San Pedro Pochutla, Oaxaca, and Marquelia, Guerrero, Mexico. However, the range of *C. ehecatl* probably overlaps with *C. simus* near Tonalá, in coastal Chiapas. As well, *C. simus* probably overlaps with *C. tzabcan* in northen Belize and the Petén region, in Guatemala (see Mcranie 1993; Campbell & Lamar 2004). Therefore, more sampling effort is necessary to correctly delimit the distribution of each species.

Within *C. durissus* three major lineages were recovered: 1) *C. d. cumanensis* (*C. d. cumanensis* + *C. d. unicolor*), 2) *C. d. vegrandis* (*C. d. cumanensis*-La Guaira + *C. d. vegrandis*), and 3) *C. d. durissus* (*C. d. durissus* + *C. d. ruruima* + *C. d. terrificus*). The phylogenetic pattern and geographically defined lineages recovered herein corroborate the pattern found by Wüster *et al*. (2005). The three subspecies from south of the Amazon areas (*C. d.*

cascavella, *C. d. collilineatus*, and *C. d. terrificus*) are closely related to *C. d. durissus* and *C. d. ruruima* but with a poor genetic resolution; so, we tentatively consider them all as one "candidate species". However, to obtain a better resolved phylogeny it is necessary to include samples of subspecies and missing localities like *C. d. marajoensis*, *C. d. trigonicus* and unassigned isolated populations in Campos de Huamaitá (Amazonas) and the Serra do Cachimbo and Santarém (Pará) (see Campbell & Lamar 2004). The clades including *C. d. cumanensis* + *C. d. unicolor* and *C. d. cumanensis*-La Guaira + *C. d. vegrandis*, also tentatively represents "candidate species", *C. cumanensis* and *C. vegrandis*, respectively. Nonetheless, we refrain to formally propose reclassification of these lineages without additional evidence, because at this moment the absence of key samples, the polymorphism in morphological characters, and lacking a comprehensive review don't support such hypotheses. However, our results highlight a cryptic lineage diversity within the *Crotalus durissus* species complex.

We recovered seven major lineages in the *Crotalus molossus* species complex: *C. m. nigrescens*, *C. m. oaxacus* Gloyd 1948, *C. totonacus*, *C. ornatus*, *C. m. molossus* Baird & Girard 1853*, C. basiliscus* North, and *C. basiliscus* South. The phylogenetic pattern recovered is similar to those in Wüster *et al*. (2005) and Anderson & Greenbaum (2012), with the exception of the position of *C. m. oaxacus*, which was recovered as intermediate between the *C. m. nigrescens* clade and the clade containing the remaining lineages. *Crotalus totonacus* was recovered as sister clade of *C. ornatus*, and *C. basiliscus* as a sister clade of *C. m. molossus. Crotalus basiliscus* was recovered with two clades from the north and south respectively, which seem to be independent evolutionary lineages that need to be tested with additional samples and evidence to prove that they represent different species. We noted that the *Crotalus molossus* species complex needs a better sampling that includes additional localities for *C. m. molossus*, *C. m. nigrescens,* and *C. m. oaxacus,* as well as *C. basiliscus,* including all contact zones between these lineages and also including sequences of *C. estebanensis* Klauber 1949 to obtain a more complete phylogeny. Therefore, we prefer not to formally propose reclassification of these lineages.

Our decision to use the nuclear gene *c-mos* was based on which is the best represented nuclear gene for *Crotalus* including the sister complex of *C. durissus*, the *Crotalus molossus* species complex (see Alencar *et al*. 2016). Analyses of *c-mos* included in this study revealed extensive incomplete lineage sorting in the genus *Crotalus*, resulting in a weak phylogenetic signal, similar to other studies that use the same and other nuclear DNA markers (Anderson & Greenbaum 2012; Reyes-Velasco *et al*. 2013; Bryson *et al*. 2014; Doan *et al*. 2016); whereas others provide a phylogenetic signal similar to mitochondrial markers (Kubatko *et al*. 2011; Meik *et al*. 2018). Phylogenetic inference of the *c-mos* data set (not shown) indicated random placement of *Crotalus* specimens, suggesting shared ancestral polymorphism. This is a common trend in lineages that have undergone a rapid diversification, or are weakly supported (Wiens *et al*. 2010; Anderson & Greenbaum 2012), like New World pitvipers, that started to radiate around the middle/late Oligocene to the early Miocene (31.05–20.99 Mya), with a rapid diversification due to the invasion of a new area free of competitors and/or predators (Alencar *et al*. 2016). The majority of diversification of *Crotalus* occurred in the Neogene (23.03–5.33 Mya) throughout the montane pine-oak forests of western Mexico (Blair & Sánchez-Ramírez 2016). The portion of the *c-mos* gene examined herein has not allowed lineage sorting among the genus *Crotalus* to reach completion. Therefore, more research involving additional localities, larger sample sizes and the addition of faster-evolving nuclear genes is needed to make further taxonomic decisions.

Although members of the *Crotalus durissus* species complex are recovered by the phylogenetic methods, our PCA and DFA analysis of traditional morphological meristic characters used to describe previous taxa (Klauber 1952) reveal morphological overlap between them. However, we find that unique combinations of morphological characters diagnosed most species. Also, this overlap of character states has been observed in other rattlesnakes like the *C. mitchellii*, *C. molossus*, and *C. triseriatus* species complex (Anderson & Greenbaum 2012; Bryson *et al*. 2014; Meik *et al*. 2018). These clades probably represent morphologically poorly differentiated species but with clear differences in their ecology and distribution, defined as semi-cryptic species (Vondrák *et al*. 2009; Pavón-Vázquez *et al*. 2018).

Additionally, it has been documented variation in the composition of venom in members of Neotropical rattlesnake complex (see Calvete *et al*. 2010; Castro *et al.* 2013; Durban *et al.* 2017); which is consistent with the taxa recognized in our study. Recently, Neri-Castro *et al.* (2019) found interspecific variation in the LD_{50} and in the percentage of β-neurotoxin (crotoxin homologs) between *C. culminatus*, *Crotalus simus* (*sensu lato*) and *C. tzabcan*: *C. culminatus* 3.37–17.3 µg/g mouse and 0 %; *C. ehecatl* (previously *C. simus*) 0.20–0.67 µg/g mouse and 6.3–14.5%; *C. mictlantecuhtli* (previously *C. simus*) 0.15–0.32 µg/g mouse and 21.6–44.3%; *C. tzabcan* 0.71–8.31 µg/g mouse and 0–7.7%. Meanwhile adult *C. simus* from Costa Rica possess ~0.889 µg/g mouse and 4.1% of crotoxin (Gutiér-

rez *et al*. 1991; Calvete *et al*. 2010); *C. d. cumanensis* 0.18–0.86 µg/g mouse and 2.6%; *C. d. ruruima* 0.03–0.23 µg/g mouse and 82.7%; *C. d. durissus* 0.88 µg/g mouse and 68% and *C. d. terrificus* 0.06–0.09µg/g mouse and 59.5% (Calvete *et al*. 2010).

In Mexico, the conservation authority regards *Crotalus durissus* (*sensu lato*, inclusive of *C. culminatus*, *C. simus* and *C. tzabcan*, and now *C. ehecatl* and *C. mictlantecuhtli*) as Special Protection (NOM-059-SEMARNAT-2010), while the IUCN consider *C. durissus*, *C. simus*, *C. tzabcan* as Least Concern species (Martins & Lamar 2010; Acevedo *et al*. 2014; Dwyer *et al*. 2014). Given our proposed taxonomic changes, we recommend that the conservation status of *Crotalus durissus* species complex be reevaluated. *Crotalus ehecatl* and *C. mictlantecuhtli* arguably represent two of the most threatened species in the *Crotalus durissus* species complex. Both species are distributed in highly degraded habitats, especially *C. mictlantecuhtli*, since much of Veracruz state has been degraded mainly for agriculture, livestock and urban settlement. However, it is possible tha they are resilient to environmental degradation, since they prefer open areas, and its main prey that are rodents, are abundant in altered habitats.

Through analyses of both mtDNA and nDNA and morphological data, we find consistent evidence for previously undocumented distinct evolutionary lineages within the *Crotalus durissus* species complex. Identifying cryptic diversity has significant implications for conservation. In this way, our study helps to clarify previous relationships within populations in *Crotalus simus* (*sensu lato*). Despite adding new sequences of mitochondrial and nuclear markers, we were not able to resolve previous problematic issues in *C. durissus*, and in the *Crotalus molossus* species complex. Our results demonstrate the existence of lineages that represent species hypotheses to be tested with additional evidence (morphology, ecology, and venom composition variation), which may reveal reliable diagnostic character states for some populations or group of populations. Furthermore, this diversity has medical relevance due to the toxicity and diversity of venom composition. Finally, we encourage additional research including multiple nuclear markers to test the species boundaries in the *Crotalus durissus* species group.

Acknowledgments

The senior author thanks the Consejo Nacional de Ciencia y Tecnología (CONACYT) and El Colegio de la Frontera Sur (ECOSUR) for providing doctoral fellowship and institutional help. The senior author also is indebted to C.M. García Balderas, T. Ramírez Valverde, and N.G. Blanco Campos for field and lab assistance. We thank J. Reyes Velasco, J. Barrientos Villalobos, L.F. Carrera Parra, S.I. Salazar Vallejo, J.C. Arenas Monroy and O. Cruz for sharing their expertise in specific analyses. We thank I.T. Ahumada Carrillo, I. Ajactle Tequiliquihua, A. Alagón Cano, H. Bahena Basave, J.A. Bañuelos Alamillo, M. Bénard, R.R. Calderón Mandujano, E. Centenero Alcalá, M.A. de la Torre Loranca, J. Domínguez Laso, H. Franz, J.M. González Villa, C.I. Grünwald, J.M. Jones, B. LaForest, F. Lara Hernández, J. Loc Barragán, F. Martínez Belmar, C. Mayoral Halla, J.A. Ortiz Medina, J.G. Paz Gutiérrez, G.E. Quintero Díaz, R. Ramírez Chaparro, E.A. Rivas Mercado, L.A. Santillán Moreno, A.E. Valdenegro Brito, and C.E. Vega Flores, who kindly donated tissues samples, photographs, or provided information. We thank D.A. May Uicab for the illustrations of the head of the rattlesnakes. We are indebted to T.R. Rainwater for the English review. We thank to the anonymous reviewers that made a number of suggestions that helped improve the manuscript. We thank the following institutions for allowing us to examine specimens or providing us with digital photographs of preserved specimens under their care: D. Lazcano (UANL), O. Flores Villela and E. Pérez Ramos (MZFC), J. Rosado and J. Martinez (MCZ), B. Hollingsworth (SDNHM), S. Rogers and J. Sheridan (CM); J.A. Campbell and C.J. Franklin (UTA), C.S. Lieb and V. Zhuang (UTEP), M. Borja (UJED-CHFCB) and M.R. Barragán Vázquez and M.A. Torres Pérez (UJAT-CART). Also to Unidad de Manejo Ambiental Barranca Honda (SEMARNAT-UMA-INT-028-MOR); Unidad de Manejo Ambiental OCTOLAB (SEMARNAT-UMA-IN-CR-0086-VER/08); Predio o Instalación de Manejo de Fauna Silvestre Palancoatl (SEMARNAT-PIMVS-CR-IN-0013-VER/13). Collecting permits were provided by Dirección General de Vida Silvestre SEMARNAT (SGPA/DGVS/02570/15, SGPA/DGVS700950/16).

References

Acevedo, M., Johnson, J. & Ariano-Sánchez, D. (2014) *Crotalus tzabcan*. *The IUCN Red List of Threatened Species*, 2014, e.T197478A2488339.
https://doi.org/10.2305/IUCN.UK.2014-1.RLTS.T197478A2488339.en

Alencar, L.R.V., Quental, T.B., Grazziotin, F.G., Alfaro, M.L., Martins, M., Venzon, M. & Zaher, H. (2016) Diversification in vipers: phylogenetic relationships, time of divergence and shifts in speciation rates. *Molecular Phylogenetics and Evolution*, 105, 50−62.
https://doi.org/10.1016/j.ympev.2016.07.029

Almeida-Santos, S.M., Abdalla, F.M.F., Silveira, P.F., Yamanouye, N.Y., Breno, M.C. & Salomão, M.G. (2004a) Reproductive cycle of the Neotropical *Crotalus durissus terrificus*: I. Seasonal levels and interplay between steroid hormones and vasotocnase. *General and Comparative Endocrinology*, 139, 143–150.
https://doi.org/10.1016/j.ygcen.2004.09.001

Almeida-Santos, S.M., Laporta-Ferreira, I.L., Antoniazzi, M.M. & Jared, C. (2004b) Sperm storage in males of the snake *Crotalus durissus terrificus* (Crotalinae: Viperidae) in southeastern Brazil. *Comparative Biochemistry and Physiology*, Part A, 139, 169–174.
https://doi.org/10.1016/j.cbpb.2004.08.004

Anderson, C.G. & Greenbaum, E. (2012) Phylogeography of northern populations of the black-tailed rattlesnake (*Crotalus molossus* Baird and Girard, 1853), with the revalidation of *C. ornatus* Hallowell, 1854. *Herpetological Monographs*, 26, 19−57.
https://doi.org/10.1655/HERPMONOGRAPHS-D-11-00012.1

Akaike, H. (1973) Information theory and an extension of the maximum likelihood principle. *In*: Petrov, B.N. & Csaki, F. (Eds.), *Second International Symposium on Information Theory.* Akadémiai Kiadó, Budapest, pp. 267–281.

Arévalo, E., Davis, S.K. & Sites, J.W.Jr. (1994) Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. *Systematic Biology*, 43, 387–418.
https://doi.org/10.1093/sysbio/43.3.387

Armstrong, B.L. & Murphy, J.B. (1979) The natural history of Mexican rattlesnakes. *University of Kansas Museum of Natural History Publications*, 5, 1–88.

Ashton, K.G. & de Queiroz, A. (2001) Molecular systematics of the western rattlesnake, *Crotalus viridis* (Viperidae), with comments on the utility of the D-loop in phylogenetic studies of snakes. *Molecular Phylogenetics and Evolution*, 21, 176–189.
https://doi.org/10.1006/mpev.2001.1013

Baird, S.F. & Girard, C. (1853) *Catalogue of North American Reptiles in the Museum of the Smithsonian Institution*. *Part 1*. Serpentes. Smithsonian Institution, Washington, 172 pp.
https://doi.org/10.5962/bhl.title.5513

Bandelt, H.J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16, 37–48.

Barros, V.A., Sueiro, L.R. & Almeida-Santos, S.M. (2012) Reproductive biology of the neotropical rattlesnake *Crotalus durissus* from northeastern Brazil: a test of phylogenetic conservatism of reproductive patterns. *Herpetological Journal*, 22, 97–104.

Blair, C. & Sánchez-Ramírez, S. (2016) Diversity-dependent cladogenesis throughout western Mexico: evolutionary biogeography of rattlesnakes (Viperidae: Crotalinae: *Crotalus* and *Sistrurus*). *Molecular Phylogenetics and Evolution*, 97,145–154.
https://doi.org/10.1016/j.ympev.2015.12.020

Bryson, R.W., Nieto-Montes de Oca, A. & Reyes-Velasco, J. (2008) Phylogenetic position of *Porthidium hespere* (Viperidae: Crotalinae) and phylogeography of arid-adapted hognosed pitvipers based on mitochondrial DNA. *Copeia*, 2008 (1), 172–178.
https://doi.org/10.1643/CH-07-043

Bryson, R.W., Linkem, C.W., Dorcas, M.E., Lathrop, A., Jones, J.M., Alvarado-Díaz, J., Grünwald, C.I. & Murphy, R.W. (2014) Multilocus species delimitation in the *Crotalus triseriatus* species group (Serpentes: Viperidae: Crotalinae), with the description of two new species. *Zootaxa*, 3826 (3), 475–496.
https://doi.org/10.11646/zootaxa.3826.3.3

Calvete, J.J., Sanz, L., Cid, P., de la Torre, P., Flores-Díaz, M., Dos Santos, M.C., Borges, A., Bremo, A., Angulo, Y., Lomonte, B., Alape-Girón, A. & Gutiérrez, J.M. (2010) Snake venomics of the Central American rattlesnake *Crotalus simus* and the South American *Crotalus durissus* complex points to neurotoxicity as an adaptive paedomorphic trend along *Crotalus* dispersal in South America. *Journal of Proteome Research*, 9, 528–544.
https://doi.org/10.1021/pr9008749

Campbell, J.A. (1998) *Amphibians and Reptiles of Northern Guatemala, Yucatán and Belize*. University of Oklahoma Press, Norman, 380 pp.

Campbell, J.A. & Lamar, W.W. (2004) *The Venomous Reptiles of the Western Hemisphere*. *Vols. 1–2*. Cornell University Press, Ithaca and London, 870 pp.

Cope, E.D. (1864) Contributions to the herpetology of tropical America. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 16,166–181.

Castro, E.N., Lomonte, B., del Carmen Gutiérrez, M., Alagón, A. & Gutiérrez, J.M. (2013) Intraspecies variation in the venom of the rattlesnake *Crotalus simus* from Mexico: different expression of crotoxin results in highly variable toxicity in the venoms of three subspecies. *Journal of Proteomics*, 87, 103–121.

https://doi.org/10.1016/j.jprot.2013.05.024.
Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) JModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9, 772.
https://doi.org/10.1038/nmeth.2109
de Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology*, 56, 879–886.
https://doi.org/10.1080/10635150701701083
Doan, T.M., Mason, A.J., Castoe, T., Sasa, M. & Parkinson, C.L. (2016) A cryptic palm-pitviper species (Squamata: Viperidae: *Bothriechis*) from the Costa Rican highlands, with notes on the variation within *B. nigroviridis*. *Zootaxa*, 4138 (2), 271–290.
httpS://doi.org/10.11646/zootaxa.4138.2.3
Durban, J., Sanz, L., Trevisan-Silva, D., Neri-Castro, E., Alagón, A. & Calvete, J.J. (2017) Integrated venomics and venom gland transcriptome analysis of juvenile and adult Mexican rattlesnakes *Crotalus simus*, *C. tzabcan*, and *C. culminatus* revealed miRNAmodulated ontogenetic shifts. *Journal of Proteome Research*, 16, 3370–3390.
https://doi.org/10.1021/acs.jproteome.7b00414
Dwyer, Q., Lamar, W., Porras, L.W., Solórzano, A., Sunyer, J. & Chaves, G. (2014) *Crotalus simus*. *The IUCN Red List of Threatened Species*, 2014, e.T197480A2488426.
https://doi.org/10.2305/IUCN.UK.2014-1.RLTS.T197480A2488426.en
Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797.
https://doi.org/10.1093/nar/gkh340
Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783–791.
https://doi.org/10.1111/j.1558-5646.1985.tb00420.x
Fetzner, Jr.J.W. (1999) Extracting high-quality DNA from shed reptile skins: a simplified method. *BioTechniques*, 26, 1052–1054.
https://doi.org/10.2144/99266bm09
Gloyd, H.K. (1936) A mexican subspecies of *Crotalus molossus* Baird and Girard. *Occasional Papers of the Museum of Zoology, University of Michigan*, 325, 1–5.
Gloyd, H.K. (1948) Description of a neglected subspecies of rattlesnake from Mexico. *Natural History Miscellanea*, 17, 1–4.
Gloyd, H.K. & Kauffeld, C.F. (1940) A new rattlesnake from Mexico. *Bulletin of the Chicago Academy of Sciences*, 6, 11–14.
Gutiérrez, J.M., dos Santos, M.C., Furtado, M.F. & Rojas, G. (1991) Biochemical and pharmacological similarities between the venoms of newborn *Crotalus durissus durissus* and adult *Crotalus durissus terrificus* rattlesnakes. *Toxicon*, 29, 1273–1277.
https://doi.org/10.1016/0041-0101(91)90201-2
Hallowell, E. (1854) Notices of new reptiles from Texas. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 1854, 192–193.
Hammer, Ø., Harper, D.A.T. & Ryan, P.D. (2001) PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4, 1–9.
Harris, H.S. & Simmons, R.S. (1978) A preliminary account of the rattlesnakes with the descriptions of four new subspecies. *Bulletin of the Maryland Herpetological Society*, 14, 105–211. [1977]
Hart, M.W. (2010) The species concept as an emergent property of population biology. *Evolution*, 65, 613–616.
https://doi.org/10.1111/j.1558-5646.2010.01202.x
Heimes, P. (2016) *Snakes of Mexico. Herpetofauna Mexicana. Vol. I*. Chimaira, Frankfurt, 572 pp.
Hoge, A.R. "1965" (1966) Preliminary account on Neotropical Crotalinae (Serpentes: Viperidae). *Memorias do Instituto Butantan*, 32, 109–184
Humboldt, A. de (1813) Sur deux nouvelles espèces de Crotales. *In*: Humboldt, A.& Bonpland, A. (Eds.), *Recueil d'observations de Zoologie et d'anatomie Compafie Faitesdans I'Octan Atlantique, dans I'interieur du Nouveau Continent et dans la Mer du Sud Pendant les années 1799,1800,1801,1802 et 1803*. *Vol. 2*. F. Schoell and G. Dufour, Paris, pp. 1–8.
Klauber, L.M. (1941) A species of rattlesnake from Venezuela. *Transactions of the San Diego Society of Natural History*, 30, 333−336.
Klauber, L.M. (1949) Some new and revived subspecies of rattlesnakes. *Transactions of the San Diego Society of Natural History*, 11 (6), 61−116.
https://doi.org/10.5962/bhl.part.28857
Klauber, L.M. (1952) Taxonomic studies on rattlesnakes of mainland Mexico. *Bulletins of the Zoological Society of San Diego*, 26, 1−143.
https://doi.org/10.5962/bhl.title.130475
Klauber, L.M. (1956) *Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind*. *Vols. 1–2*. University of California Press, Berkeley, 1476 pp.
Kubatko, L.S., Gibbs, H.L. & Bloomquist, E.W. (2011) Inferring species-level phylogenies and taxonomic distinctiveness using multilocus data in *Sistrurus* rattlesnakes. *Systematic Biology*, 60 (4), 393–409.
https://doi.org/10.1093/sysbio/syr011
Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets.

Molecular Biology and Evolution, 33, 1870–1874.
https://doi.org/10.1093/molbev/msw054
Kumazawa, Y., Ota, H., Nishida, M. & Ozawa, T. (1998) The complete nucleotide sequence of a snake (*Dinodon semicarinatus*) mitochondrial genome with two identical control regions. *Genetics*, 150, 313–329.
Laurenti, J.N. (1768) *Specimen Medicum, Exhibens Synopsin Reptilium Emendatam cum Experimentis circa Venena et Antidota Reptilium Austracorum, quod Authoritate et Consensu*. Joan, Thomae, Vienna, 217 pp.
https://doi.org/10.5962/bhl.title.5108
Lawson, R., Slowinski, J.B., Crother, B.I. & Burbrink, F.T. (2005) Phylogeny of the Colubroidea (Serpentes): new evidence from mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution*, 37, 581–601.
https://doi.org/10.1016/j.ympev.2005.07.016
Lee, J.C. (1996) *The Amphibians and Reptiles of the Yucatán Peninsula*. Comstock Publishing Associates, Cornell University Press, Ithaca, 500 pp.
Linnaeus, C. (1758) *Systema Naturæ per Regna tria Naturæ, Secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis*. *Tomus I. 10th Edition*. Laurentii Salvii, Holmiæ, 824 pp.
https://doi.org/10.5962/bhl.title.542
Maddison, W.P. & Maddison, D.R. (2017) Mesquite: a modular system for evolutionary analysis. Version 3.31. Available from: http://mesquiteproject.org (accessed 13 April 2019)
Martins, M. & Lamar, W.W. (2010) *Crotalus durissus*. *The IUCN Red List of Threatened Species*, 2010, e.T178477A7554740.
https://doi.org/10.2305/IUCN.UK.2010-4.RLTS.T178477A7554740.en
McCranie, J.R. (1993) *Crotalus durissus* Linnaeus, Neotropical rattlesnake. *Catalogue of American Amphibians and Reptiles*, 577, 1−11.
McCranie, J.R. (1984) *Crotalus vegrandis* Klauber, Uracoan rattlesnake. *Catalogue of American Amphibians and Reptiles*, 550, 1−2.
Meik, J.M., Schaack, S., Flores-Villela, O. & Streicher, J.W. (2018) Integrative taxonomy at the nexus of population divergence and speciation in insular speckled rattlesnakes. *Journal of Natural History*, 52, 898−1016.
https://doi.org/10.1080/00222933.2018.1429689
Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans*, 2010, 1−8.
https://doi.org/10.1109/GCE.2010.5676129
Murphy, R.W., Fu, J., Lathrop, A., Feltham, J.V. & Kovak, V. (2002) Phylogeny of the rattlesnakes (*Crotalus* and *Sistrurus*) inferred from sequences of five mitochondrial DNA genes. *In*: Schuett, G.W., Hôggren, M., Douglas, M.E. & Greene, H.W. (Eds.), *Biology of the Vipers*. Eagle Mountain Publishing, Eagle Mountain, Utah, pp. 69–92.
Neri-Castro, E., Hernández-Dávila, A., Olvera-Rodríguez, A., Cardoso-Torres, H., Bénard-Valle, M., Bastiaans, E., López-Gutierrez, O. & Alagón, A. (2019) Detection and quantification of a β-neurotoxin (crotoxin homologs) in the venom of the rattlesnakes *Crotalus simus*, *C. culminatus* and *C. tzabcan* from Mexico. *Toxicon*, X (2), 100007.
https://doi.org/10.1016/j.toxcx.2019.100007
Padial, J.M., Miralles, A., De la Riva, I. & Vences, M. (2010) The integrative future of taxonomy. *Frontiers in Zoology*, 7, 16.
https://doi.org/10.1186/1742-9994-7-16
Pavón-Vázquez, C.J., García-Vázquez, U.O., Bryson, R.W., Feria-Ortiz, M., Manríquez-Morán, N.L. & Nieto-Montes de Oca, A. (2018) Integrative species delimitation in practice: revealing cryptic lineages within the short-nosed skink *Plestiodon brevirostris* (Squamata: Scincidae). *Molecular Phylogenetics and Evolution*, 129, 242–257.
https://doi.org/10.1016/j.ympev.2018.08.020
Polzin, T. & Daneshmand, S.V. (2003) On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters*, 31, 12–20.
https://doi.org/10.1016/S0167-6377(02)00185-2
Pook, C.E., Wüster, W. & Thorpe, R.S. (2000) Historical biogeography of the western rattlesnake (Serpentes: Viperidae: *Crotalus viridis*), inferred from mitochondrial DNA sequence information. *Molecular Phylogenetics and Evolution*, 15, 269–282.
https://doi.org/10.1006/mpev.1999.0756
Porras, L., Wilson, L.D., Schuett, G.W. & Riserer, S. (2013) A taxonomic reevaluation and conservation assessment of the common cantil, *Agkistrodon bilineatus* (Squamata: Viperidae): a race against time. *Amphibian & Reptile Conservation*, 7 (1), 48–73.
Rambaut, A., Suchard, M. & Drummond, A.J. (2018) Tracer. Version 1.6. Available from: http://tree.bio.ed.ac.uk/software/tracer/ (accessed 15 July 2018)
Reyes-Velasco, J., Meik, J.M., Smith, E.N. & Castoe, T.A. (2013) Phylogenetic relationships of the enigmatic longtailed rattlesnakes (*Crotalus ericsmithi*, *C. lannomi*, and *C. stejnegeri*). *Molecular Phylogenetics and Evolution*, 69, 524–534.
https://doi.org/10.1016/j.ympev.2013.07.025
Rivas, G.A., Molina, C.R., Ugueto, G.N., Barros, T.R., Barrio-Amorós, C.L. & Kok, P.J.R. (2012) Reptiles of Venezuela: an updated and commented checklist. *Zootaxa*, 3211 (1), 1–64.
https://doi.org/10.11646/zootaxa.3211.1.1
Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.

https://doi.org/10.1093/bioinformatics/btg180
Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E. & Sánchez-Gracia. A. (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution*, 34, 3299–3302.
https://doi.org/10.1093/molbev/msx248
Sabaj, M.H. (Ed.) (2016) *Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference. Version 6.5*. American Society of Ichthyologists and Herpetologists, Washington, D.C. Available from: http://www.asih.org/ (accessed 13 April 2019)
Savage, J.M., Campbell, J.A. & Lamar, W.W. (2005) On names for neotropical rattlesnakes. *Herpetological Review*, 36, 369–371.
Smith, H.M. & Taylor, E.H. (1950) Type localities of Mexican reptiles and amphibians. *The University of Kansas science bulletin*, 33, 313–380.
Sonnini, M.C.S. & Latreille, P.A. (1801) *Histoire Naturelle des Reptiles, avec Figures Dessinees d'Apres Nature*. *3*. Librairie encyclopédique de Roret, Paris, 332 pp.
https://doi.org/10.5962/bhl.title.4688
Stamatakis, A. (2014) RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313.
https://doi.org/10.1093/bioinformatics/btu033
Uetz, P., Freed, P. & Hošek, J. (2019) The Reptile Database. Available from: http://www.reptile-database.org (accessed 21 October 2019)
Van Lidth de Jeude, Th.W. (1887) On a collection of reptiles and fishes from the West-Indies. *Notes from the Leyden Museum*, 9, 129–139.
Vondrák, J., Pavel, Ř., Ulf, A. & Søchting, U. (2009) The taxonomy of the *Caloplaca citrina* group (Teloschistaceae) in the Black Sea region; with contributions to the cryptic species concept in lichenology. *The Lichenologist*, 41, 571–604.
https://doi.org/10.1017/S0024282909008317
Wiens, J.J., Kuczynski, C.A. & Stephens, P.R. (2010) Discordant mitochondrial and nuclear gene phylogenies in emydid turtles: implications for speciation and conservation. *Biological Journal of the Linnean Society*, 99, 445–461.
https://doi.org/10.1111/j.1095-8312.2009.01342.x
Wüster, W., Ferguson, J.E., Quijada-Mascareñas, J.A., Pook, C.E., Graca-Salomao, M.D. & Thorpe, R.S. (2005) Tracing an invasion: landbridges, refugia, and phylogeography of the neotropical rattlesnake (Serpentes: Viperidae; *Crotalus durissus*). *Molecular Ecology*, 14, 1095–1108.
https://doi.org/10.1111/j.1365-294X.2005.02471.x
Zarza, E., Reynoso, V.H., Faria, C.M.A. & Emerson, B.C. (2019) Introgressive hybridization in a Spiny-Tailed Iguana, *Ctenosaura pectinata*, and its implications for taxonomy and conservation. *PeerJ*, 7, e6744.
https://doi.org/10.7717/peerj.6744

APPENDIX 1. Taxon, locality, and haplotype details. Name of species follows Campbell & Lamar (2004), Anderson & Greenbaum (2012), and our proposed taxonomy. Our specimens without museum vouchers are either tissues only from field specimens or in private collections. Sequences obtained in this study in bold. Museum collection acronyms follow Sabaj Pérez (2016) except for: CGA=C.G. Anderson field series; CLP=C.L. Parkinson field series; FHGO=Fundación Herpetológica Gustavo Orcés, Quito, Ecuador; IBt=Instituto de Biotecnología, UNAM; IMRS=UTEP Indio Mountains Research Station; JAC=J.A. Campbell field series; Moody=S. M. Moody field series; SBS-UWB, School of Biological Sciences, University of Wales, Bangor, UK; UJED-CHFCB=Colección Herpetologica, Facultad de Biología, Universidad Juárez del Estado de Durango; UMA-Palancoatl=Unidad de Manejo Ambiental Palancoatl.

Taxon	Locality	Voucher/ simple number	Haplotype	GenBank Accesion			
				cyt *b*	ND4	ND2	*c mos*
Crotalus culminatus	Alpuyeca, Morelos, Mexico	IBt133	15	**MN067337**	**MN067376**	**MN067413**	**MN072553**
Crotalus culminatus	3.8 Km SE Las Trojitas, Michoacán, Mexico	Wild specimen	20	**MN067338**	**MN067377**	–	**MN072554**
Crotalus ehecatl	12 Km E Chiapa de Corzo, Chiapas, Mexico	ECO-CH-H-3776	47	**MN067341**	**MN067380**	–	**MN072557**
Crotalus ehecatl	Las Margaritas, Chiapas, Mexico-1	ECO-CH-H-3777	53	**MN067345**	**MN067384**	**MN067417**	**MN072561**
Crotalus ehecatl	Las Margaritas, Chiapas, Mexico-2	ECO-CH-H-3778	54	**MN067346**	**MN067385**	–	**MN072562**
Crotalus ehecatl	Francisco Sarabia, Chiapas, Mexico	IBt084	50	**MN067344**	**MN067383**	**MN067416**	**MN072560**
Crotalus ehecatl	Copainala, Chiapas, Mexico	IBt065	48	**MN067342**	**MN067381**	**MN067414**	**MN072558**
Crotalus ehecatl	San Pedro Tepanatepec, Oaxaca, Mexico	UJED-CHFCB-0380	61	**MN067352**	**MN067391**	–	**MN072568**
Crotalus ehecatl	Santo Domingo Zanatepec, Oaxaca, Mexico	UJED-CHFCB-0379	61	**MN067351**	**MN067390**	**MN067418**	**MN072567**
Crotalus simus	25 Km S Tapachula, Chiapas, Mexico-1	Wild specimen	62	**MN067353**	**MN067392**	–	**MN072569**
Crotalus simus	25 Km S Tapachula, Chiapas, Mexico-2	Wild specimen	52	**MN067354**	**MN067393**	–	**MN072570**
Crotalus simus	25 Km S Tapachula, Chiapas, Mexico-3	Wild specimen	63	**MN067355**	**MN067394**	–	**MN072571**
Crotalus mictlantecuhtli	4.2 Km SE Mata Naranjo, Veracruz, Mexico	IBt226	56	**MN067348**	**MN067387**	–	**MN072564**
Crotalus mictlantecuhtli	Rinconada, Veracruz, Mexico-1	IBt233	58	**MN067349**	**MN067388**	–	**MN072565**
Crotalus mictlantecuhtli	Rinconada, Veracruz, Mexico-2	IBt231	59	**MN067350**	**MN067389**	–	**MN072566**
Crotalus mictlantecuhtli	Actopan, Veracruz, Mexico	IBt232	45	**MN067340**	**MN067379**	–	**MN072556**
Crotalus mictlantecuhtli	El Zapote, Veracruz, Mexico	UMA Palancoatl 46	49	**MN067343**	**MN067382**	**MN067415**	**MN072559**
Crotalus mictlantecuhtli	La Tinaja, Veracruz, Mexico	UMA Palancoatl 50	55	**MN067347**	**MN067386**	–	**MN072563**
Crotalus totonacus	Santa Ana, Nuevo León, Mexico-1	Wild specimen	80	**MN067362**	**MN067401**	–	**MN072578**
Crotalus totonacus	Santa Ana, Nuevo León, Mexico-2	Wild specimen	81	**MN067363**	**MN067402**	–	**MN072579**
Crotalus totonacus	Cerro de la Silla, Nuevo León, Mexico	Wild specimen	77	**MN067358**	**MN067397**	–	**MN072574**
Crotalus totonacus	Santa Ana, Nuevo León, Mexico-4	Wild specimen	81	**MN067364**	**MN067403**	–	**MN072580**
Crotalus totonacus	Ahuacales, Tamaulipas, Mexico	Wild specimen	75	**MN067356**	**MN067395**	**MN067419**	**MN072572**
Crotalus totonacus	Joya, Verrde, Tamaulipas-1	Wild specimen	75	**MN067359**	**MN067398**	–	**MN072575**
Crotalus totonacus	Joya, Verrde, Tamaulipas-2	Wild specimen	78	**MN067360**	**MN067399**	–	**MN072576**

.....continued on the next page

APPENDIX 1. (Continued)

Taxon	Locality	Voucher/ simple number	Haplotype	GenBank Accesion			
				cyt *b*	ND4	ND2	*c mos*
Crotalus totonacus	Joya, Verrde, Tamaulipas-3	Wild specimen	79	**MN067361**	**MN067400**	–	**MN072577**
Crotalus totonacus	Los Alamitos, San Luis Potosí, Mexico	SDSNH_HerpPC_ 05358	76	**MN067357**	**MN067396**	–	**MN072573**
Crotalus tzabcan	Muchucuxcáh, Yucatán, Mexico	ECO-CH-H-3771	87	**MN067369**	**MN067408**	**MN067422**	–
Crotalus tzabcan	Tibolón, Yucatán, Mexico	ECO-CH-H-3772	89	**MN067371**	**MN067410**	–	–
Crotalus tzabcan	Calderitas, Quintana Roo, Mexico	ECO-CH-H-3779	84	**MN067366**	**MN067405**	**MN067420**	**MN072582**
Crotalus tzabcan	Javier Rojo Gómez, Quintana Roo, Mexico	ECO-CH-H-3803	86	**MN067368**	**MN067407**	–	**MN072584**
Crotalus tzabcan	Conkal, Yucatán, Mexico	IBt254	85	**MN067367**	**MN067406**	**MN067421**	**MN072583**
Crotalus tzabcan	Puerto Morelos, Quintana Roo, Mexico	IBt214	88	**MN067370**	**MN067409**	–	**MN072585**
Crotalus tzabcan	Akumal, Quintana Roo, Mexico	IBt205	83	**MN067365**	**MN067404**	–	**MN072581**
Crotalus tzabcan	Zoh Laguna, Campeche, Mexico	IBt265	91	**MN067372**	**MN067411**	–	**MN072586**
Crotalus basiliscus	Sierra de Quila, Jalisco, Mexico	Wild specimen	3	**MN067336**	**MN067375**	**MN067412**	**MN072552**
Crotalus basiliscus	Playa Hinchahuevos, Sinaloa, Mexico	Wild specimen	4	**MN067334**	**MN067373**	–	**MN072550**
Crotalus basiliscus	Ixtlahuacán, Colima, Mexico	Wild specimen	5	**MN067335**	**MN067374**	–	**MN072551**
Crotalus ornatus	Aldama,Chihuahua, Mexico	Wild specimen	37	**MN067339**	**MN067378**	–	**MN072555**
Crotalus basiliscus	Guadalajara, Jalisco, Mexico	Zoo Gudalajara, live collection	3	AY704845	AY704895	AY704796	–
Crotalus basiliscus	San Blas, Nayarit, Mexico	822	6	AY704844	AY704894	–	–
Crotalus d. cascavella	Belém do São Francisco, Pernambuco, Brazil	IB live coll. 4112	7	AY704819	AY704869	AY704775	–
Crotalus d. cascavella	Grajau, Maranhão, Brazil-1	IB 56419	8	AY704816	AY704866	–	–
Crotalus d. cascavella	Grajau, Maranhão, Brazil-2	IB 56420	8	AY704817	AY704867	AY704773	–
Crotalus d. cascavella	Grajau, Maranhão, Brazil-3	IB 56421	8	AY704818	AY704868	AY704774	–
Crotalus d. cascavella	Guanambi, Bahia, Brazil	IB live coll. 4127	9	AY704804	AY704854	AY704776	–
Crotalus d. cascavella	São Desidério, Bahia, Brazil	IB live coll. 4872	9	AY704804	AY704854	AY704772	–
Crotalus d. collilineatus	Alto Boa Vista, Mato Grosso, Brazil-1	IB 58460	10	AY704811	AY704861	AY704767	–
Crotalus d. collilineatus	Alto Boa Vista, Mato Grosso, Brazil-2	IB 58466	10	AY704812	AY704862	AY704768	–
Crotalus d. collilineatus	Brasília, Distrito Federal, Brazil	IB live coll. 4826	11	AY704815	AY704865	AY704771	–
Crotalus d. collilineatus	Pontes e Lacerda, Mato Grosso, Brazil	IB live coll. 4610	12	AY704813	AY704863	AY704769	–
Crotalus d. collilineatus	Porto Primavera, São Paulo, Brazil-1	Unknown	13	AY196652	AY196631	–	–
Crotalus d. collilineatus	Porto Primavera, São Paulo, Brazil-2	Unknown	13	AY196651	AY196630	–	–

.....continued on the next page

APPENDIX 1. (Continued)

Taxon	Locality	Voucher/ simple number	Haplotype	GenBank Accesion			
				cyt *b*	ND4	ND2	*c mos*
Crotalus d. collilineatus	Porto Primavera, São Paulo, Brazil-3	Unknown	13	AY196650,	AY196629	–	–
Crotalus d. collilineatus	Ribeirão Cascalheira, Mato Grosso, Brazil	IB live coll. 4826	14	AY704814	AY704864	AY704770	–
Crotalus culminatus	El Aguacate, Puebla,Mexico	4	16	AY704828	AY704878	–	–
Crotalus culminatus	Mexico	ZSL, live collection	18	AY704827	AY704877	–	–
Crotalus culminatus	Morelos, Mexico	3291	17	AY704830	AY704880	AY704785	–
Crotalus culminatus	Sierra de Puebla, Mexico	RA	19	AY704829	AY704879	–	–
Crotalus d. cumanensis	Distrito Colina, Falcón, Venezuela	781	21	AY704820	AY704870	AY704778	–
Crotalus d. cumanensis	Curimagua, Falcón, Venezuela	788	22	AY704821	AY704871	AY704779	–
Crotalus d. cumanensis	La Guaira, Distrito Federal, Venezuela	775	23	AY704825	AY704875	AY704783	–
Crotalus d. cumanensis	Las Ventosas, Falcón, Venezuela-1	789	24	AY704822	AY704872	AY704780	–
Crotalus d. cumanensis	Las Ventosas, Falcón, Venezuela-2	790	25	AY704823	AY704873	AY704781	–
Crotalus d. durissus	Guyana	1092—SBS-UWB live collection	26	AY704826	AY704876	AY704784	–
Crotalus m. molossus	Payson, Arizona, United States of America	C134	29	AY704847	AY704897	AY704797	–
Crotalus m. molossus	Sonoyta, Sonora, Mexico	CMSON	30	AY704846	AY704896	–	–
Crotalus m. nigrescens	Cadereita, Querétaro, Mexico	1566	31	AY704841	AY704891	–	–
Crotalus m. nigrescens	El Pedregal, Estado de México, Mexico-1	CMN 4	33	AY704849	AY704899	–	–
Crotalus m. nigrescens	El Pedregal, Estado de México, Mexico-2	CMN 3	34	AY704840	AY704890	–	–
Crotalus m. nigrescens	San Luis Potosí, Mexico	2643	35	AY704842	AY704892	–	–
Crotalus m. oaxacus	Zapotitlán Salinas, Puebla, Mexico	3014	36	AY704843	AY704893	–	–
Crotalus ornatus	El Paso, Texas, United States of America	CLP66	38	AY223607	AY223645	–	–
Crotalus ornatus	Uvas Mountains, Nuevo Mexico, United States of America	C135	43	AY704848	AY704898	AY704798	–
Crotalus d. ruruima	Boa Vista, Roraima, Brazil	Unknown	44	AY196656	AY255083	–	–
Crotalus mictlantecuhtli	Catemaco, Veracruz, Mexico	1	46	AY704831	AY704881	AY704786	–
Crotalus simus	Estación Ecológica Selva Lacandona, Chiapas, Mexico-1	2065	51	AY704833	AY704883	AY704787	–
Crotalus simus	Estación Ecológica Selva Lacandona, Chiapas, Mexico-2	2067	52	AY704834	AY704884	AY704788	–
Crotalus simus	Bahía de Pájaros, Puntarenas, Costa Rica	1097	57	AY704835	AY704885	AY704790	–

.....continued on the next page

APPENDIX 1. (Continued)

Taxon	Locality	Voucher/ simple number	Haplotype	GenBank Accesion			
				cyt *b*	ND4	ND2	*c mos*
Crotalus simus	San Salvador, El Salvador	Sal 1, Sal 3, Sal 6	60	AY704803	AY704853	AY704789	–
Crotalus mictlantecuhtli	Los Tuxtlas, Veracruz, Mexico	CDD	64	AY704832	AY704882	–	–
Crotalus d. terrificus	Arapoti, Paraná, Brazil-1	907	65	AY704809	AY704859	AY704765	–
Crotalus d. terrificus	Arapoti, Paraná, Brazil-2	908	66	AY704810	AY704860	AY704766	–
Crotalus d. terrificus	Campo Mourão, Paraná, Brazil-1	Unknown	67	AY196645	AY196621	–	–
Crotalus d. terrificus	Campo Mourão, Paraná, Brazil-2	Unknown	67	AY196644	AY196620	–	–
Crotalus d. terrificus	Colômbia, São Paulo, Brazil	IB 55553	67	AY704801	AY704851	–	–
Crotalus d. terrificus	Guaratinguetá, Vale do Paraiba, São Paulo, Brazil	IB 555521	68	AY704807	AY704857	AY704763	–
Crotalus d. terrificus	Itu, São Paulo, Brazil-1	Unknown	67	AY196648	AY196626	–	–
Crotalus d. terrificus	Itu, São Paulo, Brazil-2	Unknown	67	AY196649	AY196628	–	–
Crotalus d. terrificus	Lorena, Vale do Paraiba, São Paulo, Brazil-1	Unknown	69	AY196655	AY196634	–	–
Crotalus d. terrificus	Lorena, Vale do Paraiba, São Paulo, Brazil-2	Unknown	68	AY196654	AY196633	–	–
Crotalus d. terrificus	Lorena, Vale do Paraiba, São Paulo, Brazil-3	Unknown	68	AY196653	AY196632	–	–
Crotalus d. terrificus	Machado, Minas Gerais, Brazil-1	Unknown	70	AY196642	AY196615	–	–
Crotalus d. terrificus	Machado, Minas Gerais, Brazil-2	Unknown	70	AY196641	AY196614	–	–
Crotalus d. terrificus	Machado, Minas Gerais, Brazil-3	Unknown	70	AY196640	AY196613	–	–
Crotalus d. terrificus	Paraná State, Brazil	Unknown	71	AY196632	AY196611	–	–
Crotalus d. terrificus	Paraná State, Brazil	Unknown	72	AY196639	AY196612	–	–
Crotalus d. terrificus	Paraná State, Brazil	Unknown	72	AY196637	AY196610	–	–
Crotalus d. terrificus	Paraná State, Brazil	Unknown	67	AY196636	AY196609	–	–
Crotalus d. terrificus	Paraná State, Brazil	Unknown	67	AY196635	AY196608	–	–
Crotalus d. terrificus	Pindamonhangaba, Vale do Paraiba, São Paulo, Brazil	IB 55600	67	AY704802	AY704852	–	–
Crotalus d. terrificus	Pindamonhangaba, Vale do Paraiba, São Paulo, Brazil	IB 55601	67	AY704801	AY704851	AY704762	–
Crotalus d. terrificus	Patrocínio Paulista, São Paulo, Brazil-1	Unknown	70	AY196647	AY196624	–	–
Crotalus d. terrificus	Patrocínio Paulista, São Paulo, Brazil-2	Unknown	70	AY196646	AY196623	–	–
Crotalus d. terrificus	Roseira, São Paulo, Brazil	IB 55522	72	AY704808	AY704858	–	–
Crotalus d. terrificus	São Luiz do Paraitinga, Vale do Paraiba, São Paulo, Brazil	IB 55552	69	AY704800	AY704850	AY704764	–

.....continued on the next page

APPENDIX 1. (Continued)

Taxon	Locality	Voucher/ simple number	Haplotype	GenBank Accesion			
				cyt *b*	ND4	ND2	*c mos*
Crotalus totonacus	30 Km SW Ciudad Victoria, Tamaulipas, Mexico	SA	74	AY704839	AY704889	–	–
Crotalus totonacus	Tomajo, Querétaro, Mexico	3102	82	AY704838	AY704888	AY704795	–
Crotalus totonacus	Tamaulipas, Mexico	SD	75	AY704837	AY704887	AY704794	–
Crotalus tzabcan	Xaibe, Corozal, Belize-1	255—Peter Singfield, live collection	86	AY704806	AY704856	AY704791	–
Crotalus tzabcan	Xaibe, Corozal, Belize-2	258—Peter Singfield, live collection	86	AY704806	AY704856	AY704792	–
Crotalus tzabcan	Xaibe, Corozal, Belize-3	259—Peter Singfield, live collection	90	AY704836	AY704886	AY704793	–
Crotalus d. unicolor	Aruba, Netherlands Antilles	211, 212—ZSL, live collection	92	AY704805	AY704855	AY704777	–
Crotalus d. vegrandis	Venezuela	833—ZSL, live collection	93	AY704824	AY704874	AY704782	–
Agkistrodon contortrix	Athens Co., Ohio, United States of America	Moody 338	1	AY223612	AF156576	–	–
Agkistrodon piscivorus	South Carolina, United States of America	CLP 30	2	AY223610	AF156578	–	–
Sistrurus catenatus	Haskel Co., Texas, United States of America	Moody 502	96	AY223610	AY223648	–	–
Sistrurus miliarius	Lee Co., Fort Myers, Florida, United States of America	UTA-Live	97	AY223611	U41889	–	–
Crotalus tigris	Pima Co., Arizona, United States of America	CLP 169 (ND4, cytb), MVZ150244 (ND2)	73	AY223606	AF156574	AY016240	–
Crotalus viridis	Sherman Co., Texas, United States of America	UTEP 15872	94	AF147869	AF194160	AY704799	–
Crotalus m. molossus	Maricopa Co., Arizona, United States of America	UTEP 19091	28	JN620845	JN620995	–	JN620924
Crotalus m. molossus	Grant Co., New Mexico, United States of America	UTEP 19982	27	JN620840	JN620990	–	JN620919
Crotalus ornatus	Hudspeth Co., Texas, United States of America	UTEP 18677	40	JN620820	JN620970	–	JN620902
Crotalus ornatus	Luna Co., New Mexico, United States of America	UTEP 16179	42	JN620836	JN620986	–	JN620917
Crotalus ornatus	Jeff Davis Co., Texas, United States of America	CGA 06	41	JN620825	JN620975	–	JN620907
Crotalus ornatus	Hays Co., Texas, United States of America	UTEP 17515	39	JN620846	JN620996	–	JN620925
Crotalus m. nigrescens	Durango, Mexico	JAC 29296	32	JN620849	JN620999	–	–

APPENDIX 2. Specimens examined

***Crotalus culminatus* (*n*=25)**. MEXICO: GUERRERO: Chilpancingo de los Bravo, municipality of Chilpancingo de los Bravo (MCZ-R 34353). MORELOS: Barranca Honda, municipality of Tlaltizapan (UMABH 1–14). MICHOACÁN: Coyahuana de Hidalgo, municipality of Coyahuana (OCTOLAB Cc149, Cc263F, Cc263H, Cc263K, Cc263Ñ, Cc263M, Cc263G); El Sabino, municipality of Uruapan (FMNH 126616, MCZ-R 56021); PUEBLA. 3.4 km NE Zacapala, municipality of Zacapala (field specimen).

***Crotalus ehecatl* (*n*=16).** MEXICO: CHIAPAS: 12.2 km west of Chiapa de Corzo, municipality of Chiapa de Corzo (ECO-CH-H-3776); San José Tintonishac, municipality of Las Margaritas (ECO-CH-H-3777–3778); Galecio Narcía, Chiapa de Corzo (IBt 084); Las Maravillas, Villa de Corzo (MZFC 23990); Tonalá, municipality of Tonalá (MCZ-R 5036). OAXACA: Salina Cruz, municipality of Salina Cruz (UJED-CHFCB 2363); Santo Domingo, Zanatepec, municipality of Santo Domingo Zanatepec (UJED-CHFCB 3079); San Pedro Tapanatepec, minicipality of San Pedro Tapanatepec (UJED-CHFCB 0380, SDNHM 24383, MCZ-R 27819, 27821); La Ventosa, municipality of Juchitán de Zaragoza (field specimen); Thuantepec, municipality of Santo Domingo Tehuantepec (MCZ-R 46485); 2mi E Puerto Escondido, municipality of Santa María Colotepec (CM-Herps 69448); 5mi W of Tehuantepec, municipality of Santo Domingo Tehuantepec (CM-Herps 69449).

***Crotalus mictlantecuhtli* (*n*=35).** MEXICO: VERACRUZ: Playas del Conchal, municipality of Boca del Río (IBt 224, 225); 4.2 Km SE Mata Naranjo, municipality of Cuitláhuac (IBt226); Rinconada, municipality of Emiliano Zapata (IBt 231, 233, 230); Actopan, municipality of Actopan (IBt 268, 270, UMA-OCTOLAB Cs11,12, 61,93, 114, 124,132, 142, 152, 153, 156, 157, 159, 161, 166); El Zapote, municipality of Alvarado (UMA-Palancoatl 46); La Tinaja, municipality of Cotaxtla (UMA-Palancoatl 50, IBt Cs1–7); Tierra Blanca, municipality of Tierra Blanca (SDNHM 24026); Veracruz, municipality of Veracruz (SDNHM 22416); 5.3 mi W Anton Lizardo, municipality of Alvarado (UTEP H-4710).

***Crotalus simus* (*n*=10).** GUATEMALA: El Arenal, San Vicente, Departamento de Zacapa (UTA-R 52032); EL SALVADOR: Hacienda Los Angeles, municipality of San Antonio el Pajonal (MZFC 574, 808). MEXICO: CHIAPAS: Miguel Alemán Valdez (Jericó), municipality of Pijijiapan (MZFC 19013, 19031); Tapachula, municipality of Tapachula de Córdova y Ordoñez (4 field specimens); 48 km NW Tapachula, municipality of Villa Comaltitlán (MCZ-R 147425).

***Crotalus tzabcan* (*n*=56).** MEXICO: CAMPECHE: municipality of Calakmul (ECO-CH-H-0861, 0862, 0884, 1071, 1147, 1168, 1419, 1441, 1549, 1751, IBt 265). QUINTANA ROO: municipality of Benito Juárez (ECO-CH-H 3782); municipality of Felipe Carrillo Puerto (ECO-CH-H 2006, 2087); municipality of José María Morleos (ECO-CH-H 2954); municipality of Othón P. Blanco (ECO-CH-H-0104, 0175, 0294, 0381, 1131, 2192, 2710, 2873, 3126, 3143, 3144, 3145, 3354, 3355, 3356, 3357, 3763, 3775, 3779, 3780, 3781, 3783, 3802, 3803, 3827, 3846, 3976, 3977; 2 wild specimens); municipality of Tulum (IBt 206); one uncatalogued specimen. YUCATÁN: municipality of Sotuta (ECO-CH-H 3772); municipality of Sudzal (ECO-CH-H 3773; one wild specimen); municipality of Tekom (ECO-CH-H-3771); municipality of Tinum (one wild specimen); municipality of Yaxcabá (ECO-CH-H 3774, 3804); municipality of Kantunil (FMNH 36168); 2 field specimen. TABASCO: municipality of Balancán (UJAT-CART 00520).