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ORIGINAL ARTICLE

Not withering on the evolutionary vine: systematic revision of the Brown Vine Snake (Reptilia: Squamata: *Oxybelis*) from its northern distribution

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Abstract
The genus *Oxybelis* currently is composed of four taxa despite numerous studies suggesting and describing multiple taxa within the *O. aeneus* complex. Here, we utilize a multilocus molecular dataset (i.e., cyt *b*, ND4, 12S, 16S, cmos, PRLR, 3663 bp) to conduct phylogenetic analyses to assess the evolutionary history of *Oxybelis*. Our molecular analyses find three major lineages of *Oxybelis* (i.e., *O. aeneus* complex, *O. brevirostris*, *O. fulgidus* complex) with a sister relationship between *O. brevirostris* and the *O. aeneus* complex to the exclusion of the *O. fulgidus* complex. More specifically, *O. aeneus* appears to harbor at least five taxa currently unrecognized while *O. fulgidus* was found to be paraphyletic with respect to *O. wilsoni*, suggesting cryptic diversity and novel taxa in that clade as well. Additionally, we use morphological data in concert with our molecular analyses and the literature to support removing *Oxybelis microphthalmus* Barbour and Amaral, 1926; *Oxybelis potosiensis* Taylor, 1941; and *Dryophis vittatus* Girard, 1854 from the synonymy of *O. aeneus*. Finally, we describe two new species from Central America and northern South America.

Keywords Bayesian analysis · Biodiversity · Cryptic species · Serpentes · Species delineation · Taxonomy

Introduction

Arboreal lifestyles in snakes have evolved in numerous clades, and some arboreal lineages have exploded into radiations that exploit numerous niches in the forest canopies of Asia (e.g., *Ahaetulla*), Africa (e.g., *Thelotornis*), and the New World (e.g., *Oxybelis*). Species with extremely attenuated, slender bodies, an elongated head, large eyes capable of binocular vision, a long tail, and diurnal behavior collectively are often called "vine snakes." This ecomorph exploits the smallest branches in the canopy, their low mass-to-length ratio allowing them to cantilever, or bridge, distances between branches that pass 50% of their body length to reach prey, often lizards (Henderson 1980; Ray 2012). Additionally, the

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ability to move from tree to tree instead of descending to the ground to climb again likely saves energy (Ray 2012).

The colubrid genus *Oxybelis* currently contains four species of vine snakes, all of which have populations in Central America (Köhler 2008; Uetz et al. 2018). *Oxybelis brevirostris* (Cope 1861) and *O. fulgidus* (Daudin 1803) occur in Central and northern South America, while *O. wilsoni* Villa and McCranie, 1995 is known only from Isla de Roatán, a Caribbean island located 48 km from mainland Honduras (McCranie et al. 2005). On the other hand, the Brown Vine Snake, *O. aeneus* (Wagler, 1824), is found at low and high elevational ranges and throughout contrasting habitats, from semi-desert to tropical rainforest (Keiser 1982; Van Devender et al. 1994). *Oxybelis aeneus* exhibits a vast distributional range, one of the most widespread species of terrestrial snakes worldwide, ranging from southern USA to southeastern Brazil. The colonization capability of *O. aeneus* (sensu Keiser 1974) becomes apparent by its presence throughout the mainland and on various islands. More specifically, the species spans both coasts of Mexico and Central America; the island populations in Central America are known from the Tres Márias Island (Nayarit, Mexico), Isla de la Blanca (Quintana Roo, Mexico), Islas de la Bahía (Honduras), Los Blancos Islands (El Salvador), Corn Islands (Nicaragua), and the Pearl Islands and Coiba (Panama). In South America, it occurs on both sides of the Andes, extending to the Guiana shield and Atlantic coast southward to Central Bolivia and southeastern Brazil, and most likely Paraguay (Keiser 1982), although Keiser (1991) mentioned the southern limits of its range are still poorly known. Other island populations are present on both Trinidad and Tobago (Murphy et al. 2018), as well as the Venezuelan islands of Margarita and Los Testigos (Roze 1966). Keiser also reported *O. aeneus* from Aruba (Southern Antilles), though there is no specimen record to confirm it.

Oxybelis aeneus can be defined as having an elongate head and body with a low mass-to-length ratio, a brown to gray dorsum, and a cream to gray to tan ventral surface, often with narrow stripes or fine mottling. Dorsal scales are usually in 17–17–13 rows, keeled dorsal scales are absent in most populations, and when present they are on the posterior portion of the body. Ventrals can number 173 to 205, subcaudals 137 to 203; the anal plate is divided. The head width/head length ratio is 0.22–0.47 and varies with the size of the specimen and population. Despite these similarities, the large distribution and morphological variation in *O. aeneus* have resulted in populations being described as "distinct" numerous times. Keiser's (1974, 1982) are the most recent reviews of the species, and given that polytypic species were accepted widely as the norm at the time, Keiser considered *O. aeneus* a single, extremely variable species. Therefore, although numerous important geographic barriers (e.g., Isthmus of Tehuantepec, Trans-Mexican Volcanic Belt, Isthmus of Panama, Andes Mountains) and biogeographic areas (e.g., Atlantic forest, Chocó, Llanos, Cerrado) occur across its wide range and many morphological distinctions exist for specific populations (see Fig. 1), the Brown Vine Snake currently is considered a single species (Keiser 1974, 1982).

Jadin et al. (2019) assembled a multilocus molecular dataset of *Oxybelis* from populations in the northern part of

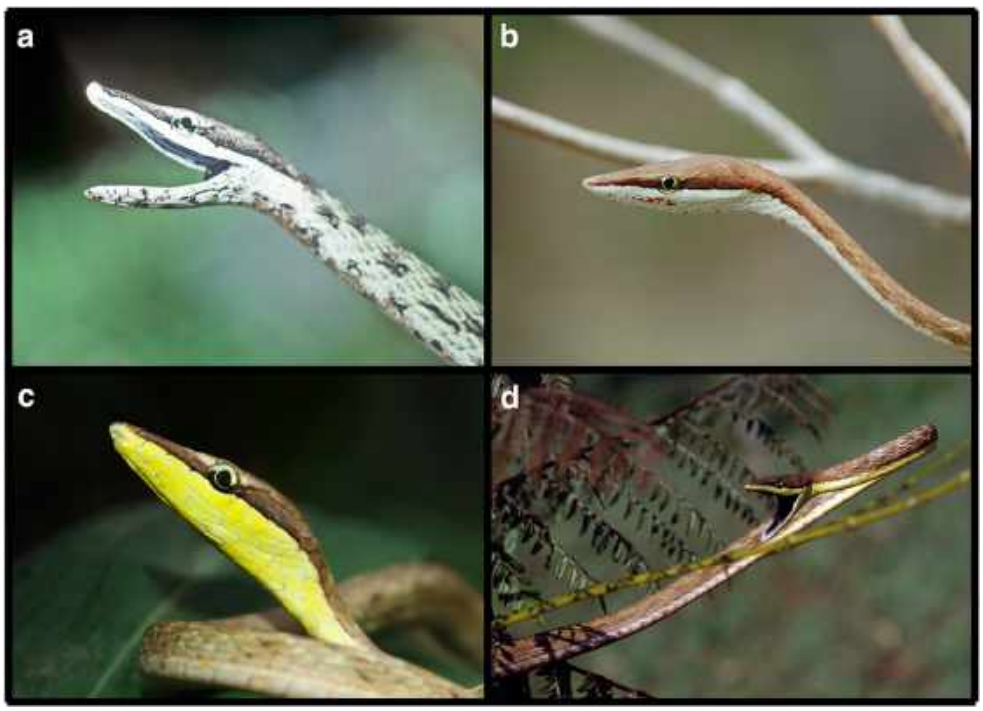


Fig. 1 In life photographs of *Oxybelis aeneus* (sensu Keiser 1974) from throughout its distribution showing tremendous morphological variation. **a** Reserva Amazonica, Peru (W.E. Duellman); **b** Santa Rosa, Costa Rica (L. Porras); **c** Venezuela (D.A. Briceño C.); **d** Jalapão, Tocantins, Brazil (LJV). Photos **a** and **d** show a gaping mouth that is a typical defense behavior for members of the *Oxybelis aeneus* complex

their range to examine its evolutionary history and test for evidence of cryptic lineages using Bayesian and maximum likelihood criteria. This study showed evidence that *O. aeneus* is likely a complex of species showing relatively deep species-level divergences initiated during the Pliocene. Here, we expand on that work incorporating an increased molecular dataset and population sampling of the New World vine snakes throughout most of their northern range. More specifically, we implement multiple phylogenetic analyses using multigene datasets, including both mitochondrial (mtDNA) and nuclear (nDNA) DNA sequences, to assess the evolutionary history within the genus *Oxybelis*. Our analyses recovered numerous distinct species-level clades, and we combined these results with morphological data to revise the species-level taxonomy of *O. aeneus*. This study resurrects several synonyms as distinct species while also describing two novel taxa.

Materials and methods

Morphological data

Museum specimens were examined from across the range of *Oxybelis aeneus* (Appendix 1). Specimen examination was conducted at the Field Museum of Natural History, the University of Arizona's herpetology collection, the University of Wisconsin – Eau Claire, the University of Wisconsin – Stevens Point Museum of Natural History, and Arizona State University. Scale counting methodologies generally follow those of Peters (1964) with some minor exceptions. Dorsal scales were counted on the neck at about the 10th ventral, at mid-body, and about 10 ventral scales anterior to the vent, and they were counted on the diagonal. Dorsal scale rows expressed here as 17–17–13 refers to the number of rows on the anterior body, at mid-body, and at posterior body, respectively. Scale counts and scale measurements on most specimens were done under a dissection microscope. Measurements were taken with a meter stick, metric tape, and dial calipers. Scale counts separated by a dash (−) represent a range taken from different individuals. Scale counts separated by a slash (/) represent scale counts taken from a single individual; the number on the left is the number of scales on the snake's left, and the number on the right is the number of scales on the specimen's right side. We also rely heavily on Keiser (1974) for his excellent data and analysis of *Oxybelis aeneus*.

Photographs of scale arrangements were taken with Canon EOS cameras and macro lenses. Sex was determined by probing, tail shape, dissection, and/or visual inspection of the hemipenes, testes, and/or ovaries.

Ventral count data were obtained from museum specimens as well as published data (Taylor 1941; Bogart and Oliver 1945; Keiser 1974), and counting methods used for this study follow Dowling (1951). Previous authors (Bogert and Oliver 1945; Keiser 1974) as well as this study found no evidence of sexual dimorphism in the ventral scale counts, so male and female data are combined to compare populations. However, sexual dimorphism was found in the subcaudal counts. Complete subcaudal counts are rarely available for these snakes because so many have broken tails.

To determine whether geographic regions harbored distinct units within *Oxybelis aeneus*, we conducted a discrimination analysis (DA) using the following sixteen morphological and morphometric characters: (1) eye diameter/frontal length, (2) eye diameter/head length, (3) eye diameter/internasal length, (4) eye diameter/prefrontal length, (5) eye diameter greater or lesser than preocular length, (6) eye–nostril distance/eye diameter, (7) head width/head length, (8) internasal length/prefrontal length, (9) prefrontal length/frontal length, (10) supraoculars longer or shorter than prefrontal, (11) second pair of chin shields in contact or separated for most of their length, (12) presence or absence of mid-ventral stripe, (13) number of upper labials, (14) number of upper labials contacting orbit, (15) number of upper labials contacting post orbitals, and (16) the snout shape varying among three types of tapering (Fig. 2).

We lumped our examined specimens into groups representing six geographically distinct regions corresponding to potentially distinct species based on molecular data (e.g., Jadin et al. 2019). These regions include western region (Southern Arizona through Western Mexico and into Southern Mexico), Eastern Mexico, Central America, Panama, northern South America (Tobago, Trinidad, and Venezuela), and Central Brazil.

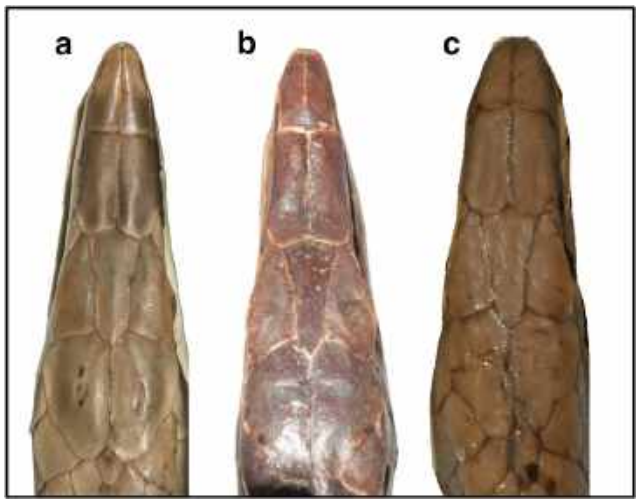


Fig. 2 Image showing the variability in snout shape among populations of *Oxybelis aeneus*. **a** A slender taper from the occipital region to rostral scale (UAZ 16787, Arizona, USA); **b** tapered, but snout in front of eyes is slightly constricted (FMNH 64417, Brazil); **c** a taper from the occipital region but the area in front of the eyes is broad and the rostal is rounded (UIMNH 25069, San Luis Potosí, Mexico)

Statistical analyses were completed with Excel (2019 v16) and XLstats (v 2020.1), (alpha = 0.05). Abbreviations are as follows: n = number of specimens, x = mean value, SD = standard deviation, and SVL = snout–vent length. ANOVA single-factor tests were used to compare ventrals and subcaudals between males and females.

Molecular data

Genomic DNA was extracted from tissues of specimens of *Oxybelis* (Table 1, Fig. 3) using a Qiagen DNeasy extraction kit and protocol. Four mitochondrial [cytochrome *b* (cyt *b*), NADH dehydrogenase subunit 4 (ND4), ribosomal RNA (12S rRNA, 16S rRNA)] and two nuclear [oocyte maturation factor mos (cmos) and prolactin receptor (PRLR)] gene fragments were independently amplified using GoTaq Green master mix by Promega, (Madison, WI, USA) with the primer pairs L14910 + H16064 (cyt *b*), ND4 + LEU (ND4), L1091 + 12E (12S), L2510 + H3059 (16S), S77 + S78 (cmos), and PRLR_f1 + PRLR_r3 (PRLR) described in previous studies (i.e., cyt *b*: Burbrink et al. 2000; ND4: Arévalo et al. 1994; 12S and 16S: Knight and Mindell 1993; cmos: Lawson et al. 2005; PRLR: Townsend et al. 2008). For PCR of five fragments (excluding PRLR), we implemented thermocycler conditions of an initial denaturation of 3 min at 95 °C followed by 40 cycles 40 s at 95 °C, 40 s at 48 °C, 1 min at 72 °C, and a final elongation at 72 °C for 5 min. Annealing temperature for PRLR was 50 °C. Sequencing was performed in both forward and reverse directions using the PCR primers, and sequence chromatographs were edited using Geneious R6 6.1.6. No internal stop codons were found in protein-coding gene fragments, and indels were treated as missing data. Novel sequences generated were deposited in GenBank (MT969178–MT969331) and combined with sequences of several other *Oxybelis* and other colubrid taxa previously published on GenBank (Table 2). We selected our outgroup taxa based on recent studies that found *Oxybelis* in a clade of New World colubrids (Pyron et al. 2013; Jadin et al. 2014, 2019; Figueroa et al. 2016; Zaher et al. 2019). Sequence alignments for each gene fragment were conducted separately, first automatically using the program MUSCLE (Edgar 2004) and then manually rechecked using Se-Al v.2.0a11 (Rambaut 2002).

Phylogenetic and species delimitation analyses

We used IQ-TREE v.1.6.12 (Nguyen et al. 2015) to estimate maximum likelihood (ML) phylogenies and determine the evolutionary history of *Oxybelis*. We used ModelFinder (Kalyaanamoorthy et al. 2017) to perform substitution model selection (using BIC) and tree inference in a single command. We implemented a partition model (Chernomor et al. 2016) for the concatenated analysis using the MFP+MERGE command in IQ-TREE. We created a partition file specifying different data partitions by gene and by codon position (for the protein-coding genes). This initial partitioning scheme was chosen because ModelFinder can only merge partitions (there is no additional splitting). However, to quantify potential issues with data partitioning, we compared our results to an unpartitioned analysis. The results were virtually identical with conflict only at poorly supported nodes, suggesting that the choice of partitioning scheme had little influence on the analysis. Support for nodes was assessed using both 1000 SH-aLRT replicates (Guindon et al. 2010) and 10,000 ultrafast bootstrap (UFboot) replicates (Hoang et al. 2017). Nodes with SH-aLRT > 80% and UFboot > 95% were indicative of strong support. Unrooted trees were rooted with *Drymarchon corais* (Jadin et al. 2014, 2019).

Additionally, we conducted Bayesian Markov chain Monte Carlo [MCMC] mixed-model analyses in MrBayes v.3.0b4 (Ronquist and Huelsenbeck 2003), partitioning by gene as well as by codon (Table 3). Two simultaneous runs of four MCMC analyses, consisting of one cold and three incrementally heated chains, were initiated with random trees for a total of 8×10^6 generations per run, sampling trees and parameters every 100 generations. We used potential scale reduction factor values (output by MrBayes), together with plots of cold-chain likelihood values and parameter estimates visualized in TRACER v.1.6 (Rambaut et al. 2014) to confirm stationarity and convergence of MCMC runs. Based on this evaluation, the first 3×10^6 generations from each run were discarded as burn-in.

We also performed a coalescent-based species tree analysis using SVDquartets (Chifman and Kubatko 2014, 2015) in PAUP*v.4.0b10 (Swofford 2002). SVDquartets uses site pattern frequencies and algebraic statistics to estimate relationships among quartets. A subsequent quartet assembly step is then used to estimate a species tree containing all taxa. We assigned individuals to species based on a combination of current taxonomy and the results from the concatenated ML and Bayesian analyses. All quartets were evaluated (i.e., exhaustive sampling), and the full species tree was assembled using QFM (Reaz et al. 2014). The unrooted species tree was rooted using *Drymarchon corais*, and support for nodes was determined using 100 non-parametric bootstrap replicates.

To further estimate the number of species/populations among *Oxybelis* lineages, we used the program BPP v.4.1.3 (Yang 2015; Flouri et al. 2018). BPP uses the multispecies coalescent model (MSC) in a Bayesian framework for the estimation of species limits, species trees, and associated parameters (i.e., divergence times and population sizes). Given

Table 1 Genbank numbers for DNA sequences analyzed in this study, new sequences in italics

Species	Voucher	Locality	Cyt *b*	ND4	12S	16S	cmos	PRLR
Oxybelis brevirostris	JMR 2013-020	Panama	MT969178	MT969199	MT969241	MT969275	MT969311	–
	UTA R-55952	San Lorenzo, Esmeraldas, Ecuador	MT969179	–	–	–	MT969312	–
	LSUMZ H-12770	Imbabura Province, Ecuador	MT969180	MT969200	MT969242	MT969276	MT969313	–
O. fulgidus	UTA R-52506	Veracruz, Mexico	MK497173	MT969201	MT969243	MT969277	MK497197	MK497219
	UTA R-53002	Oaxaca, Mexico	MK497174	MT969202	MT969244	MT969278	MK497198	MK497220
	JAC 24318	Chiapas, Mexico	MK497175	MT969203	MT969245	MT969279	MK497199	MK497221
	UTA R-53417	Yucatan, Mexico	MK497176	MT969204	–	MT969280	MK497200	MK497222
	UTA R-45292	Izabal, Guatemala	MK497177	MT969205	–	MT969281	MK497201	MK497223
	MSM 439	Comayagua, Honduras	MK497178	MT969206	MT969246	MT969282	MK497202	–
	USNM 565820	Olancho, Honduras	MK497179	MT969207	MT969247	MT969283	MK497203	MK497224
	LSUMZ H-6358	Honduras	MT969181	MT969208	MT969248	MT969284	MT969314	–
	LSUMZ H-6352	Unknown	MT969182	MT969209	MT969249	MT969285	MT969315	–
	MBLUZ 1480	Bolivar, Venezuela	MT969183	MT969210	MT969250	MT969286	–	–
O. koehleri sp. nov.	UTA R-46846	Zacapa, Guatemala	MK497189	MT969211	MT969251	MT969287	MK497212	–
	UTA R-46865	Comayagua, Honduras	MK497190	MT969212	MT969252	MT969288	MK497213	MK497232
	UTA R-44838	Jinotega, Nicaragua	MK497191	MT969213	MT969253	MT969289	MT969316	MK497233
	KU R-288907	El Salvador	MT969184	MT969214	MT969254	MT969290	MT969317	–
	LSUMZ H-6353	Unknown	MT969185	MT969215	MT969255	MT969291	MT969318	–
O. microphthalmus	JAC 30618	Sinaloa, Mexico	MT969186	MT969216	MT969256	–	MK497204	MK497225
	UTA R-53331	Nayarit, Mexico	MK497180	MT969217	–	MT969292	MK497205	MK497226
	UTA R-53373	Jalisco, Mexico	MK497181	MT969218	–	MT969293	MK497206	MK497227
	UTA R-57658	Colima, Mexico	MK497182	MT969219	–	–	–	–
	UTA R-57659	Colima, Mexico	MK497183	MT969220	–	MT969294	MK497207	–
	UTA R-53374	Jalisco, Mexico	MK497184	MT969221	–	–	MK497208	MK497228
	MZFC 19224	Guerrero, Mexico	MK497185	MT969222	MT969257	MT969295	MK497209	MK497229
	UTA R-53024	Guerrero, Mexico	MK497186	MT969223	MT969258	MT969296	MK497210	MK497230
	UTA R-53026	Oaxaca, Mexico	MK497187	MT969224	MT969259	MT969297	–	–
	UTA R-52648	Oaxaca, Mexico	MK497188	MT969225	MT969260	MT969298	MK497211	MK497231
	UTA R-53021	Michoacan, Mexico	MT969187	MT969226	MT969261	MT969299	MT969319	–
	UTA R-53427	Oaxaca, Mexico	MT969188	MT969227	MT969262	–	MT969320	–
O. potosiensis	MZFC 17581	Yucatan, Mexico	MT969189	MT969228	MT969263	MT969300	MT969321	–
	UTA R-52600	Puebla, Mexico	MT969190	MT969229	MT969264	MT969301	MT969322	MT969330
O. rutherfordi sp. nov.	UWIZM.2011.18.10	Tobago	MK497193	MT969230	–	–	MK497215	–
	UWIZM.2012.27.49	Tobago	MK497194	–	–	–	MK497216	–
	UWIZM.2011.20.14	Trinidad	MK497195	MT969231	MT969265	MT969302	MK497217	MT969331
	UWIZM.2016.23.5	Trinidad	MK497196	–	–	–	MK497218	–
	LSUMZ H-6608	Sucre, Venezuela	MT969191	MT969232	MT969266	MT969303	MT969323	–
	MBLUZ 1268	Isla de Margarita,Venezuela	MT969192	MT969233	MT969267	MT969304	–	–
O. vittatus	ENS 11259	Isla de Coiba, Panama	MK497192	MT969234	MT969268	MT969305	MK497214	MK497234
	JMR 2013-016	Panama	MT969193	MT969235	MT969269	MT969306	MT969324	–
	JMR 2013-019	Panama	MT969194	MT969236	MT969270	MT969307	MT969325	–
	JMR UNK020	Panama	MT969195	MT969237	MT969271	–	MT969326	–
	JMR 2013-025	Panama	MT969196	MT969238	MT969272	MT969308	MT969327	–
	JMR 2013-105	Panama	MT969197	MT969239	MT969273	MT969309	MT969328	–

Table 1 (continued)

Species	Voucher	Locality	Cyt *b*	ND4	12S	16S	cmos	PRLR
	JMR UNK015	Panama	MT969198	MT969240	MT969274	MT969310	MT969329	–

Abbrevations of institutions and individuals for voucher specimens are as follows: *ENS* Eric N. Smith field series; *JAC* Jonathan A. Campbell field series; *JMR* Julie M. Ray field series; *KU* University of Kansas, Biodiversity Institute and Natural History Museum; *LSUMZ* Louisiana State University, Museum of Natural Sciences; *MBLUZ* Museo de Biología, Universidad del Zulia; *MSM* Mahmood Sasa Marín field series; *MZFC* Museo de Zoología de la Facultad de Ciencias, Universidad Nacional Autónoma de México; *UTA* Amphibian and Reptile Diversity Research Center, University of Texas, Arlington; *USNM* Smithsonian National Museum of Natural History; *UWIZM* The University of the West Indies Zoology Museum

the recent concerns that BPP may oversplit (Sukumaran and Knowles 2017; Leaché et al. 2018), we took a conservative approach to our analysis. We initially defined 10 species based on the results of the concatenated ML and Bayesian analyses. We also used the concatenated Bayesian topology as the starting topology for all BPP runs. We implemented four joint species delimitation/species tree (A11) analyses, testing the impact of species delimitation algorithm used (Alg 0, Alg 1) and the theta prior on the results. For all analyses, we specified an inverse gamma prior of (3, 0.04) for tau. For theta, we tested the influence of a large (3, 0.04) and small (3, 0.004) prior on our results. All analyses used a uniform rooted tree prior. To accommodate the combined mitochondrial and nuclear data, we turned on the locus rate option ($\alpha = 2.0$) and introduced heredity multipliers (G 4,4]). Analyses used an initial burn-in of 100,000 generations followed by a total of 50,000 posterior samples taken every 10 generations. Convergence was assessed through visual inspection of results from independent runs.

Results

Morphology

Our morphological analyses show distinction among lineages across geographically separated populations of *Oxybelis aeneus*. The discrimination analysis results found Wilks' lambda test, Pillai's test, Hotelling–Lawley trace, and Roy's greatest root all had p values < 0.0001 with alpha set at < 0.05 rejecting the null hypothesis of no discriminating ability. The eigenvalue for F1 accounted for 62.1% of the total variance (Table 4). The factor/correlations for the traits in the analysis

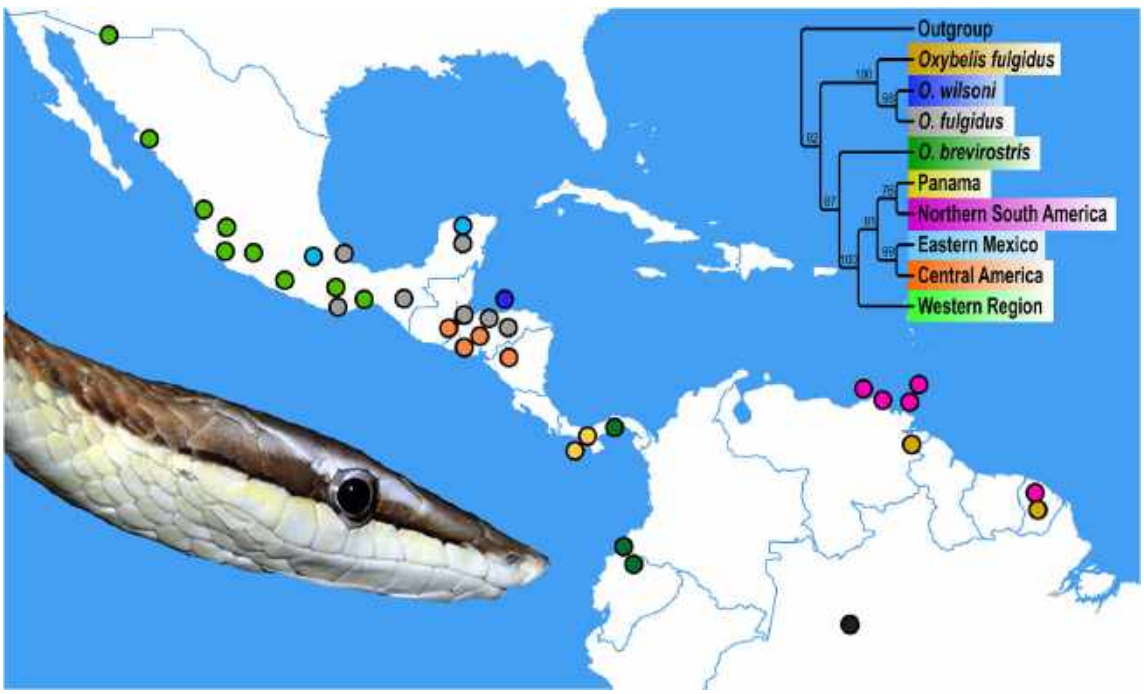


Fig. 3 Localities from which we obtained tissues for molecular work, other than the type locality represented by a black circle. Colors represent tissues of brown vine snakes from Central America (orange), Eastern Mexico (light blue), Panama (yellow), northern South America (purple), and the western region (light green); other taxa with molecular data represented are *O. brevirostris* (dark green), *O. fulgidus* (gray and dark yellow), and *O. wilsoni* (dark blue). Also shown is the locality of *O. aeneus* (black, not currently sequenced). Inserts are a brown vine snake from Pima Co. AZ, USA, taken by JCM (left) and SVDquartets species tree (right). Node values on the species tree represent support values from 100 non-parametric bootstrap replicates

Table 2 GenBank numbers for DNA sequences of New World colubrids analyzed in this study

Species	cyt *b*	ND4	12S	16S	cmos
Coluber constrictor	AY486914	AY487041	AY122819	L01770	AY486938
Drymarchon corais	AF471064	DQ902314	HM565758	HM582218	AF471137
Leptophis depressirostris	KR814686	KR814724	KR814617	KR814643	KR814682
Oxybelis aeneus Arizona	AF471056	–	–	–	AF471148
Oxybelis aeneus French Guiana	–	–	AF158416	AF158498	–
Oxybelis aeneus	–	–	HM565765	HM582225	HQ157829
Oxybelis fulgidus	MK209278	–	MK209203	MK209316	–
Oxybelis fulgidus French Guiana	–	–	AF158432	AF158497	–
Oxybelis wilsoni	KR814689	KR814710	KR814626	KR814647	KR814669

are shown in Table 4. Figure 4 shows the observations on the factor axes and confirms that the species are discriminated on the basis on the morphometric and meristic variables. The group means are designated by the centroids that allow for visualization of how the functions discriminate between groups by plotting the individual scores for the discriminant functions.

Phylogeny and species diversity

The BI, ML, and SVDquartets analyses recovered nearly identical tree topologies with mostly strong node support, especially towards the tree tips (Figs. 3 and 5). ModelFinder in IQ-TREE selected the following partition scheme and substitution models: (1) cyt *b*-1st + ND4-1st + 12S + 16S + PRLR-2nd + PRLR-3rd (TIM2 + F + I + G4); (2) cyt *b*-2nd + ND4-2nd + cmos-3rd (HKY + F + R2); (3) cyt *b*-3rd + ND4-3rd (TIM3 + F + R2); (4) cmos 1st + cmos 2nd + PRLR 1st (K2P + I). All our molecular phylogenetic analyses strongly support the presence of three major lineages (i.e., the brown vine snakes, *O. brevirostris*, and the green vine snakes) (Figs. 3 and 5). Furthermore, we

Table 3 Results from a priori best-fit model selections of nucleotide substitution based on Akaike information criterion (AIC) conducted in MrModeltest 2.2 (Nylander 2004) run in PAUP*v4.0b10 (Swofford 2002)

Partitions	Total characters	AIC model
Cyt *b* 1st pos	359	GTR + I + gamma
Cyt *b* 2nd pos	359	HKY + I
Cyt *b* 3rd pos	359	GTR + gamma
ND4 1st pos	222	GTR + gamma
ND4 2nd pos	222	HKY + I
ND4 3rd pos	222	HKY + gamma
12S	373	GTR + I + gamma
16S	491	GTR + I + gamma
cmos 1st pos	186	HKY
cmos 2nd pos	186	HKY
cmos 3rd pos	186	HKY
PRLR 1st pos	166	F81 + I
PRLR 2nd pos	166	HKY + I
PRLR 3rd pos	166	HKY + I

Table 4 The eigenvalues for the discrimination analysis and the variable and factor correlations from the sixteen morphometric characteristics

	F1	F2	F3	F4	F5
Eigenvalue	12.627	5.252	1.634	0.537	0.255
Discrimination (%)	62.188	25.868	8.046	2.643	1.256
Cumulative %	62.188	88.056	96.102	98.744	100.000
Variables					
1	−0.379	0.511	0.137	0.412	0.053
2	−0.240	−0.887	0.136	−0.088	0.158
3	−0.478	0.672	−0.202	−0.047	0.212
4	−0.830	0.308	0.387	−0.112	−0.042
5	0.207	0.452	−0.462	0.250	0.196
6	0.392	−0.678	−0.039	0.246	−0.044
7	−0.401	0.235	0.334	0.017	0.575
8	−0.013	−0.171	0.465	−0.376	0.112
9	0.728	0.101	−0.198	0.480	0.065
10	−0.227	0.149	0.194	−0.297	−0.351
11	−0.153	0.207	0.613	0.264	0.154
12	0.739	−0.465	0.040	0.229	−0.014
13	0.909	−0.121	0.369	−0.035	−0.021
14	0.798	0.079	−0.411	0.069	−0.063
15	0.314	−0.214	0.811	−0.105	0.035
16	−0.184	−0.326	−0.363	0.421	−0.154

Morphological variables described in the methods as characters

1 eye diameter/frontal length, *2* eye diameter/head length, *3* eye diameter/internasal length, *4* eye diameter/prefrontal length, *5* eye diameter greater or lesser than preocular length, *6* eye–nostril distance/eye diameter, *7* head width/head length, *8* internasal length/prefrontal length, *9* prefrontal length/frontal length, *10* supraoculars longer or shorter than prefrontal, *11* second pair of chin shields in contact or separated for most of their length, *12* presence or absence of mid-ventral stripe, *13* number of upper labials, *14* number of upper labials contacting orbit, *15* number of upper labials contacting postorbitals, *16* the snout shape

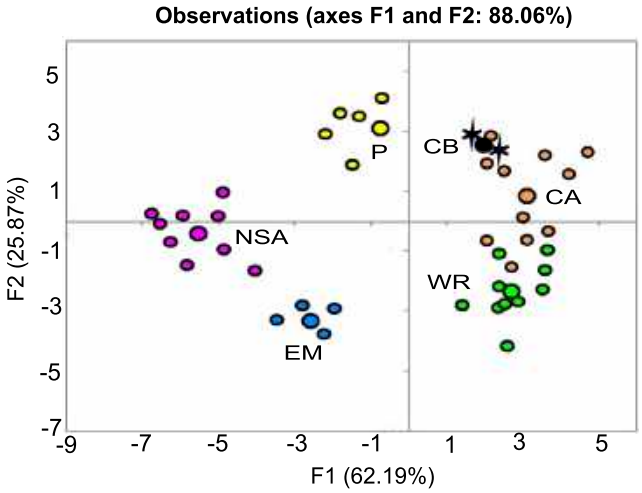


Fig. 4 Plot of discrimination analysis of sixteen morphological characters, showing clustering among geographically defined groups. Colors represent specimens from Central America (CA, orange), Central Brazil (CB, black stars), Eastern Mexico (EM, blue), northern South America (NSA, purple), Panama (P, yellow), and western region (WR, green). Colors correspond to those in Fig. 3

found strong support that *O. brevirostris* is sister to the *O. aeneus* complex, which together were sister to the green vine snakes (i.e., *O. fulgidus*, *O. wilsoni*). Additionally, our analyses show a paraphyletic *O. fulgidus* with respect to *O. wilsoni* (Figs. 3 and 5). Within the brown vine snakes, all our analyses strongly support a sister relationship between lineages in Central America and those from Eastern Mexico. Incongruence among analyses occurs with the position of the western region, which is found as sister to the Eastern Mexico–Central America clade in the BI and both ML analyses (though with low support) whereas the SVDquartets analysis strongly supports a sister relationship between the western region and the remaining brown vine snake populations (Fig. 3). Finally, the unpartitioned ML analysis placed the South American clade sister to the rest of the brown vine snakes and the Panama clade sister to a western region–Eastern Mexico–Central America clade. However, these relationships had low support (not shown).

Our BPP results indicated support for eight species-level lineages within *Oxybelis*. Species delimitation results were similar among algorithms, but differed based on the prior specified for theta (Fig. 6). For example, when utilizing a smaller prior, there was > 95% posterior support for seven species and relatively weak support for the distinction of *O. wilsoni* and *O. fulgidus* from Central America. When using a larger prior, the best delimitation model merged these three taxa with moderate posterior probability (0.87). In all BPP analyses, each *O. aeneus* lineage was supported as distinct with high posterior probability. These geographically defined lineages correspond to our morphological analyses above, strongly supporting species recognition, and we therefore describe and diagnose the taxa below.

Systematics

***Oxybelis aeneus* (Wagler, 1824)**

Brown Vine Snake

Dryinus aeneus – Wagler 1824

Oxybelis aeneus – Duméril et al. 1854: 819

Dryophis acuminata – Günther 1858: 156

Oxybelis aeneus aeneus – Bogert and Oliver 1945: 391

Oxybelis aeneus – Keiser 1974: 7

Lectotype ZSM 2645/0 from the forest along the Solimões River, near Ega, now Tefé, Amazonas, Brazil (~ 03° 21′ S, 64° 42′ W). Keiser (1974) considered ZSM 2645/0 the holotype but Hoogmoed and Gruber (1983) gave it lectotype status suggesting that Wagler, 1824 had seen more than one specimen.

Diagnosis Using data from Keiser (1974) and our examination of specimens from Central Brazil (Fig. 7), we constructed the following description for *Oxybelis aeneus*. A vine snake with (1) three upper labials (4–5–6) bordering the orbit; (2) black bars or spots present on the anterior body; (3) no stripes on the ventral surface; venter is mottled; (4) eye diameter greater than preocular; (5) second pair of chin shields separated by smaller scales for most of its length; (6) nine upper labials, three located behind the orbit; (7) snout from above narrow, tapered, and flat at rostral (snout type B); (8) supraocular slightly longer than prefrontals; (9) last upper labial longer than primary temporal; (10) lower surface of head uniform in color; and (11) second upper labial does not contact the preocular.

Tail is 0.7 of the SVL; the eye diameter is 1.4 times the length of the preocular scale and 0.93 of the internasal length. Primary temporal contacts both postoculars, the parietal, and two secondary temporals. Upper labials 6–7–8–9 contact the primary temporal. Ventral counts in males 179–197 ($n = 15$, $x = 188.8$, SD = 9.00). In females, ventral counts ranged from 184 to 203 ($n = 20$, $x = 192.1$, SD = 9.54). Subcaudal counts 154–188 in males ($n = 12$, $x = 169.5$, SD = 17.16) and 146–184 ($n = 15$, $x = 168.2$, SD = 19.08) in females. It has 17–20 maxillary teeth.

Variation The rostral is visible from above and followed by nine plate-like scales on the crown: a pair of internasals, a pair of prefrontals, the frontal and two larger supraoculars, and a pair of parietals. The preoculars extend slightly on to the crown between the prefrontals and supraoculars. In profile, the nasal scale is elongate, extending from the edge of the rostral, beyond the posterior edge of the internasal to the anterior border of the fused prefrontal–loreal. The preocular scale is short and less than the length of the eye's diameter. The eye diameter/internasal ratio for one specimen is 0.93. Scales bordering the

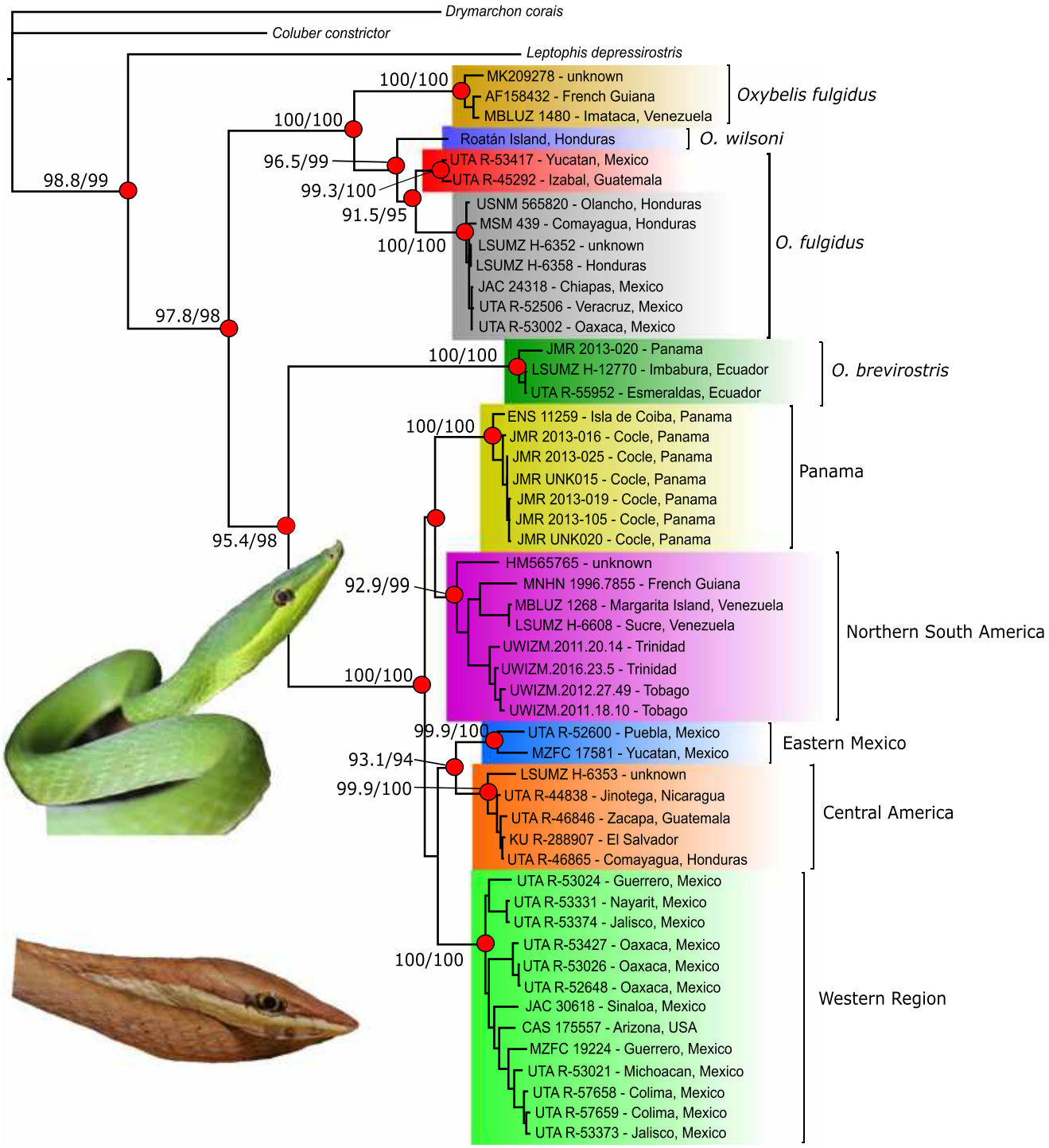


Fig. 5 Phylogenetic estimate of relationships within *Oxybelis* estimated from a Bayesian 50% majority-rule consensus phylogram using a multilocus dataset (cyt *b*, ND4, 12S, 16S, cmos and PRLR; total of 3663 bp) with posterior probabilities (≥95) represented at the node (red circles). Values adjacent to nodes represent additional support values (SH-aLRT > 80% and UFboot > 95%) from maximum likelihood (ML) analyses of the partitioned dataset obtained from IQ-TREE. Inserts are *O. fulgidus* (MBLUZ 1480; above) from the Sierra de Imataca, Bolivar, Venezuela, and *O. aeneus* NSA (below) photographed at Quebrada Chacaito, El Avila National Park, north of Caracas, Venezuela. Photographs were taken by D.A. Briceño C. and L.A. Rodríguez J., respectively. Colors correspond to those in Figs. 3 and 4

orbit are the preocular, the supraocular, two small postoculars, and upper labials 4–5–6. The primary temporal contacts both postoculars, the parietal, and two secondary temporals; upper labials 6–7–8–9 contact the primary temporal. Upper labials are

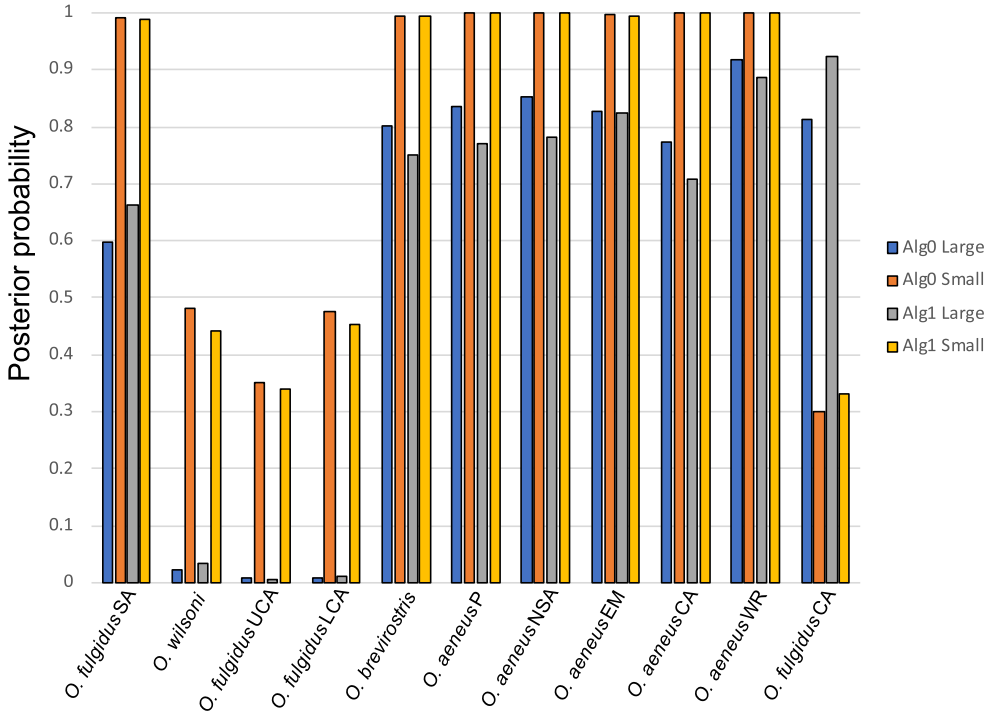


Fig. 6 Bayesian posterior probability support for species/populations within *Oxybelis* from BPP analyses of the combined mtDNA and nDNA data. Four joint species delimitation/species tree analyses (A11) were performed to test the impact of species delimitation algorithm used (Alg 0, Alg 1) and the theta prior ((large = IG [3, 0.04]); (small = IG [3, 0.004])) on the results. Abbreviations after *O. fulgidus* refer to populations from South America (SA), upper Central America (UCA = Guatemala and Yucatan), lower Central America (LCA = Honduras and Mexico), and Central America (CA = UCA, LCA, and *O. wilsoni*) while those after *O. aeneus* refer to populations from Panama (P), northern South America (NSA), Eastern Mexico (EM), western region (WR), and Central America (CA)

usually nine but range from 8 to 10. The eighth upper labial is the shortest. The ninth upper labial is the longest. Upper labials 1–2 contact the nasal, 2–3 contact the prefrontal–loreal, and 3–4 contact the preocular. The tallest upper labial can be the sixth or seventh. Lower labials range from 8 to 10 (usually nine). The first four (rarely five) contact the anterior chin shields, a total of six contact both pair of chin shields. The anterior pair of chin shields are shorter (about 50%) than the length of the second pair of chin shields; the second pair are completely separated by smaller scales. Ventral counts in males vary from 179 to 197 ($n = 15$, $x = 188.8$, SD = 9.00). Ventral counts in females vary from 184 to 203 ($n = 20$, $x = 192.1$, SD = 9.54). Subcaudal counts 154–188 in males ($n = 12$, $x = 169.5$, SD = 17.16) and 146–184 in females ($n = 15$, $x = 168.2$, SD = 19.08). Maxillary teeth vary from 17 to 20.

Fig. 7 Specimen of *O. aeneus* (FMNH 64417) from Manaus, Amazonas, Brazil. This specimen was collected c. 518 km from the type locality

Coloration and pattern The crown of the head and upper face are golden brown to tan. The upper labials and ventral surface of the head are a uniform cream. The transition in color is separated by a preocular dark brown stripe extending from the nasal scale, under the eye, and onto the anterior body. This stripe may continue as a series of spots onto the body. On the anterior body, the first two scale rows are the same yellow color as the ventral surface and form a ventrolateral stripe. A series of black marks occurs on some scales scattered on the sides of the body. An indistinct mid-line stripe occurs on the ventral surface.

Geographic distribution This species appears restricted to the Amazon Basin.

Comparison A vine snake with the combinations of the second pair of chin shields mostly separated by smaller scales, three upper labials bordering the orbit, four upper labials in contact with the primary temporal, and the eye diameter is about equal to the length of the internasal. Specimens from populations in Central America, Panama, and the western region have the second pair of chin shields in contact and two or three upper labials in contact with the primary temporal. Populations from Eastern Mexico have the second pair of chin shields in contact for most of their length and an eye diameter that is about 0.8 the length of the internasal. Those from northern South America also have three upper labials bording the orbit but have supraocualrs longer than the prefrontal.

***Oxybelis koehleri* sp. nov.**

Köhler's Vine Snake
Oxybelis aeneus – Duméril et al. 1854: 819
Dryophis acuminata – Günther 1858: 156
Oxybelis acuminata – Boulenger 1896: 192
Oxybelis aeneus aeneus – Bogert and Oliver 1945: 381
Oxybelis aeneus – Keiser 1974: 7

Holotype UTA R-46846 (ENS 9858), a female from Guatemala: El Arenal (circa 560 m, 14° 53′ 1.788″ N, 89° 46′ 31.799″ W) of the Municipio Cabañas in the Department of Zacapa. Collected by a local between Feb and May 1998, preserved 24 Aug 1998.

Paratypes UTA R-44838 Nicaragua: Jinotega, El Paraiso Km 152.5, carretera Jinotega-Matagalpa, 1490 m; UTA R-46865 Honduras: Comayagua, Playitos: Aldea "Lo de Reina", 785 m. UTA R-53176–77 Honduras: Gracias a Dios, Mocorón, 30–50 m.

Diagnosis Using data from our examination of specimens from Central America, we constructed the following description for *Oxybelis koehleri*. A vine snake with (1) three upper labials (4–5–6) bordering the orbit; (2) black spots or bars on anterior body, brown uniform brown with little black pigment; (3) labials white and underside of head red–brown with a medial red–brown stripe in females; (4) eye diameter greater than preocular length; (5) second pair of chin shields in contact for most of their length; (6) nine upper labials, three located behind the orbit; (7) snout from above is narrow, tapered, and rounded (snout type A); (8) supraocular is longer than the prefrontal; (9) last upper labial and primary temporal about the same length; and (11) second upper labial does not contact the preocular.

Description of holotype (UTA R-46846, Fig. 8) A female, total length 905 mm, tail length 439 mm. Rostral broader than tall; barely visible from above; upper labials 8 (5 + 6 fused)/9; internasals paired, extending past the posterior border of the first upper labial but not the nasal, which is longer than both; prefrontals paired, contact upper labials 2–3; triangular frontal and supraoculars elongated and circa 8 mm long, paired parietals slightly longer circa 9 mm; supraoculars and parietals,

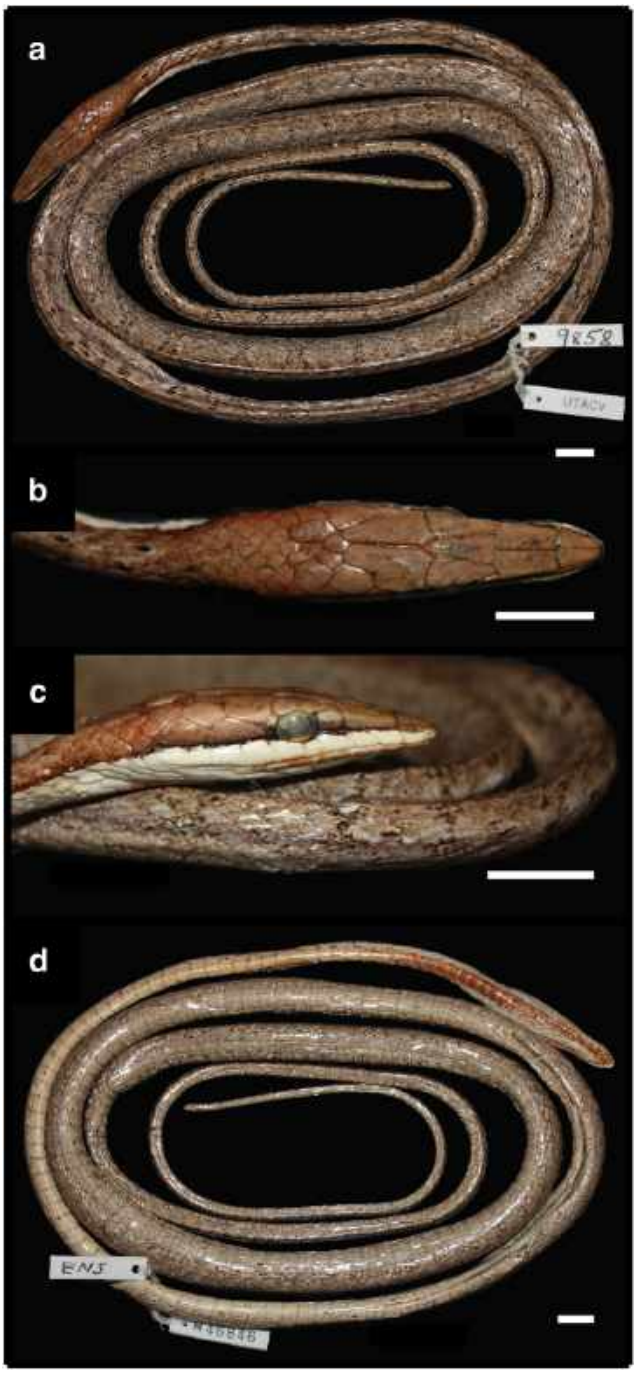


Fig. 8 Holotype of *Oxybelis koehleri* sp. nov., UTA R-46846 preserved; **a** dorsal; **b** top of the head; **c** profile; **d** ventral views. Scale bar = 1 cm

contact upper postocular; postoculars 2/2, upper larger; upper labials 3–4 contact the preocular; 4–5–6 are in the orbit (4 + 5 on left side because of fusing); 7–8–9 contact the primary temporal; 9 interrictals; one preocular less than the diameter of the eye; lower labials 10/10, first four contact the first pair of chin shields; second pair of chin shields longest; four paired gulars. Dorsal scales smooth in 17–17–13 rows. Ventrals 198, 119 divided subcaudals, anal plate divided.

Variation Rostral is not visible from above and followed by nine plate-like scales on the crown: a pair of internasals, a pair of prefrontals, the frontal, two larger supraoculars, and a pair of parietals. Preoculars extend slightly on to the crown between the prefrontals and supraoculars. The internasals are 75% ($r = 0.63–1.0$) of the prefrontal length. Average eye diameter is 1.19 ($r = 0.83–1.53$) the preocular length. In profile, the nasal scale is elongate extending from the edge of the rostral, beyond the preocular scale is short and less than the diameter of the eye. Scales bordering the orbit are the preocular, the supraocular, two small postoculars, and upper labials 4–5–6. Primary temporal contacts both postoculars, the parietal, and two secondary temporals, as well as upper labials 6–7–8–9. Upper labials number nine (rarely eight or ten). The shortest upper labial can be the first or the eighth. The last (usually the ninth) upper labial is the longest. Upper labials 1–2 contact the nasal, 2–3 contact the prefrontal–loreal, 3–4 contact the preocular. Lower labials vary from eight through 10, usually 9; the first four contact the anterior chin shields, a total of six contact both pair of chin shields. The anterior pair of chin shields are shorter (about 60%) than the length of the second pair; the second pair are in contact for most of their length. Dorsal scales are in 17–17–13 rows.

In males, total length ranged from 1135 to 1432 mm ($n = 6$, $x = 1329.83$, SD = 106.68), SVL 673–835 mm ($n = 7$, $x = 783.71$, SD = 62.55), tail lengths 462–600 mm ($n = 7$, $x = 554.0$, SD = 47.75), tail/SVL ratios ($n = 7$, $r = 0.67–0.79$, $x = 0.71$, SD = 0.04). In females, total lengths ranged from 1137 to 1300 mm ($n = 8$, $x = 1221.43$, SD = 63.72); tails ranged from 425 to 527 mm ($n = 7$, $x = 478.14$, SD = 31.22). Tail/SVL ratios in females 0.53–0.73, $x = 0.65$, SD = 0.06.

Ventrals in males vary from 176 to 191 ($n = 7$, $x = 183.83$, SD = 4.88); ventrals in females vary from 184 to 191 ($n = 7$, $x = 187.57$, SD = 2.26). Subcaudals in males vary from 164 to 186 ($n = 5$, $x = 177$, SD = 8.12); in females, subcaudals vary from 176 to 189 ($n = 5$, $x = 184.8$, SD = 5.91).

Coloration and pattern (Fig. 9) Head usually a uniform brown (it may have some darker pigmented spots), body brown with light mottling and some dark spots anteriorly, posteriorly indistinct transverse blotches that are wide on the vertebral line and narrow laterally; upper labials cream to white and separated from the brown by a black stripe on the dorsal edge of the second labial that extends past the eye to the last labial; lower labials have some red–brown pigmentation; ventral surface of head tan laterally with a red–brown medial stripe that extends from the mental onto the first 15 ventrals. Longitudinal ventral stripes absent. All adult females we have examined (including sequenced material) have a red–orange stripe on the underside of the head and onto the first 12 anterior ventrals, and the coloration is present as spots on some upper labial scales.

In alcohol, (FMNH 27050) the coloration and pattern are much reduced. The head is gray–brown, the labials are white, and the marking on the lower labials, along with the reddish-brown chin, is usually absent. The ventral side of the head is uniform cream. The dorsum is gray with black lateral spots where the transverse blotches were in life. The ventrals are pale with dense mottling.

Geographic distribution This species occurs from Guatemala to Costa Rica in Central America.

Etymology The specific epithet is a patronym honoring Gunther Köhler, who has contributed greatly to our knowledge on the systematics and natural history of amphibians and reptiles, with particularly impressive contributions in Central America. Dr. Köhler has published more than 200 scientific articles and numerous books in English, Spanish, and German, greatly increasing scientific and public access to Central American herpetology.

Fig. 9 *Oxybelis koehleri* sp. nov. in life. **a** Mocorón, Gracias a Dios, Honduras (C.J. Franklin); **b** Santa Rosa, Costa Rica (L. Porras)

Comparison A vine snake with nine upper labials, three of which border the orbit, an eye diameter that is about equal to the length of the internasal, three upper labials in contact with the primary temporal, and the second pair of chin shields in contact for most of their length. Specimens of *O. aeneus* and those from northern South America have the second pair of chin shields separated for most of their length. Specimens from the western region have an eye diameter that is less than the length of the internasals while those from Panama usually have eight upper labials.

***Oxybelis microphthalmus* Barbour and Amaral, 1926**
Thrornscrub Vine Snake
Dryinus aeneus – Wagler, 1824
Oxybelis aeneus – Duméril et al. 1854: 819
Dryophis acuminata – Günther 1858: 156
Oxybelis acuminata – Boulenger 1896: 192
Oxybelis microphthalmus – Barbour and Amaral 1926
Oxybelis aeneus auratus – Bogert and Oliver 1945: 381
Oxybelis aeneus auratus – Zweifel and Norris 1955
Oxybelis aeneus – Keiser 1974: 7

Holotype MCZ 22417 from Calabasas Canyon, Arizona (circa 31° 28′ N, 110° 58′ W) designated by Barbour and Amaral 1926: 80

Diagnosis Using data from our examination of specimens from the western region, we constructed the following description for *Oxybelis microphthalmus*. A vine snake with (1) three upper labials (4–5–6) bordering the orbit; (2) black spots or bars on anterior body, dorsum mostly uniform brown with little black pigment; there are small scattered black spots on the dorsum; (3) venter is finely mottled and it can have a dark lateral stripe on the outer edge of each ventral, and a pale mid-ventral stripe; (4) eye diameter shorter than preocular; (5) second pair of chin shields in contact for most of their length; (6) eight upper labials in most Arizona and Sonora specimens, nine upper labials in other Mexican populations, but all tend to have three labials behind orbit; (7) snout from above is narrow, tapered, and rounded at the tip (snout type A); (8) supraoculars are longer than the prefrontals; (9) lower surface of the head is uniform white or yellow in color (not mottled); (10) last upper labial shorter than primary temporal; and (11) second upper labial does not contact the preocular.

The rostral is visible from above and followed by nine plate-like scales on the crown: a pair of internasals, a pair of prefrontals, the frontal and two larger supraoculars, and a pair of parietals. Preoculars extend slightly on to the crown between the prefrontals and supraoculars. In profile the nasal scale is elongate extending from the edge of the rostral, beyond the posterior edge of the internasal to the anterior border of the fused prefrontal–loreal. Eye diameter/internasal ratio in this species averages 0.82 ($n = 34$, $r = 0.58$–0.97, SD = 0.10). Preocular scale is long and greater in length than the diameter of the eye. Scales bordering the orbit are the preocular, the supraocular, two small postoculars, and upper labials 4–5–6 (rarely 5–6–7). Primary temporal contacts both postoculars, the parietal, and two secondary temporals, upper labials 7–8 or 7–8–9 or 6–7–8–9. Upper labials vary from 8 to 10. Of 66 sides of heads examined, 30 (45%) had 8 upper labials; 30 (45%) had 9 upper labials, and six (10%) had 10 upper labials. The shortest upper labial can be the first or the fifth. The longest upper labial is the eighth or ninth. Upper labials 1–2 contact the nasal, 2–3 contact the prefrontal–loreal, and 3–4 contact the preocular. The tallest upper labial can be the sixth or seventh. Lower labials range from 8 to 10, (usually 9). The first four labials (rarely five) contact the anterior chin shields; a total of six contact both pair of chin shields. The anterior pair of chin shields are shorter (about 50%) of the length of the second pair of chin shields; the second pair are in contact anteriorly and partially separated by a pair of scales posteriorly. Dorsal scales are in 17–17–13 rows with the posterior scales being weekly keeled.

Variation Geographic variation in the upper labial and ventral counts exists. Arizona (USA) and Sonoran (Mexico) populations tend to have eight upper labials, while Oaxaca and Guerrero (Mexico) populations have nine upper labials. Ventral counts in Arizona and Sonora tend to be at the high end of the range; populations to the south have lower numbers.

In males, total length varies from 1197 to 1337 mm ($n = 10$, $x = 1262.13$, SD = 73.17), SVL varies from 713 to 834 mm ($n = 10$, $x = 766.68$, SD = 46.37), tail lengths vary from 484 to 538 mm ($n = 8$, $x = 502.21$, SD = 35.99); tail/SVL ratios vary from 0.63 to 0.74 ($n = 8$, $x = 0.68$, SD = 0.03). Female total lengths vary from 667 to 1407 mm ($n = 14$, $x = 1238.0$, SD = 195.99); tails vary from 250 to 544 mm ($n = 12$, $x = 475.83$, SD = 107.19). Tail/SVL ratios in females vary from 0.60 to 0.72 ($x = 0.64$, SD = 0.04).

Ventrals in males vary from 184 to 202 ($n = 14$, $x = 192.0$, SD = 5.92); ventrals in females vary from 184 to 204 ($n = 12$, $x = 193.58$, SD = 5.2). Subcaudals in males vary from 163 to 175 ($n = 5$, $x = 168.4$, SD = 4.18); in females, subcaudals vary from 170 to 183 ($n = 7$, $x = 177.2$, SD = 4.22).

Coloration and pattern The crown of the head and upper face are brown to tan (Fig. 10). The upper labials and ventral surface of the head are a uniform cream. The transition in color is separated by a preocular dark brown stripe extending from the nasal scale, under the eye, and onto the anterior body. This stripe may continue as a series of spots onto the body. The first two scale rows on the anterior body are the same yellow color as the ventral surface, and form a ventrolateral stripe. At mid-body, the first four dorsal scale rows and the lower half of the

Fig. 10 *Oxybelis microphthalmus* in-life. **a** and **b** show the profiles while **c** shows the gaping mouth defensive behavior. Photograph **a** by JCM and **b** and **c** by J. Reyes-Velasco

fifth scale row are mottled heavily with dark pigment; the upper half or row five and rows 6–8 lack the dense mottling, giving the overall impression of a series of lateral stripes. On the ventral surface is an indistinct mid-ventral stripe.

In alcohol (UAZ 39545), the coloration has often faded but the elements of the pattern and the colors are still detectable.

Geographic distribution Southeastern Arizona southward to Oaxaca, Mexico

Comparison A vine snake with eight (Arizona and Sonora) or nine (remainder of distribution in Mexico) upper labials with three behind the orbit, an eye diameter that is about 0.8 of the internasal (no other species of *Oxybelis* has an eye diameter this small). It also has two or three upper labials in contact with the primary temporal and the second pair of chin shields are in contact for most of their length. *Oxybelis aeneus* and those from northern South America have the second pair of chin shields separated for most of their length. *Oxybelis koehleri* and those from Panama usually have two upper labials behind the orbit.

***Oxybelis potosiensis* Taylor,** 1941

Gulf Coast Vine Snake

Dryinus aeneus – Wagler 1824: 12

Oxybelis aeneus – Duméril et al. 1854: 819

Dryophis acuminata – Günther 1858: 156

Oxybelis acuminata – Boulenger 1896: 192

Oxybelis potosiensis – Taylor 1941

Oxybelis aeneus auratus – Bogert and Oliver 1945: 381

Oxybelis aeneus – Keiser 1974: 7

Holotype UIMNH 25069 (Fig. 11), a female from 36 km northwest of Ciudad Maíz (circa 22° 30′ N, 99° 56′ W), San Luis Potosí, Mexico, designated by Taylor, 1941: 128

Diagnosis Using data from our examination of specimens from Eastern Mexico, we constructed the following description for *Oxybelis potosiensis*. A vine snake with (1) two or three upper labials (4–5 or 4–5–6) bordering the orbit; (2) transverse black bars on the anterior body; (3) venter finely mottled, a stripe or stripes are not apparent; (4) eye diameter longer than preocular; (5) second pair of chin shields are in contact for most of their length; (6) nine upper labials, two or three upper labials behind the orbit; (7) snout from above is very broad, slightly tapered, and rostral is very rounded (snout type C); (8) supraocular and prefrontals about the same length; (9) last upper labial equal or greater in length than the primary temporal; (10) underside of head uniform white or cream; and (11) second upper labial does not contact the preocular.

Fig. 11 **a**–**d** Holotype of *Oxybelis potosiensis* Taylor 1941 (UIMNH 25069) from San Luis Potosí, Mexico; **a** whole specimen; **b** the crown, note the lack of a constriction anterior to the eyes, rostral not visible from above, and the relatively broad snout; **c** the profile; **d** arrangement of the chin shields

Description of holotype (Fig. 11) Rostral is visible from above; preoculars extend slightly on to the crown between the prefrontals and supraoculars, and the postoculars can be seen from above. Preocular scale is shorter than eye diameter. Scales bordering the orbit: preocular, the supraocular, two small postoculars, and upper labials 5–6 on the left and 4–5 on the right. The primary temporal is in contact with both postoculars, the parietal, two secondary temporals, and upper labials 6–7–8–9. Eight upper labials on the right and nine on the left; the shortest upper labial can be the first or the eighth; the longest upper labial is the last (eighth or ninth). Upper labials 1–2 contact the nasal, 2–3 contact the prefrontal-loreal, and 3–4 contact the preocular. The tallest upper labial can be the fifth or sixth. Lower labials 9–10; the first four contact the anterior chin shields, a total of six contact both pair of chin shields. The anterior pair of chin shields are shorter (about 60%) than the length of the second pair of chin shields; the second pair are in contact anteriorly and partially separated by a pair of scales posteriorly. Dorsal scales are in 17–17–13 rows. Ventrals 196, subcaudals 160.

Variation In males, total lengths vary from 1175 to 1535 mm ($n = 4$, $x = 1371.75$, SD = 164.08) and tails vary from 500 to 628 mm ($n = 4$, $x = 564.75$, SD = 58.09). Tail/SVL ratios in males vary from 0.67 to 0.69 ($x = 0.68$, SD = 0.01). In females, total lengths vary from 804 to 1272 mm ($n = 3$, $x = 1083.33$, SD = 201.53), SVL vary from 734 to 804 mm ($n = 3$, $x = 761.67$, SD = 30.40), and tail lengths vary from 440 to 525 mm ($n = 2$, $x = 482.5$, SD = 42.50); one female had a tail/SVL ratio that was 0.70.

Ventrals in males vary from 174 to 190 ($n = 6$, $x = 185.67$, SD = 5.73); ventrals in females vary from 186 to 195 ($n = 3$, $x = 191.67$, SD = 4.03). No subcaudal counts were taken because of broken or questionable tail tips.

Size: To at least 1290 mm total length

Coloration and pattern In alcohol (UIMNH 25069), head and body gray–brown, upper labials cream and separated from the gray–brown by a black stripe; ventral surface of head cream transitioning to yellow posteriorly. Transverse black bars on the anterior body. No ventral stripes (Fig. 11).

Geographic distribution This species occurs in San Luis Postosí and northern Veracruz, southward to Yucatan, Mexico, and Belize.

Comparison A vine snake with nine upper labials, two bordering the orbit, eye diameter greater than the length of the preocular, and second pair of chin shields in contact for most of their length. Specimens of *Oxybelis aeneus* and those from northern South America have the second pair of chin shields separated by smaller scales. Specimens of *O. aeneus*, *O. koehleri*, *O. microphthalmus*, and those from Panama have three upper labials bordering the orbit.

***Oxybelis rutherfordi* sp. nov.**

Rutherford's Vine Snake

Dendrophis auratus – Court 1858: 411

Dryiophis aeneus – Garman 1887: 284

Oxybelis acuminatus – Mole and Urich 1894: 86

Oxybelis aeneus aeneus – Bogert and Oliver 1945: 381

Oxybelis aeneus – Beebe 1952: 175

Oxybelis A. aeneus – Wehekind 1960: 75

Oxybelis ae. aeneus – Mertens 1972: 18

Holotype UTA R-64851 (Figs. 12 and 13), from Trinidad, Arima Valley, William Beebe Tropical Research Centre, circa 6 km N Arima, 247 m, 10° 41′ 32″ N, 61° 17′ 22″ W. Collected by Mike G. Rutherford 20:00 h, 31 March 2018. Measurement: SVL 745 mm, total length 1245 mm

Paratypes FMNH 215839, Trinidad, circa 2 miles south of Simla-Quarry Rd., on Arima-Blanchisseuse Rd. (10° 39′2 1.73″ N, 61° 17′ 22.77″ W), JCM, M. Dloogatch, and Reznick; FMNH 49978 and 49982 Trinidad, San Rafael (10° 33′ 59 ″ N, 61° 15′ 59″ W); FMNH 215838, circa 3 mi. south of Simla-Quarry Rd., on Arima-Blanchisseuse Rd., egg farm (10° 39′ 38″ N, 61° 17′ 22″ W), collected by JCM, M. Dloogatch, and R. Humbert; MBLUZ 1268, between San Francisco de Macanao and Cerro Los Cedros, Isla de Margarita, Nueva Esparta, Venezuela (11° 01′ 34″ N, 64° 17′ 30″ W) by Gilson Rivas, Eusebio Millán, Ángel Fernández, and Reina Gonto on 10 October 2013. FMNH 17839–40, Puerto Viejo, Península de Paria, Sucre, Venezuela.

Diagnosis Using data from our examination of specimens from northern South America, we constructed the following description for *Oxybelis rutherfordi*. A vine snake with (1) two upper labials (4–5) bordering the orbit; (2) black spots or bars on anterior body, dorsum mostly uniform brown with little black pigment; there are small scattered black spots on the dorsum; (3) venter finely mottled with a pale mid-ventral stripe; (4) preocular shorter than the diameter of the eye; (5) second pair of chin shields in contact for most of their length; (6) eight upper labials with three labials behind orbit; (7) snout from above narrow, tapered, and rounded at the rostrum (snout type B); (8) supraoculars longer than the prefrontals; (9) last upper labial longer than the primary temporal; (10) lower surface of head uniform in color; (11) second upper labial does not contact the preocular.

Description of holotype (UTA R-64851, Fig. 12) A female, SVL 735 mm, tail length 510 mm. Rostral broader than tall, barely visible from above; upper labials 8/8; internasals paired, extending to the anterior border of the second upper labial but not past the nasal, which is longer than both and extends to the middle of the second upper labial; prefrontals paired, contact upper labials 2–3 and nasal; frontal (circa 6.5 mm long) and supraoculars (circa 6 mm long) elongated; paired parietals circa 7.5 mm; supraoculars and parietals, contact upper postocular; postoculars 2/2, upper larger; upper labials 3–4 contact the

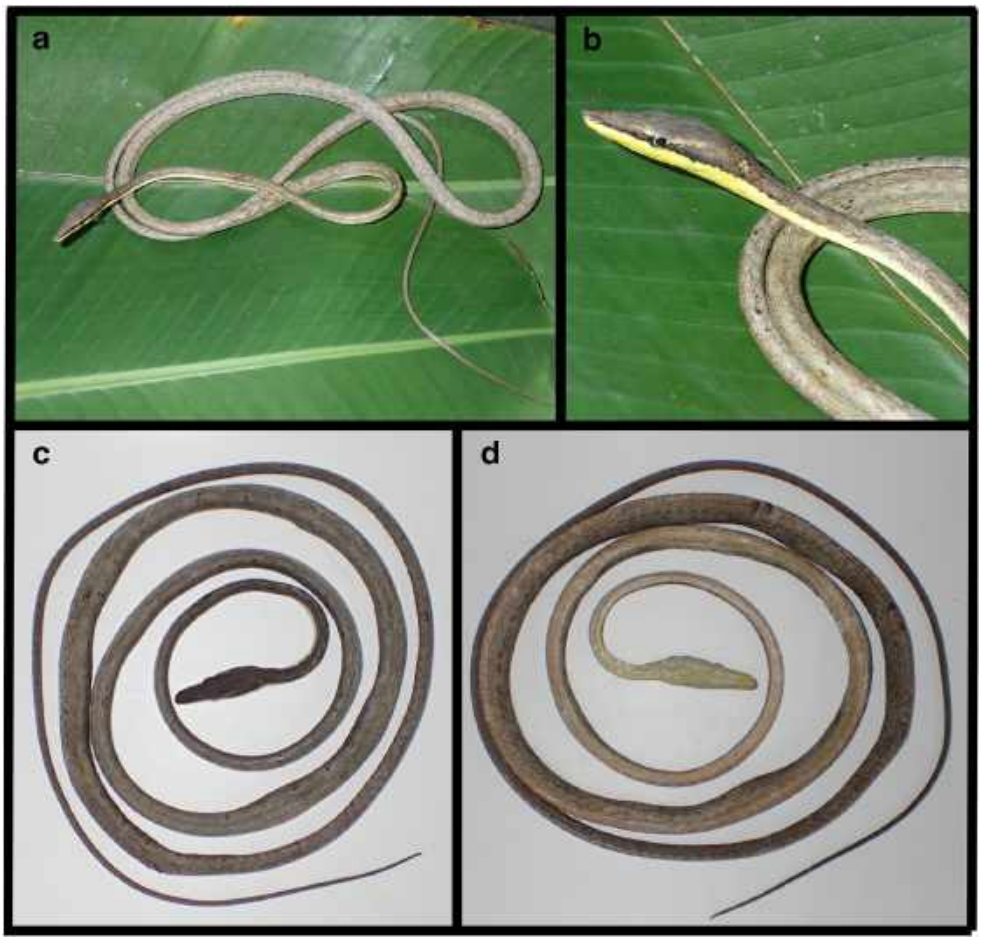
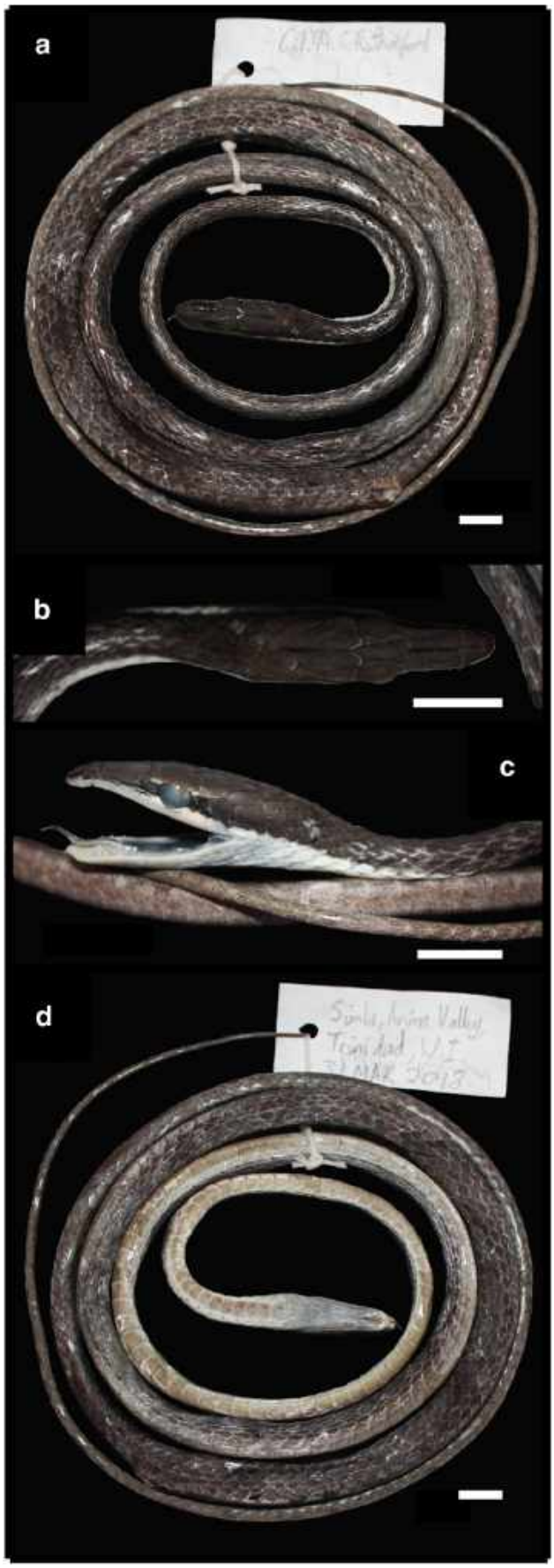


Fig. 12 Holotype (UTA R-64851) of *Oxybelis rutherfordi* sp. nov. in life prior to preservation collected in the Arima Valley, Trinidad (M.G. Rutherford)



◀ **Fig. 13** Holotype *Oxybelis rutherfordi* sp. nov., UTA R-64851, preserved; **a** dorsal; **b** top of the head; **c** profile; **d** ventral views. Scale bar = 1 cm

preocular, 4–5 are in the orbit; 6–7–8 contact the primary temporal; 6 interrictals; one preocular less than the diameter of the eye; lower labials 8/8, first four contact the first pair of chin shields; second pair of chin shields longest; three paired gulars. Dorsal scales smooth in 17–17–13 rows. Ventrals in eight males vary from 183 to 188 ($x = 185$, SD = 1.87); ventrals in eight females vary from 180 to 190 ($x = 184.63$, SD = 3.42).

Variation: Rostral visible from above and followed by nine plate-like scales on the crown: a pair of internasals, a pair of prefrontals, the frontal and two larger supraoculars, and a pair of parietals. The preoculars extend slightly on to the crown between the prefrontals and supraoculars. Internasals are about 0.83 of the prefrontals. Preoculars are about 55% of the eye diameter. In profile, the nasal scale is elongate extending from the edge of the rostral, beyond the posterior edge of the internasal to the anterior border of the fused prefrontal–loreal. The preocular scale is short and less than the diameter of the eye. Scales bordering the orbit are the preocular, supraocular, two small post oculars, and upper labials 4–5 or 5–6. The primary temporal contacts both postoculars, the parietal, and two secondary temporals, as well as upper labials 6–7–8. Upper labials can be seven to nine, but most often eight. The shortest upper labial can be the first or the eighth. The longest upper labial is the last (eighth or ninth). Upper labials 1–2 contact the nasal, 2–3 contact the prefrontal–loreal, and 3–4 contact the preocular. The tallest upper labial can be the fifth or sixth. Lower labials can number seven through 10, usually eight; the first four contact the anterior chin shields; a total of six contact both pair of chin shields. The anterior pair of chin shields are shorter (about 50%) than the length of the second pair of chin shields; the second pair are in contact anteriorly and partially separated by a pair of scales posteriorly. Dorsal scales are in 17–17–15 rows.

In males, total lengths vary from 671 to 1475 mm ($n = 8$, $x = 1075.25$, SD = 210.45), SVL varies from 391 to 860 mm ($n = 8$, $x = 658.75$, SD = 128.1), tail lengths vary from 280 to 449 mm ($n = 7$, $x = 422.14$, SD = 65.94), and tail/SVL ratios vary from 0.64 to 0.73 ($n = 7$, $x = 0.70$, SD = 0.03). In females, total lengths vary from 831 to 1274 mm ($n = 8$, $x = 1090.63$, SD = 166.01) and tails vary from 382 to 521 mm ($n = 7$, $x = 464.0$, SD = 56.15). Tail/SVL ratios in females vary from 0.643 to 0.715 ($x = 0.68$, SD = 0.027).

Ventrals in males vary from 183 to 188 ($n = 8$, $x = 185.0$, SD = 4.88); ventrals in females vary from 180 to 190 ($n = 10$, $x = 184.63$, SD = 3.42). Subcaudals in males vary from 163 to 175 ($n = 5$, $x = 168.4$, SD = 4.18). Ventrals in females vary from 180 to 190 ($n = 8$, $x = 184.63$, SD = 3.42); subcaudals in females, 162–171 ($n = 6$, mean = 166.33, SD = 3.25).

Size: To at least 1668 m in total length based on UWIZM.2012.27.49.

Coloration and pattern (Figs. 12 and 14) Crown is brown sometimes with small black spots. Overall head and body brown or gray–brown, upper and lower labials intense yellow and separated from the brown by a black stripe on the dorsal edge of the second labial that extends past the eye to the last labial; ventral surface of head yellow transitioning to cream posteriorly. Small black dash-like marks on scale rows 1–7 on the upper edge of each scale, separated by two scale rows. Ventral surface is yellow to cream with finely stippled pigment most restricted to the outer edges of the ventrals leaving an indistinct mid-ventral stripe. Ventrals may have small black marks with irregular borders. In preservative, the brown pigment fades to gray.

Geographic distribution It occurs on both islands of Trinidad and Tobago and on the adjacent mainland of Venezuela, including Margarita Island and Los Testigos Archipelago. The species also is present in most of Northern Venezuela from the eastern Andes to the Peninsula de Paria, including the coastal ranges, and into French Guiana. It also occurs in lowland areas such as the Llanos and the Orinoco Delta in the Venezuelan Guayana.

Remarks Population from the Maracaibo-like basin and those from the Cordillera de Merida on the Venezuelan Andes need to be evaluated to assess if these are conspecific with *O. rutherfordi*. We believe it is likely that the population from southern and Amazonian Venezuela are *O. aeneus*.

Habitat The holotype was resting coiled on a branch of *Miconia* sp. (family Melastomataceae) shrub at night, in a garden approx. 5 m from a building. Top of the head and dorsum light brown with occasional dark flecks, lateral stripe on head darker brown, venter yellow on head and first few centimeters of body then fading to light tan. This species has been found in relatively open habitats at Aripo Savanna in Trinidad as well as in secondary forests and in forest edge situations. On Tobago, it was found in roadside vegetation and in old cacao

Fig. 14 *Oxybelis rutherfordi* sp. nov. in life. **a** and **b** Tobago specimens. JCM; **c** Aripo Savanna, Trinidad. JCM; **d** an exceptionally large specimen (2.1 m) from Tobago (M. Patrikeev)

plantations. In Venezuela, MBLUZ 1268 was collected active in a bush circa 1.5 m above the ground on a dry creek.

Etymology This snake is named in honor of Mike G. Rutherford, collector of the holotype and curator of the Zoology Museum at the University of the West Indies, for his contributions to the zoology and natural history of Trinidad and Tobago.

Comparison A vine snake with eight upper labials, two of them border the orbit, three are behind the orbit, and the shortest is the seventh. All other brown vine snake species have eight or nine upper labials with three bordering the orbit and the shortest is the seventh. *Oxybelis aeneus*, *O. koehleri*, *O. microphthalmus*, and those from Panama have eight or nine upper labials and the fifth is the shortest.

***Oxybelis vittatus* (Girald 1854)**

Striped Vine Snake

Dryinus aeneus – Wagler 1824: 12

Dryophis vittatus – Girard 1854: 226

Oxybelis aeneus auratus – Bogert and Oliver 1945: 381

Oxybelis aeneus – Keiser 1974: 7

Holotype USNM 7315 from Taboga Island, Bay of Panama, Panama. Girard (1854) reports the holotype has 193 ventral scales and 165 plus subcaudal scales (the tail is broken).

Diagnosis Using data from our examination of specimens from Panama, we constructed the following description for *Oxybelis vittatus*. A vine snake with (1) three upper labials (4–5–6) bordering the orbit; (2) transverse black bars or spots on the anterior body; (3) a pale, indistinct stripe on the mid-ventral, and black mottling; (4) eye diameter greater than length of preocular; (5) second pair of chin shields in contact for most of their length; (6) eight upper labials, three behind the orbit; (7) from above the snout is tapered and terminally rounded (snout type A); (8) supraoculars longer than the prefrontals; (9) last upper labial longer than primary temporal; (10) lower surface of head uniform and pale; and (11) second upper labial does not contact the preocular (Fig. 15).

Variation Rostral is barely visible from above and followed by nine plate-like scales on the crown: a pair of internasals, a pair of prefrontals, the frontal and two larger supraoculars, and a pair of parietals. Preoculars extend slightly on to the crown between the prefrontals and supraoculars. In profile, the nasal scale is elongate extending from the edge of the rostral, beyond the posterior edge of the internasal to the anterior border of the fused prefrontal–loreal. Preocular scale is long and greater in length than the diameter of the eye. The eye diameter/internasal ratio in this species averages 1.16 ($n = 9$, $r = 1.05–1.31$, SD = 0.17). Scales bordering the orbit are the preocular, the supraocular, two postoculars (the upper one is usually the largest), and upper labials 4–5–6. The primary temporal contacts both postoculars, the parietal, two secondary temporals, and upper labials 5–6–7–8, 6–7–8, or 6–7–8–9 are common arrangements. Upper labials range from 8 to 10. Of 20 sides examined, 13 (65%) had 8 upper labials, six (30%) sides had 9 upper labials, and one side (0.05%) had 10 upper labials. The shortest upper labial can be the fourth or the fifth. The longest upper labial is the last eight, nine, or ten. Upper labials 1–2 contact the nasal, 2–3 contact the prefrontal–loreal, and 3–4 contact the preocular. The tallest upper labial can be the sixth or seventh. Lower labials range from 7 to 9 (usually 8 or 9). The first four (rarely five) contact the anterior chin shields; a total of six contact both pair of chin shields.

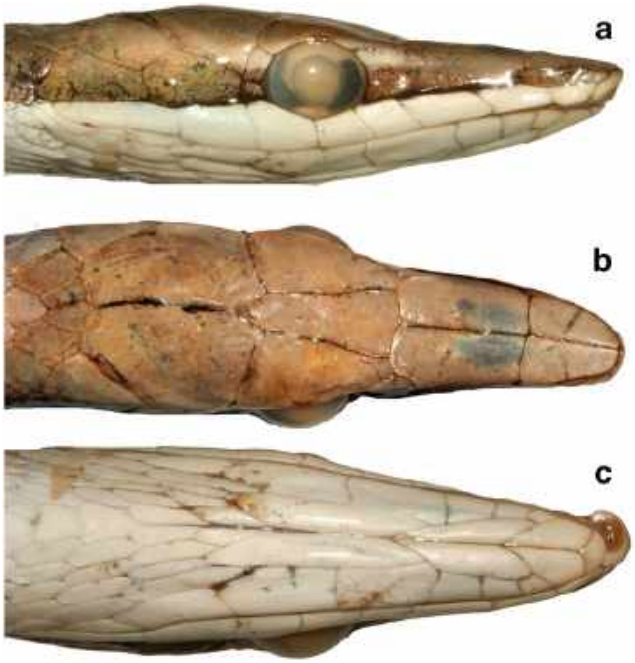


Fig. 15 *Oxybelis vittatus*. FMNH 170133, from the Canal Zone of Panama. **a** Profile; **b** crown; **c** underside of head

In males, total lengths vary from 1149 to 1323 mm ($n = 2$, $x = 1236.0$ SD = 87.00), SVL varies from 653 to 763 mm ($n = 2$, $x = 708$, SD = 55.00), tail lengths vary from 460 to 560 mm ($n = 2$, $x = 505.33$, SD = 41.35), and tail/SVL ratios vary from 0.73 to 0.76 ($n = 2$, $x = 0.74$, SD = 0.01). In females, total lengths vary from 940 to 1345 mm ($n = 7$, $x = 1138.57$, SD = 128.85), SVL varies from 510 to 832 ($n = 7$, $x = 670$ mm, SD = 95.53), and tails vary from 410 to 518 mm ($n = 7$, $x = 505.33$, SD = 41.35). Tail/SVL ratios in females vary from 0.62 to 0.84 ($x = 0.74$, SD = 0.01).

Ventrals in males vary from 179 to 187 ($n = 5$, x = 183.4, SD = 3.38); ventrals in females vary from 182 to 193 (n = 7, x = 186.83, SD = 4.02. Subcaudals not counted because of damaged tails.

Coloration and pattern The crown of the head and upper face are brown to tan; they may be uniform or slightly mottled. Upper

labials and ventral surface of the head are a uniform cream. The transition in color is separated by a preocular dark-brown stripe extending from the nasal scale, under the eye, and onto the anterior body. This stripe may continue as a series of spots onto the body. On the anterior body, the first two scale rows are the same yellow color as the ventral surface, and form a ventrolateral stripe. At mid-body, the first four dorsal scale rows and the lower half of the fifth scale row have a dark pigment streak in the middle of the scale giving the impression of a faint stripe on each scale row; scale rows 6–8 lack the dark pigmentation. The ventral surface is often flecked with dark pigment and indistinct lateral stripes on the edges of the ventrals.

Geographic distribution Panama likely southward into the Chocoan region of Colombia

Comparison A brown vine snake with eight upper labials, two behind the orbit, and the fifth is the shortest; the second pair of chin shields are in contact for most of their length, and the primary temporal is shorter than the last upper labial. *Oxybelis aeneus* and *O. rutherfordi* have the second pair of chin shields separated; *O. koehleri* and *O. microphthalmus* usually have three upper labials behind the orbit, and the last upper labial is equal to the length of the primary temporal.

Discussion

Biologists traditionally have relied on morphological traits to sort species, resulting in an underestimation of diversity and incomplete understanding of the lineages' evolutionary history (Beheregaray and Caccone 2007). Many cryptic taxa have remained unrecognized because morphological variation may be subtle and overlooked or interpreted as individual variation or variation associated with a cline (Oliver et al. 2009; Padial et al. 2010). With the increase in molecular phylogeographic studies, numerous wide-ranging "species" are found to be complexes harboring cryptic species-level lineages (e.g., Bickford et al. 2006; Jadin et al. 2012; Murphy et al. 2016; Ruane et al. 2018).

Vine snakes in the *Oxybelis aeneus* complex appear incredibly diverse (Fig. 1) yet are conservative in much of their external morphology, making diagnoses difficult. Like other vine snakes, they are attenuated with an elongated head distinct from the forebody with a stereotypical nine plate-like scales on the crown: a pair of internasals, a pair of prefrontals that fuse with the loreal, the frontal, two supraoculars, and a pair of parietals. In populations of the *O. aeneus* complex, scales bordering the orbit include the preocular, the supraocular, two small postoculars, and upper labials 4–5–6 in most taxa, but 4–5 or 5–6 in others. Most taxa have upper labials 1–2 contacting the nasal, 2–3 contacting the prefrontal–loreal, and 3–4 contacting the preocular. The crown of the head and upper face are usually golden brown to tan with upper labials and the ventral surface of the head usually a uniform cream. The color change is separated by a dark-brown or black preocular stripe extending from the nasal scale, under the eye, and onto the anterior body.

Despite the similarities listed above, our morphological examination of specimens from across the range suggests the *O. aeneus* complex is composed of numerous species that do possess some distinguishing features (Table 5). Subsequently, our phylogenetic analyses of *Oxybelis* lineages (Figs. 3 and 5) support the presence of at least eight distinct species under the general lineage concept of species (de Queiroz 1998), a considerably greater number than the four taxa previously recognized. Finding diagnostic characters for each species is challenging but possible, such as specimens of *O. rutherfordi* consistently showing the fourth and fifth upper labial bordering the orbit. Likewise, some species are defined by having combinations of characters not found in their congeners such as *O. microphthalmus* having a preocular longer than the diameter of the eye and a narrow, tapered snout with a barely rounded rostrum, while *O. potosiensis* has a preocular less than the diameter of the eye and a broad, only slightly tapered snout with a very rounded rostrum. These features, and others, are described for distinguishing among the taxa we recognize above.

Future prospects

Recently, Hillis (2019) reviewed the importance of proximate sampling in taxonomy and the need for proper interpretation of multispecies coalescent models. Similarly, Chamber and Hillis (2020) argue that new species designations for widely distributed taxa should address multiple evidences of geographic variation (i.e., behavior, genes, morphology) as well as data on reproductive isolation and gene flow. In concert with these recommendations, this work advances our understanding of the species diversity and evolutionary history of New World vine snakes beyond the publication of Jadin et al. (2019), which strictly focused on molecular analyses suggesting multiple taxa. In this study, we followed, where possible, such observations by limiting our systematic analyses to the northern distribution of the genus, as southern population sampling remains deficient. Our increased molecular sampling has added described taxa (e.g., *O. brevirostris* and *O. potosiensis*) and new populations to our phylogenetic analyses, while novel morphological data have allowed us to justify several lineages of brown vine snakes as distinct. Additional molecular and morphological sampling of populations within the range of what was formerly considered *O. aeneus*, especially throughout South America, surely will recover novel taxa not identified in this study.

Table 5 A morphological comparison of six species in the *Oxybelis aeneus* complex

	O. aeneus	*O. koehleri*	*O. microphthalmus*	*O. potosiensis*	*O. rutherfordi*	*O. vittatus*
Labials in orbit	4–5–6	4–5–6	4–5–6	4–5 or 5–6	4–5-6	4–5–6
Number of labials (*x*), no. behind orbit	(9) 3	(9) 3	(8) 3 AZ/Sonora (9) 3 s. Mexico	(9) 2 (9) 3	(8) 3	(8–10) 3
Stripes on venter	None	Mid-ventral stripe or none	One mid-ventral, one on outer edge of each ventral	One or none	Mid-ventral stripe	Variable
Preocular shorter than eye diameter	Yes	Yes	No	Yes	Yes	Usually
Posterior border of internasals extends beyond posterior edge of first upper labial	Yes	Yes	Yes	Yes	No	No
Chin heavily mottled	No	Yes, in females	No	No	No	No
Supraocular longer than prefrontal	No, equal in length	No, equal in length	Yes	Yes	Yes	Yes

Beyond the addition of species, the broad geographic distribution of these taxa, particularly those populations formerly identified as *O. aeneus*, indicates that greater sampling of these taxa will not only allow for a more in-depth understanding of the species diversity and evolutionary history of this complex but also provide a more complete understanding of the geological and biological processes driving diversification across the Neotropics. In line with this, molecular dating and genetic analyses of *O. vitattus* in South America may further explain the role of the Panamanian isthmus closing and the colonization of South America by New World vine snakes. Genetic assessment of Trans- and Cis-Andean populations will elucidate vicariant speciation events and the timing of such. Furthermore, establishing more accurate species distribution ranges will aid in the understanding of habitat boundaries and habitat selection pressures between populations and species contact zones (e.g., *O. koehleri* and *O. brevirostris*). For example, sampling in Costa Rica will clarify the southern range of *O. koehleri* and likely northern range of *O. vittatus*.

Finally, our findings include strong support for a paraphyletic *O. fulgidus* with respect to *O. wilsoni* and that the Isthmus of Panama is the likely dividing point between two divergent sister clades. Although our analyses support the splitting of *O. fulgidus* north and south of the Isthmus of Panama, we recommend maintaining the current taxonomy until a more thorough taxonomic revision of the green vine snakes is complete.

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Data availability The molecular data generated during the current study are available in the GenBank repository [https://www.ncbi.nlm.nih.gov/genbank/] while the morphological datasets are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Appendix 1. Specimens examined

Museum acronyms follow Sabaj (2019).

Oxybelis aeneus—($n = 8$) *Brazil*: FMNH 64417 Amazonas; FMNH 19203 Pará; KU R-124605, 124606, 140173, MCZ R-2582, 2778, and 53211 Pará

Oxybelis brevirostris—($n = 2$) *Ecuador*: UTA R-55952-53 Canton San Lorenzo: Parroquia Santa Rita, Esmeraldas

O. koehleri—($n = 34$) *Costa Rica*: FMNH 179061 Cartago, Turrialba; *El Salvador*: FMNH 10997 Chalatenango; San Jose del Sacare, 3600′; FMNH 10998 Morazán, Divisadero; FMNH 64955, La Libertad, Volcan San Salvador, 1917 Lava, 500 m; FMNH 64956 La Paz, Los Blancos; KU 289907 Usulutan: Isla San Sebastian; *Guatemala*: FMNH 20088 Izabal: Bobos Plantation, near Playitas; FMNH 20171 and 20418 Sololá: Olas de Moca; UTA R-46795 Chiguimula; UTA R-45880 Huehuetenango; UTA R-22182–83, 33,040, 33,042, 37,256, 39,236, 42,433 Izabal; UTA R-37258 Peten; UTA R-46846 Zacapa, El Arenal; *Honduras*: FMNH 22231 Tela; FMNH 27050 San Pedro Sula; FMNH 34565, 34571, 34574, 34576, Bay Islands: Roatan, near Coxen Hole; UTA R-55231 Bay Islands: Roatan; FMNH 34770 Yoro, Portillo Grande; FMNH 40872 Gracias; UTA R-46865 Comayagua, Playitos: Aldea "Lo de Reina," 785 m; UTA R-53176–78 Honduras: Gracias a Dios, Mocorón, 30–50 m. *Nicaragua*: UTA R-44838 Jinotega, El Paraíso Km 152.5, carretera Jinotega-Matagalpa, 1490 m

O. microphthalmus—($n = 36$) *USA*: Arizona: UAZ 47314 2.8 mi west of Sycamore Canyon; UAZ 519,225 miles east Sycamore Canyon, Ruby Rd.; UAZ 39544 Patagonia Mts.; Santa Cruz County: ASU 33314, ASU 33364, ASU 35069, ASU 35563, UAZ 16787, UAZ 39545; no specific locality: UMMZ 75779. *Mexico*: Colima: UTA R-57658; Guerrero UAZ 106056, 106058, 38448, 38451, 38455, 38461, 38467, 106051, 106057, 106059, 106054; Oaxaca: UAZ 106055, 117841–43, 178707, 178708; Sonora: UAZ 26972 0.5 miles West Alamos; UAZ 28279 8.8 miles east Alamos; Alamos UAZ 16797, UAZ 26973, ASU 06735, ASU 68990, ASU 88990; 35 miles east of Cannansa junction w/ Aqua Prieta Rd. UAZ 16796

O. potosiensis—($n = 6$) *Mexico*: UIMNH 25069 San Luis Potosí; UTA R-6107–10, 8752, and 12,368 S of Zapotitl, Puebla; UTA R-9014 6.0 mi E San Rafael, road to Rancho Nuevo, Tamaulipas

O. rutherfordi—($n = 20$) *Tobago*: FMNH 251213 Bloody Bay Rd., between Roxborough and Bloody Bay; *Trinidad*: FMNH 49973 no specific locality; FMNH 49974–75 Brickfield; FMNH 49976 Mount Harris, FMNH 49977–85 San Rafael; FMNH 215838 circa 3 miles S Simla-Quarry Rd., on Arima-Blanchisseuse Rd., egg farm; FMNH 215839 circa 2 miles S Simla-Quarry Rd., on Arima-Blanchisseuse Rd.; UTA R-64851 Arima Valley, William Beebe Tropical Research Centre, c. 6 km N Arima, 247 m; *Venezuela*: FMNH 17839–40 Puerto Viejo, Península de Paria, Sucre; MBLUZ 1268 between San Francisco de Macanao and Cerro Los Cedros, Isla de Margarita, Nueva Esparta.

O. vittatus—($n = 16$) *Panama*: FMNH 152067 Almirante; FMNH 83552, 130674, 131314 Canal Zone: Summit; FMNH 161478 Canal Zone: Barro Colorado Island; FMNH 153665 Coiba Island; FMNH 170132 San Blas Territory: Soskantupu, 8° 57′ N, 77° 44′ W, 1 m; FMNH 154043 Bocas del Toro, 11 km NW Almirante 600 ft.; FMNH 154478, 154517 no locality data; MCZ R-22274, 22231, and 25118 Canal Zone; UF 65037, 65038, and 170469 Canal Zone.

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