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Unravelling a tangle of Mexican serpents: a systematic revision of highland pitvipers

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As most recently recognized, the name *Cerrophidion barbouri* Dunn, 1919, refers to a highland species of pitviper endemic to Guerrero, Mexico, of which *Agkistrodon browni* Shreve, 1938, is considered a junior synonym. This species is rarely collected and prior to recent decades it was known from only a few specimens. A careful re-examination of nearly all known specimens of *C. barbouri* and the type series of *A. browni* reveals that both names represent valid species and we therefore resurrect *A. browni*. Both species are extremely variable with respect to cephalic scalation and colour pattern, which has previously confounded efforts to identify them. We provide phylogenetic analyses using both Bayesian and maximum parsimony criteria of New World pitvipers to investigate the phylogenetic position of *A. browni* and *C. barbouri*. Our phylogenetic tree, based on 2235 bp of mitochondrial data [12S, 16S, cytochrome *b* (cyt *b*), NADH dehydrogenase subunit 4 (ND4)], strongly supports a clade consisting of *A. browni*, *C. barbouri*, and *Ophryacus melanurus*, which has a distant sister relationship to *Ophryacus undulatus*. Based on the deep phylogenetic divergences amongst these species and distinctive morphology we recommend that a new genus be recognized for *A. browni*, *C. barbouri*, and *O. melanurus*. Finally, we revise the genera *Cerrophidion* and *Ophryacus* in accordance with our new classification.

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ADDITIONAL KEYWORDS: Bayesian – *Cerrophidion* – hemipenes – morphology – new genus – *Ophryacus* – parsimony – phylogeny – snake.

INTRODUCTION

Mexico is well known for its herpetofaunal diversity of approximately 1200 known amphibian and reptile species (Flores-Villela & Canseco-Márquez, 2004), including many endemic genera (e.g. *Barisia*, *Charadrahyla*, *Chiropterotriton*, *Rhadinophanes*). In particular, pitvipers are extremely diverse in Mexico, with at least 56 species and nine genera of the total 115 species and 14 genera recognized in the New World (Campbell & Lamar, 2004; Campbell & Flores-Villela, 2008; Fenwick *et al*., 2009). These numbers of New World pitvipers have increased greatly from the 90 species in nine genera recognized only two decades ago (Campbell & Lamar, 1989). Our knowledge of pitviper diversity and relationships is constantly being refined as independent geographical lineages are distinguished and new species are discovered. With rapidly advancing phylogenetic methodologies, we are proceeding toward a more thorough understanding of the evolutionary histories of this remarkable group (see Gutberlet & Harvey, 2004; Castoe & Parkinson, 2006; Fenwick *et al*., 2009).

Cerrophidion barbouri is a pitviper restricted to the Sierra Madre del Sur in southern Mexico at elevations above 2000 m. Prior to some recent survey work, this rare species was known from only a few

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individuals and its evolutionary and natural history remains poorly known. Dunn (1919) described *Lachesis barbouri* from Omilteme, Mexico, and distinguished it from other vipers by its undivided subcaudals, 17 dorsal scale rows, and enlarged frontal plate. Specimens were obtained in the state of Guerrero for North American collections by Mr Wilmot W. Brown during the early decades of the 1900s (Campbell & Flores-Villela, 2008), and Shreve (1938) named *Agkistrodon browni* from Omilteme on the basis of two specimens collected by Brown. Shreve (1938) diagnosed the species only from Old World members of the genus *Agkistrodon* (*sensu lato*), with which he assumed it was allied, presumably based on its large cephalic plates. After observing variation in head scales and number of ventrals in *Cerrophidion godmani*, but lacking examination of *Cerrophidion barbouri* specimens, Smith (1941) concluded that similar trends present in *A. browni* could be attributed to sexual dimorphism, stating 'there is no reasonable doubt that *browni* and *barbouri* are synonymous.' *Agkistrodon browni* and *C. barbouri* possess external features, such as numbers of ventral and subcaudal scales, which overlap considerably. The most comprehensive works on *C. barbouri*, with *A. browni* considered as a synonym, have been those of Campbell (1977, 1988) and Campbell & Lamar (1989, 2004), who noted considerable variation amongst the morphology of *C. barbouri* but did not suggest the possibility of multiple species.

In this study we examine type material and all but three known specimens of *A. browni* and *C. barbouri*. We demonstrate the distinctiveness of *A. browni* from *C. barbouri* and provide detailed descriptions for both species. Additionally, we conduct a phylogenetic analysis using four mitochondrial gene fragments under Bayesian and parsimony criteria in order to assess the phylogenetic position of both *A. browni* and *C. barbouri* amongst the New World pitvipers. Our findings render the endemic Mexican pitviper genus *Ophryacus* paraphyletic and we therefore propose a new genus and systematic revisions of *Cerrophidion* and *Ophryacus*. Finally, we review the published natural history information attributed to *C. barbouri* (and by implication *A. browni*) and segregate this information for the two species.

MATERIAL AND METHODS

External morphological data

Based on the type series and original descriptions of *A. browni* (Shreve, 1938) and *C. barbouri* (Dunn, 1919), we separated the 27 *C. barbouri* (*sensu lato*) specimens into two morphotypes, '*Cerrophidion barbouri*' and '*Agkistrodon browni*'. External morphology of 14 *C. barbouri* and 13 *A. browni* was examined (Appendix S1; Table 1). Institutional abbreviations of specimens follow Leviton *et al.* (1985). Descriptions and nomenclature for characters are mostly from Klauber (1972), Campbell (1977, 1988), and Campbell & Lamar (1989, 2004) but particular methods for counting these characters have been described in the following morphological phylogenetic studies: Werman (1992), Wüster *et al.* (1996), Gutberlet (1998), Gutberlet & Harvey (2002), Jadin (2010), and Jadin, Gutberlet & Smith (2010). Scale count abbreviations follow citations: scales contacting third supralabial (Jadin Gutberlet & Smith no. 14, modified Wüster *et al.* no. 28; C3SL), counted as scales directly contacting third supralabial from rostral; scales contacting supraoculars (Wüster *et al.* no. 27; CSupOc); gulars (Gutberlet & Harvey no. 8; GLR); infralabials (IL); interoculabials (Gutberlet & Harvey no. 1; IOL); interrictals (Gutberlet & Harvey no. 7; IR); intersupraoculars (Werman no. 25; ISO); dorsal scale rows at midbody (Gutberlet & Harvey no. 10; NMSR); subcaudals (Gutberlet & Harvey no. 62; NSC); ventral scales (Gutberlet & Harvey no. 9; NVEN); prefoveals (Gutberlet & Harvey no. 2, Werman no. 37, in part; PF); postoculars (Jadin no. 19, Jadin, Gutberlet & Smith no. 16; PO); subfoveals (Gutberlet & Harvey no. 16; SF); supralabials (Werman no. 26; SL); suboculars (Gutberlet & Harvey no. 3; SO).

Additional characters were examined in the type specimens of *A. browni* and *C. barbouri* (Table 2) to firmly establish species allocation. Meristic characters and their abbreviations are as follows: canthals (Werman no. 32; CAN); dentary teeth (Gutberlet & Harvey no. 30; DNT); dorsal scale arrangement (DSA), number of dorsal scale rows one head length behind the head, at midbody, and one head length anterior to the vent; intercanthals (Jadin no. 21, Jadin, Gutberlet & Smith no. 18; IC); internasals (Jadin no. 20, Jadin *et al.* no. 15; IN); palatine teeth (Gutberlet & Harvey no. 28; PAL); prefrontal scales (PFR); pterygoid teeth (Gutberlet & Harvey no. 29, Werman no. 51, in part; PTY); preventral scales (PV), as defined in Dowling (1951); scales forward of frontal scale (SFF), number of dorsal head scales between frontal and rostral scales (i.e. prefrontals, canthals, and internasals); snout shape (SS), curvature of snout defined as being either pointed or round. The following mensural characteristics were obtained from the type series using a digital calliper or dissecting microscope with an optical micrometer, and were taken to the nearest 0.1 mm. Descriptions and abbreviations follow Grismer, Grismer & McGuire (2006) and Vogel, David & Pauwels (2004): distance between nostrils (DBN); distance from lower eye margin to bottom edge of the fourth supralabial, directly below (modified from Grismer *et al.*, 2006; DEL); distance from

anterior margin of eye to the posterior margin of the nostril (DETN); distance from the anterior edge of the eye to the posterior edge of the pit cavity (DETP); distance from the anterior edge of the eye to the rostral scale (DER); second supralabial height (H2SL); third supralabial height (H3SL); horizontal eye diameter (HED); head length (HL); head width (HW); second supralabial length (L2SL); third supralabial length (L3SL); length of frontal scale (LFS); loreal scale height (LH); loreal scale length (LL); length of supraocular scale (LSupOc); parietal scale length (PL); parietal scale width (PW); vertical eye diameter (VED); width of frontal scale (WFS); width of supraocular scale (WSupOc). The snout-to-vent length (SVL), tail length (TaL), and total body length (TL) were taken using a metre stick to the nearest millimetre.

Hemipenial preparations

We dissected and examined the left hemipenes from specimens deposited at MZFC and UTA (MZFC 2881 and UTA R-4450). We removed by dissection at the base. We fully everted hemipenes by filling them with warm water using a blunt-tipped syringe needle. We removed water and then injected hot liquid petroleum jelly with blue wax-dye until maximum expansion was achieved. Finally, we tied the organs at the base and stored them in 70% ethanol. This procedure is modified from that of Myers & Cadle (2003), Zaher & Prudente (2003), and Smith & Ferrari-Castro (2008). Hemipenial terminology follows Dowling & Savage (1960), Keogh (1999), and Savage (2002).

Molecular data

Genomic DNA from muscle tissue or ventral scale clips from three *A. browni* and one *C. barbouri* (Table S1) was isolated using a Qiagen DNeasy extraction kit and protocol. Four mitochondrial gene fragments – 16S rRNA, NADH dehydrogenase subunit 4 (ND4), 12S rRNA, and cytochrome *b* (cyt *b*) – were independently PCR amplified as described in (Knight & Mindell, 1993; Arévalo, Davis & Sites, 1994; Parkinson, Moody & Ahlquist, 1997; Parkinson, Campbell & Chippindale, 2002) using Promega GoTaq Green master mix, the primer pairs: 16SF + 16SR, ND4 + LEU, L1091 + 12E, and Gludg + AtrCB3, and annealing temperatures 45, 48, 50, and 48 °C, respectively. Either AMPure magnetic beads (Agencourt, Bioscience, Beverly, Massachusetts, USA) or ExoSap It (USB Corporation, Cleveland, Ohio, USA) were used to clean amplified fragments. Post PCR cleanup sequencing protocols were performed by SeqWright Inc. (Houston, Texas, USA; http://www.seqwright.com) or the University of Texas at Arlington genomics core facility (Arlington, Texas, USA; http://gcf.uta.edu). Sequencing was performed in both forward and reverse directions and sequence chromatographs were edited together using SEQUENCHER 4.2. Novel sequences from this study were deposited in GenBank (HM363639–HM363653). Previously published sequences of ingroup – 50 additional New World pitviper taxa – and outgroup taxa – *Deinagkistrodon acutus*, *Gloydius halys*, and *Protobothrops jerdonii* – were downloaded from GenBank (Table S1). *Deinagkistrodon acutus* was used to root every analysis. This taxonomic sampling includes all 14 previously recognized New World genera. Sequences for each gene were aligned separately, first automatically using the program MUSCLE (Edgar, 2004) and, then manually using Se-Al v. 2.0a11. This data set was further edited manually or transformed using GeneDoc (Nicholas & Nicholas, 1997). The entire 16S fragment was trimmed to 100% representation; the ND4 fragment contained nine taxa with 15 bp missing at one end and had one complete missing operational taxonomic unit (OTU) of *A. browni*, although this same taxon was represented by two other sequences; 12S was allowed to have only one 25 bp stretch of sequence missing from one taxon; and cyt *b* was allowed one sequence with missing data, fewer than 100 bp. In general there was never more than 3% of the total data per gene missing. More than 80% of the OTUs were always represented by the data. Gaps in alignments were treated as missing data and internal stop codons were not found in the two protein-coding gene fragments.

Phylogenetic analyses

Bayesian inference and maximum parsimony (MP) were implemented to reconstruct phylogenies. Model likelihoods for each gene fragment were independently calculated and models were chosen using the Akaike information criterion (AIC) in MrModelTestv2.2 (Nylander, 2004) and PAUP* v4.0b10 (Swofford, 2002). AIC scores for each gene fragment were found to best fit the general time reversible + invariant sites + gamma-distribution rate variation (GTR + I + Γ) model of evolution. The four gene fragments were concatenated into one NEXUS file (2235 total bp) and protein coding genes cyt *b* and ND4 were partitioned into three codon positions and ribosomal RNA loci 12S and 16S were partitioned into stems and loops, resulting in a total of ten partitions (model 10× in Castoe & Parkinson, 2006), and implemented for phylogenetic analyses.

Bayesian Markov chain Monte Carlo (MCMC) phylogenetic analyses were conducted using MrBayes v. 3.0b4 (Ronquist & Huelsenbeck, 2003). Two simulta-

neous runs of four MCMC analyses, consisting of one cold and three incrementally heated chains, were initiated with random trees for a total of 5.0×10^6 generations (sampling every 100 generations). The first 1.5×10^6 generations from each run were discarded as burn-in. We used TRACER v. 1.5 (Rambaut & Drummond, 2009) to detect stationarity in the Markov chain within the burn-in period.

Parsimony-based analysis of molecular data was conducted using PAUP* v. 4.0b10 (Swofford, 2002) under a heuristic search criterion using tree bisection-reconnection branch swapping and ten random addition sequence replicates with all characters weighted equally. A weighted parsimony (WP) analysis was conducted utilizing a tri-level weighting scheme (Benabib, Kjer & Sites, 1997; Flores-Villela *et al*., 2000) with gaps coded as a fifth base or 21st amino acid. Tri-level weighting incorporates three different levels of information on the structure and inferred function of nucleotide substitutions. Under this WP scheme, transitions have a weight of 1, transversions are weighted 2, and any nucleotide substitution that is inferred to cause an amino-acid substitution is weighted +1 more. For our parsimony analysis, the extremes of the gene fragments were further trimmed to exclude additional missing data that could potentially affect analysis when using gaps as an extra character state. This data set of 2235 bp – 16S (486 bp), ND4 (684 bp), 12S (411 bp), and cyt *b* (654 bp) – was coded for transition/transversion analysis (doubling the number of original characters), and the protein coding genes were transformed to amino acid (aa) sequence, ND4 – 227 aa, and cyt *b* – 217 aa in length. The coded sequence comprised a total of 4914 characters, 1272 parsimony informative. All raw DNA characters were independent but with different weights according to their biochemical properties. Weighted parsimony employed accelerated transformation optimization of character state changes. Bootstrap analysis (Felsenstein, 1985) involved 1×10^4 pseudoreplicates obtained via random addition sequence. The parsimony tri-level weighting approach of the combined gene data sets is justified by the comparative study of Kjer *et al*. (2007), who showed that this method outperforms all other methods, including MP, unpartitioned maximum likelihood, and Bayesian likelihood analyses.

Finally, to obtain an estimate of genetic distances we computed pairwise comparisons of the cyt *b* gene fragment between and within the various genera according to our classification. We calculated these distances with MEGA v. 4.1 (Tamura *et al*., 2007) and in accord with previous studies (e.g. Fenwick *et al*., 2009) incorporated the Kimura two-parameter model with Γ–distributed rate variation.

Natural history

Description of the habitat and natural history of *C. barbouri* and *A. browni* is from published accounts (i.e. Davis & Dixon, 1959; Campbell, 1977, 1988; Campbell & Lamar, 1989, 2004) and personal observations.

RESULTS

Morphological analyses

While examining 27 specimens of *C. barbouri* (*sensu lato*), including type material, we found distinct morphological differences that may be attributable to two species. *Agkistrodon browni* is readily distinguished from *C. barbouri* in having greater numbers of mid-dorsal scale rows, and fewer interoculabials, inter-supraoculars, inter-rictals, prefoveals, postoculars, scales contacting the third supralabial and supraocular, and subfoveal rows (Fig. 2, Table 1) and a prehensile tail (Fig. 3). Additionally, the type series of these two species differs greatly in these and additional characters providing further distinctions (see Table 2).

Furthermore, hemipenial features of *A. browni* and *C. barbouri* differ greatly (Fig. 4). A thorough description of the hemipenis of *A. browni* and *C. barbouri* is lacking and therefore included here. The everted left hemipenes of *A. browni* (UTA R-4450; SVL 397 mm, TaL 53 mm, subcaudals 31, Fig. 4A) and *C. barbouri*

Table 1. Morphological comparisons between *Cerrophidion barbouri* and *Agkistrodon browni*

	C. barbouri $N = 14$	*A. browni* $N = 13$
C3SL	5.15 (5 & 6)	4 (4)
CSupOc	10.25 (9–11)	8 (7–9)
GLR	3.44 (2–5)	3.85 (3–5)
IL	9.36 (8–10)	9.15 (9 & 10)
IOL	1 (1)	0 (0)
IR	24.53 (22–26)	20.09 (19–22)
ISO	4.36 (3–5)	1 (1)
NMSR	17.29 (17 & 19)	19 (19)
NSC	30.14 (27–32)	30.31 (27–35)
NVEN	140.14 (130–148)	138.91 (134–145)
PF	2.78 (1–6)	1.1 (0–2)
PO	3.3 (2–4)	1.92 (1 & 2)
SF	1 (1)	0 (0)
SL	8.61 (8–10)	8.04 (7–10)
SO	3.63 (3–5)	2.58 (1–3)

See Material and methods for character abbreviations.
Counts of bilateral characters were taken from each side and averaged.
Means are reported with ranges in parentheses.

Table 2. Measurements and counts of the type series of *Cerrophidion barbouri* and *Agkistrodon browni*

	C. barbouri USMN R-46347 female holotype	*A. browni* MCZ R-42678 male holotype	*A. browni* MCZ R-42679 female paratype
CAN	2/2	2/2	2/2
CSupOc	10/10	7/7	7/7
DBN	4.65	5.1	4.9
DEL	2.7/2.7	2.8/2.7	2.7/2.8
DETN	3.6/3.7	4.6/4.5	3.8/3.7
DETP	1.2/1.0	1.1/1.1	0.8/0.8
DER	5.0/5.2	6.3/6.2	5.0/5.0
DNT*	10	10	12
DSA	19–17-15	19–19-15	17–19-15
GLR	2/2	4/4	5/4
H2SL	1.2/1.1	1.5/1.7	1.4/1.4
H3SL	2.4/2.4	2.1/1.9	2.0/2.1
HED	2.9/2.9	3.1/3.2	2.8/2.8
HL	19.45	24.8	20.77
HW	13.11	18.0	14.95
IC	4	2	2
IL	9/9	9/9	9/9
IN	4	6	4
IR	24	19	19
ISO	3	1	1
L2SL	1.3/1.7	1.1/1.2	1.3/1.4
L3SL	2.0/2.3	2.4/2.4	2.0/1.8
LFS	2.6	4.6	4.5
LH	1.3/1.3	1.5/1.5	1.4/1.5
LL	1.8/1.9	2.3/2.3	2.0/2.0
LSupOc	4.6/4.7	6.0/6.0	5.1/5.1
PAL*	3	3	3
PF	3/5	0/0	0/0
PFR	?	2	2
PL	?/?	4.0/5.5	3.4/4.3
PO	3/3	2/2	2/2
PTY*	12	11	10
PV	4	3	2
PW	?/?	3.5/3.4	2.9/3.0
SC	31	31	27
SFF	16	9	8?
SL	8/9	8/8	8/8
SO	4/4	2/1	2/2
SS	Pointed	Round	Round
SVL	360	425	355
TL	404	480	391
TaL	44	55	36
VED	1.8/1.7	2.0/2.0	1.8/1.7
VEN	148	134?	141
WFS	2.9	3.8	3.0
WSupOc	2.4/2.3	3.7/3.5	2.9/2.9

*Right side only.
See Material and methods for character abbreviations.
Counts and measurements are written as right/left side, measurements taken in mm.

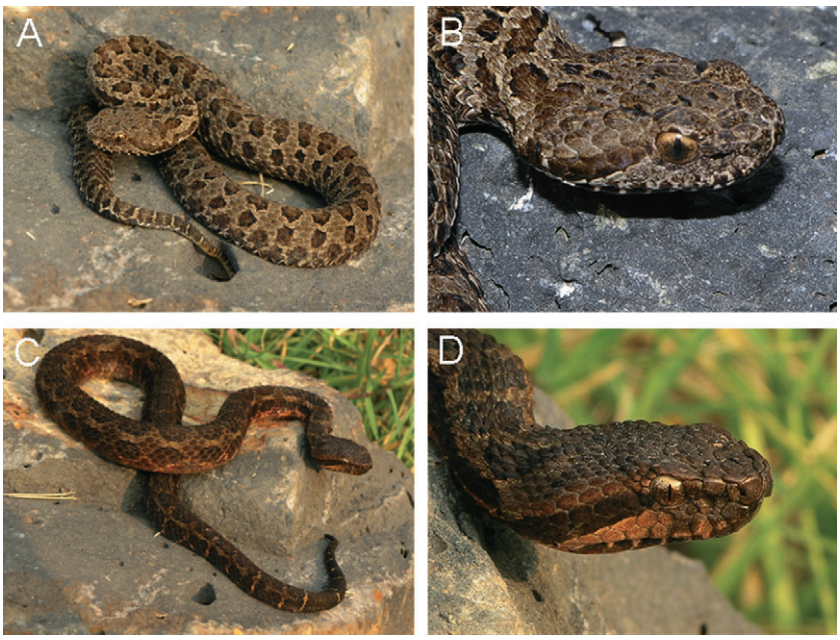

Figure 1. *Agkistrodon browni* (A, B; UTA R-56265) and *Cerrophidion barbouri* (C, D; MZFC 21432) in life, showing differences in head scalation and colour pattern.

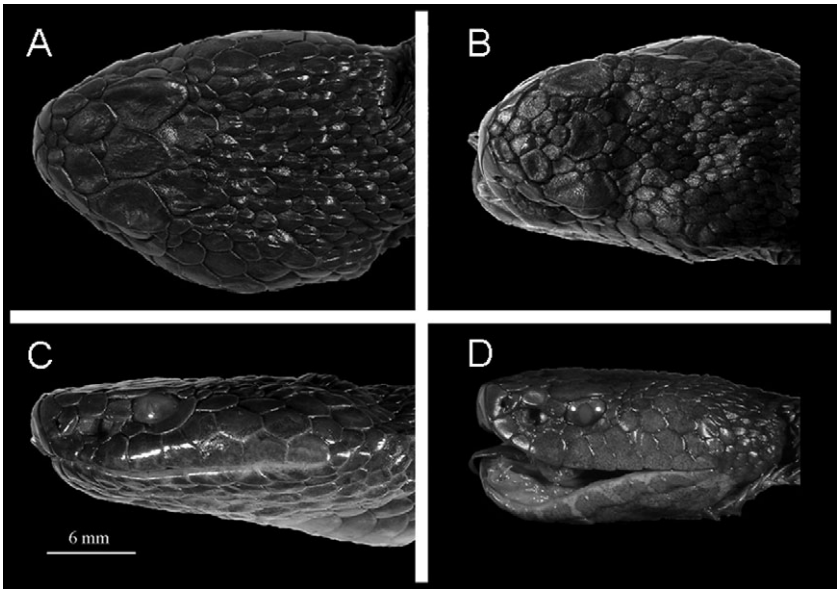

Figure 2. Dorsal view (A, B) and left side view (C, D) of *Agkistrodon browni* (holotype, MCZ R-42678; left) and *Cerrophidion barbouri* (holotype, USMN R-46347; right).

(MZFC 2881; SVL 445 mm, TaL 60 mm, subcaudals 32, Fig. 4B) are, respectively, 14 and 16 mm in length and 8 and 9.5 mm in maximum width at point of bilobation; on sulcate side base with several rows of small spines (< 0.6 mm) for 2 and 1.5 mm, then rows of larger spines and hooks extending for 3 and 4.5 mm, largest protruding 3.5 and 2.5 mm; asulcate side with naked base up to 3 and 2 mm before level of bilobation and then with 3.5 and 2 mm section of small spines (< 0.5 mm) arranged in rows followed by 2.5 and 3 mm section of larger spines; each lobe with 60 and > 35 spines and 20 and ~ eight hooks; 15 and 12 spines and hooks around each lobe at the lower rim of calyces; calyces follow spines and hooks distally; calyces scalloped and spinous or slightly scalloped, 14 rows extending > 4 and 6 mm to apex of the

Figure 3. Photo in life of *Agkistrodon browni* (UTA R-56264) showing its prehensile tail.

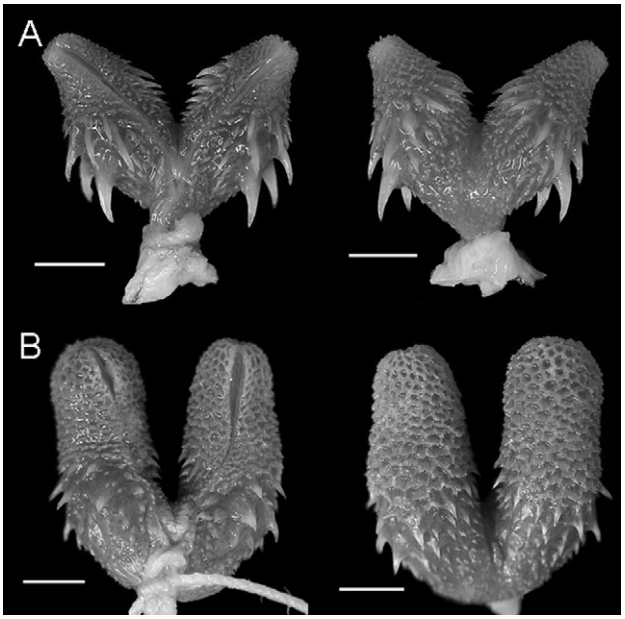

Figure 4. Sulcate (left) and asulcate (right) views of the left hemipenis of: A, *Agkistrodon browni* (UTA R-4450) and B, *Cerrophidion barbouri* (MZFC 2881). Scale bars = 3 mm.

hemipenis on the asulcate side; sulcus spermaticus bifurcates *c.* 2 mm before site of bilobation and extending upwards through spines and calyces to tip of each lobe; border of sulcus spermaticus naked to point of bilobation where small spines occur for 2 and 4 mm to level of calyces and forming the border to the apex of the lobe.

The most prominent feature of the hemipenes in *A. browni* are dramatically enlarged hooks, the largest being more than one-quarter of the length of the entire organ. The hooks of *A. browni* are not located at the base of the hemipenis, the condition characterizing most viperids, but rather are on the proximal portion of the hemipenial lobes, with smaller spines at the base of the organ. We are not aware of another pitviper species in which the spines are so disproportionally large or that have such large spines on the lobes. The hemipenes of *A. browni* further differ from those of *C. barbouri* by having much larger and nearly twice the number of spines and hooks. *Agkistrodon browni* has a smaller relative area of calyces covering the lobes, and the calyces are more scalloped and spinous than in *C. barbouri*.

Phylogenetic analyses

Our Bayesian and parsimony phylogenetic hypotheses are congruent with each other and no strongly supported conflicts exist. The weighted parsimony analysis recovered three optimal trees of 8165 steps each (Fig. 5). We chose the first of these trees presented by PAUP* with no a priori preference because the three optimal trees differed only in the arrangement of the closely related samples of '*Agkistrodon browni*', one of them lacking ND4. These hypotheses are mostly congruent with that preferred by Castoe & Parkinson (2006), showing strong nodal support for the monophyly of rattlesnakes (i.e. *Crotalus*, *Sistrurus*), the *Porthidium* group (i.e. *Atropoides*, *Cerrophidion*, *Porthidium*), the South American group (i.e. *Bothriopsis*, *Bothrocophias*, *Bothropoides*, *Bothrops*, and *Rhinocerophis*), and the genera *Agkistrodon* (excluding *A. browni*), *Bothriechis*, and *Lachesis*; although obtaining little or no support for both the backbone of the phylogeny and the monophyly of *Atropoides*. *Agkistrodon browni*, *C. barbouri*, and *Ophryacus melanurus* form a very strongly supported clade sister to *Ophryacus undulatus*. This phylogeny renders the endemic Mexican pitviper genus *Ophryacus* paraphyletic.

Genetic distances within New World pitviper genera range from 7.2 to 17.1% whereas distances between genera range from 11.6 to 25.5% (Table S2).

DISCUSSION

Taxonomic status of *Agkistrodon browni*

As a result of the distinctive morphological features (see Results; Tables 1, 2) and genetic differentiation (Fig. 5), we conclude that *A. browni* is a distinct species separate from *C. barbouri*. The large, flat head plates in *A. browni* readily distinguish it from the more moderately sized and usually keeled scales in *C. barbouri* (Figs 1, 2). This feature alone serves to distinguish *A. browni* from all other New World pitvipers except *Agkistrodon*, *Sistrurus*, and *Crotalus ravus*. As the description that Campbell & Lamar

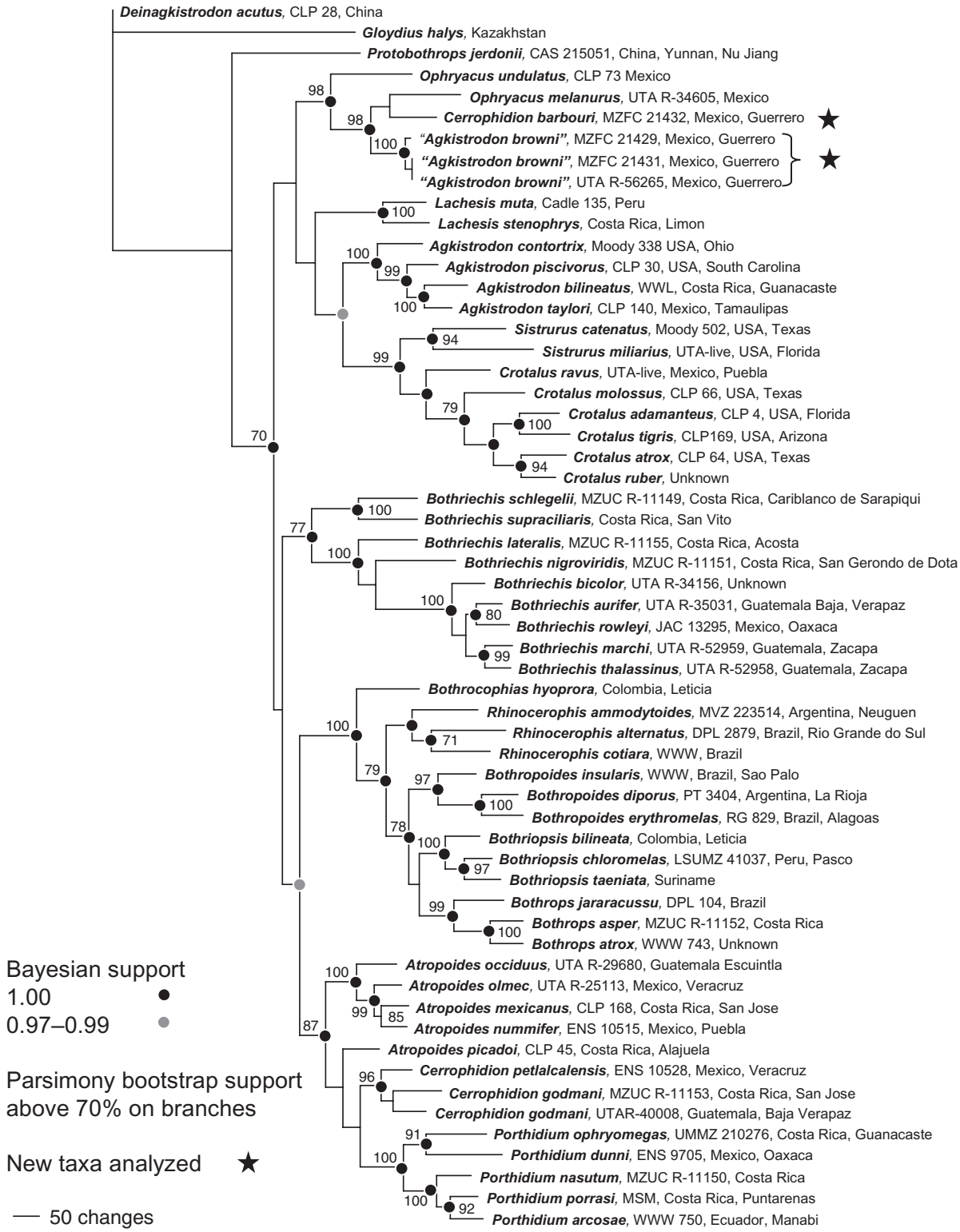

Figure 5. One of three equally parsimonious trees (8165 steps) recovered from heuristic maximum parsimony analysis of 2235 bp of four mitochondrial gene fragments (12S, 16S, cytochrome *b*, and NADH dehydrogenase subunit 4). Nodal support of posterior probability distributions from a separate Bayesian Markov chain Monte Carlo analysis. Owing to on-going taxonomic change and to help comparing phylogenies the unique ID and locality data for each operational taxonomic unit are provided next to the species names.

(2004: 431) provided was a composite of *A. browni* and *C. barbouri*, they were moved to state that 'The number and arrangement of the scales on top of the head appear to be more variable than those reported for any other snake'. With the recognition of *A. browni* as a taxon separate from *C. barbouri*, the misconception of this statement is now apparent. This discovery prompts a number of questions, the first that of relationship. Only two phylogenetic analyses have been conducted on *C. barbouri* (Campbell, 1988; Jadin, 2010). Both were morphological analyses of *Cerrophidion* and include specimens of *A. browni* as *C. barbouri*. These two analyses lacked taxonomic sampling beyond *Cerrophidion* species and therefore it is not surprising that they found a basal split between *C. barbouri* and the other *Cerrophidion*. In the original description of *A. browni*, Shreve (1938) mentioned that the species was probably most closely related to members of New World *Agkistrodon*, but was allocated to the genus because of its similarity to the Asian pitviper *Hypnale* [*Agkistrodon*] *hypnale*. Therefore, its relationships were unclear prior to our study.

Our phylogenetic analyses reveal that *A. browni* and *C. barbouri* form a clade with *O. melanurus*, a sister-group to *O. undulatus*. Additionally, *A. browni*, *C. barbouri*, and *O. melanurus* share several seemingly derived features from *O. undulatus* (e.g. entire subcaudals, long and curved tail spine, flat canthals, presence of palatine teeth, and separated splenial and angular bones). The paraphyly of the genus *Ophryacus* with respect to *A. browni* and *C. barbouri* (Fig. 5) was not anticipated but provides a feasible biogeographical scenario, as all of these taxa are highland endemics to southern Mexico. We believe that the ancestor of these four species inhabited the area between the Isthmus of Tehuantepec and the Balsas River drainage. An *O. undulatus* ancestor appears to have separated first during the mid-Miocene within the more mesic forests to the south and eventually invaded all other mesic areas of Oaxaca, Guerrero, Veracruz, and Puebla. The ancestor of *A. browni* and *C. barbouri* probably speciated within the Sierra Madre del Sur of Guerrero, west of Chilpancingo, giving an offshoot into the dry valleys of Oaxaca, Morelos, and Puebla, *O. melanurus*, what could be considered the high southern Balsas area and associated valleys and drainages.

Basis for systematic revision

When Gutberlet (1998) removed *O. melanurus* from the genus *Porthidium* and placed it in *Ophryacus*, based on careful morphological analyses, he recognized that it differed greatly from *O. undulatus* in several highly conserved characteristics (e.g. respectively, terrestrial vs. semi-arboreal habits, typically three vs. zero palatine teeth, entire vs. divided subcaudals). Initially, Gutberlet sought to describe *O. melanurus* as a monotypic genus because of these numerous divergent features (R. Gutberlet, pers. comm.). Now, more than a decade later, relative divergence estimates by Castoe *et al*. (2009) and Daza, Castoe & Parkinson (2010) suggest that the *O. melanurus* lineage (now including *A. browni* and *C. barbouri*) and the *O. undulatus* lineage diverged from each other during the mid-Miocene. Although the confidence estimates overlap, their mean estimates of divergences for this separation predates the splitting of the *Porthidium* group into three genera, the rattlesnakes into *Sistrurus* and *Crotalus*, and the *Bothriopsis–Bothrocophias–Bothropoides–Bothrops–Rhinocerophis* clade.

Additionally, our pairwise comparisons show a divergence of 12.3% within these three species, which falls within the range of intrageneric divergence (Table S2). Divergence among these three species and *O. undulatus* is 14.6%, which falls within the range of intergeneric divergence. Moreover, these three species share several seemingly derived features from *O. undulatus* (e.g. terrestrial habits, entire subcaudals, long and curved tail spine, flat canthals, presence of palatine teeth, fewer intersupraoculars, and separated splenial and angular bones). Therefore, on the basis of genetic distance and distinctive morphology there appears little doubt that *A. browni*, *C. barbouri*, and *O. melanurus* warrant allocation to their own new genus, rendering *Ophryacus* monophyletic. We hereby propose a new genus and summarize the morphological features for each of the species placed in this genus. Finally, the removal of *C. barbouri*, and thus *A. browni*, and *O. melanurus* from the genera *Cerrophidion* and *Ophryacus*, respectively, requires a revision of our concepts of these two genera. We therefore revise the genera *Cerrophidion* and *Ophryacus*.

Systematic account

Mixcoatlus gen. nov.

Type species: Agkistrodon browni Shreve, 1938, by present designation.

Etymology: The generic name is derived from the Náhuatl word *Mixcoatl*, meaning 'cloud serpent,' a god of the Aztecs and several Mesoamerican civilizations. The name alludes to the restriction of this clade to high elevations. The gender of this name is masculine.

Content: The genus *Mixcoatlus* contains *Mixcoatlus barbouri*, *Mixcoatlus browni*, and *Mixcoatlus melanurus*. Similar to *Ophryacus*, *Mixcoatlus* is a pitviper

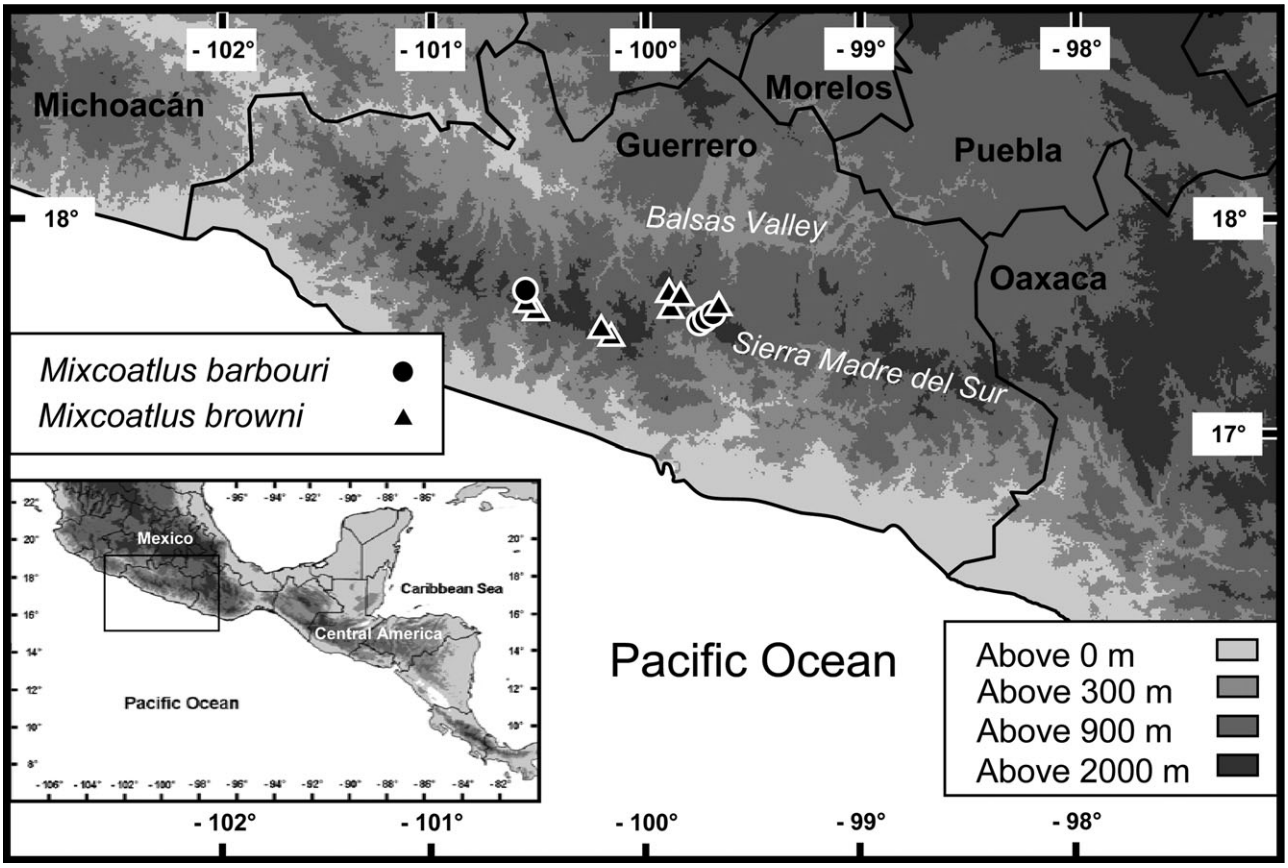

Figure 6. Distribution map of *Mixcoatlus barbouri* and *Mixcoatlus browni*.

genus endemic to the highlands of southern Mexico. *Mixcoatlus barbouri* and *M. browni* are restricted to highland humid pine-oak and cloud-forest habitats of the Sierra Madre del Sur in Guerrero, Mexico (Fig. 6), whereas *M. melanurus* occurs in highland arid tropical scrub, high deciduous forest, and seasonally dry pine-oak forest in southern Puebla and northern Oaxaca (Campbell & Lamar, 2004: map 83). This limited distribution of southern Mexico makes this genus the most restricted of New World pitvipers.

Common name: Mexican montane pitvipers

Definition and diagnosis: Rostral broader than high, front surface flat to moderately concave (*M. melanurus*); preoculars two (*M. barbouri* and *M. browni*) or three (*M. melanurus*), upper preocular largest and squarish. In *M. melanurus*, middle preocular separate from supralacunal, lower forming posterior border of pit and excluded from orbit; single, large, flat, platelike supraocular above eye (*M. barbouri and M. browni*) or two to three supraoculars along dorsal margin of eye including supraocular horn (single scale above eye forming flattened horn, dorsoventrally compressed in cross section, occupying most of dorsal margin of orbit, tip broadly rounded; adjacent scales along dorsal ocular margin slightly modified, projecting slightly or not); seven to 14 supralabials (usually eight in *M. barbouri* and *M. browni* and 11 in *M. melanurus*); lip margin strongly scalloped in *M. melanurus*; eight to 13 infralabials; canthals and internasals relatively large, flat to rounded; crown of head covered with relatively large, flat scales with keeling beginning in parietal area (*M. barbouri*, *M. browni*) or covered by small keeled scales (*M. melanurus*); intersupraoculars one (*M. browni*), three to four (*M. barbouri*), or nine to 13 (*M. melanurus*); second supralabial discrete from prelacunal (these scales may be separated by two rows of small subfoveals in *M. melanurus*); supralabial and subocular series in contact (*M. barbouri*, *M. browni*) or separated by two to four rows of small, roundish scales (*M. melanurus*); one to two postoculars; 17–21 middorsal scale rows; mid-dorsal scales at midbody moderately slender and pointed in *M. barbouri* and *M. browni* and broad and obtusely rounded in *M. melanurus*; keel generally extending to tip of scale or nearly so, apical pits not apparent; free portion of

apex of dorsal scales moderate in extent; 129–148 ventrals in *M. barbouri* and *M. browni*, 137–169 in *M. melanurus*; subcaudals undivided, 26–35 in *M. barbouri* and *M. browni* and 42–64 in *M. melanurus*; tail spine straight or distally curved upwards, moderately long. In *M. barbouri* and *M. browni* dorsum usually with ill-defined zigzag stripe bordered narrowly with black, sometimes broken into discrete blotches; 25–28 dark brown lateral body blotches; dorsal ground colour reddish brown. In *M. melanurus* dorsum with zig-zag pattern; ground colour reddish brown, olive brown, or grey; dorsal scales usually finely mottled or speckled with black, although this pattern may be apparent only under microscopic examination.

In *M. barbouri* and *M. browni* lateral edge of nasal bone expanded into roughly triangular shape; frontal bones mostly flat, dorsal surface with slightly elevated margins, longer than wide; postfrontal moderate in size, reaching frontal; transverse distance of postfrontal about equal to its distance along parietal bone; posterolateral edges of dorsal surface of parietals forming moderately distinct raised ridge continuing posteriorly on parietal to about level posterior to quadrate; junction between parietal and pro-otic rounded; squamosal extending posteriorly to level about equal to posterior edge of exoccipital; ectopterygoid much shorter than expanded, flattened base of pterygoid (posterior to the articulation with ectopterygoid), with flat shaft gradually tapering posteriorly; dorsal edge of palatine rounded. Three palatine teeth; ten to 12 pterygoid teeth; eight to 12 dentary teeth; pterygoid teeth not extending posterior to level of articulation of pterygoid with ectopterygoid; maxillary fang relatively short, being about equal in length to height of maxilla; fang at rest extending to level of about middle of supralabial 5.

In *M. melanurus* frontal bones with concave dorsal surface, strongly elevated margins, moderately longer than wide; postfrontals relatively small, not contacting frontal, comprising considerably less of dorsal perimeter of orbit than parietals; posterolateral edges of dorsal surface of parietals forming distinct flat shelf not continuing onto the parietal as a raised ridge; junction between parietal and pro-otic irregular, not particularly angular; anterior portion of ectopterygoid possessing shallow depression on medial side accommodating attachment of ectopterygoid retractor muscle; ectopterygoid noticeably longer than expanded, flattened base of pterygoid (posterior to articulation with ectopterygoid) with flat shaft tapering posteriorly; apex of choanal process positioned at about midlength on palatine, process greatly reduced in height, apex broadly rounded; dorsal surface of parietal roughly triangular; three palatine teeth, seven to ten pterygoid teeth, seven to nine dentary teeth; pterygoid teeth extending to level of articulation of pterygoid with ectopterygoid; maxillary fang relatively short, only slightly longer than height of maxilla, at rest extending to level of suture between supralabials 6–7 or supralabial 7; splenial and angular bones separate; haemapophyses separate distally.

The highland isolation of *Mixcoatlus* results in its allopatry to most species of pitvipers. However, these three species are sympatric with *O. undulatus* throughout parts of their range but are distinguished by morphological features listed above. Additionally, *M. barbouri* and *M. browni* may be broadly sympatric with *Crotalus intermedius* and *Crotalus ravus* but are distinguished from these species by not having a rattle at the end of their tail.

CERROPHIDION CAMPBELL & LAMAR, 1992

Type species: Bothriechis godmanni Günther, 1863, by subsequent designation of Campbell & Lamar (1992).

Etymology: The generic name comes from the Spanish *cerro*, meaning mountain, an allusion to the habitat, and the Greek *ophidion*, meaning small snake (Campbell & Lamar, 1992).

Content: The genus *Cerrophidion* contains three species: *Cerrophidion godmani*, *Cerrophidion petlalcalensis*, and *Cerrophidion tzotzilorum*. These species occur in pine-oak and cloud forests from Veracruz (Mexico) southward through the highlands of Central America to Panama (Campbell, 1985; Campbell & Lamar, 2004: maps 79, 80) with a vertical distribution from *c.* 1400–3491 m.

Common name: Middle American montane pitvipers.

Definition and diagnosis: Rostral wider than high, front surface flat; three preoculars, upper largest, entire, and squarish, lower forming posterior border of pit and excluded from orbit; single, large, flat, plate-like supraocular above eye; seven to 11 supralabials; eight to 12 infralabials; canthals and internasals relatively large and flat; two to seven intersupraoculars; crown of head covered with variably sized, flat or keeled scales; keeling prominent in parietal area; second supralabial discrete from prelacunal; supralabial and subocular series in contact or separated by single row of scales; 19–23 (mode 21) mid-dorsal dorsal scale rows; mid-dorsal scales at midbody moderately slender and pointed; 120–150 ventrals; 22–36 undivided subcaudals; tail spine straight, moderately long.

Lateral edge of nasal broadly expanded, bone roughly quadrangular; frontal bones mostly flat,

dorsal surface with slightly elevated margins, longer than wide; postfrontal large, not reaching frontal; transverse distance of postfrontal greater than its distance along parietal bone; posterolateral edges of dorsal surface of parietals forming low to moderately distinct raised ridge continuing posteriorly on parietal as low ridge; junction between parietal and pro-otic rounded to almost flat; squamosal extending to level posterior to posterior edge of exoccipital; ectopterygoid about same length as expanded, flattened base of pterygoid (posterior to the articulation with ectopterygoid) with flat shaft gradually tapering posteriorly; dorsal surface of parietal roughly triangular to sometimes rounded; three to five palatine teeth; seven to 18 pterygoid teeth; eight to 16 dentary teeth; pterygoid teeth extending just posterior to level of articulation of pterygoid with ectopterygoid in *C. godmani*, but not reaching this far back in congeners; maxillary fang relatively short, being about equal in length to height of maxilla; fang at rest extending to level of about middle of supralabial 5 or suture between supralabials 5–6 (mostly after Campbell & Lamar, 2004).

Ophryacus Cope, 1887

Type species: Trigonocephalus [*Atropos*] *undulatus* Jan, 1859, by monotypy.

Etymology: The generic name is derived from the Greek *ophrys*, meaning brow, and the Latin *acus*, meaning pointed, obviously in reference to the distinctive supraocular spine-like scale.

Content: The genus *Ophryacus* contains only *O. undulatus* confined to the highlands of the Sierra Madre Oriental (Hidalgo, Veracruz, Puebla), the Mesa del Sur (Oaxaca), and the Sierra Madre del Sur (Oaxaca, Guerrero), where it occurs in pine-oak and cloud forest (Campbell & Lamar, 2004: map 84).

Common name: Mexican horned pitviper.

Definition and diagnosis: Rostral broader than high, moderately to distinctly concave; three preoculars, upper largest and undivided, middle not fused with supralacunal, lower small, somewhat excluded from margin of orbit; three to four supraoculars along dorsal margin of eye including supraocular spine; ten to 13 supralabials; lip margin not scalloped; nine to 14 infralabials; single scale above eye forming long, relatively slender spine, slightly compressed to subcircular in cross section, not occupying most of dorsal margin of orbit, tip pointed; adjacent scales along dorsal ocular margin often also modified, projecting slightly; canthals and internasals often raised into short spines or with especially high keels; scales in the supraocular region small and keeled; ten to 20 (usually 12–18) intersupraoculars; top of head covered with small scales, most having tubercular keels; second supralabial usually separated from prelacunal by single small subfoveal; subocular and supralabial series separated by two to four rows of small, roundish scales; 21 mid-dorsal scale rows; mid-dorsals at midbody not noticeably broad, obtusely rounded; keel generally extending to tip of scale or nearly so, apical pits not apparent; free portion of apex of dorsal scales moderate in extent, barely overlapping contiguous scale; interstitial epidermal fold at cranial end of scale well developed; 157–178 ventrals; 37–57 subcaudals, divided; tail spine straight, about as long as preceding two to three subcaudals, pointed or obtusely rounded.

Frontal bones with concave dorsal surface, strongly elevated margins, moderately longer than wide; postfrontals moderate in size, not contacting frontal, comprising about equal amount of dorsal perimeter of orbit as parietals; posterolateral edges of dorsal surface of parietals forming distinct flat shelf continuing onto parietal as a raised ridge; junction between parietal and pro-otic irregular, not particularly angular; anterior portion of ectopterygoid possessing a shallow depression on medial side accommodating attachment of ectopterygoid retractor muscle; ectopterygoid noticeably longer than expanded, flattened base of pterygoid (posterior to articulation with ectopterygoid) with flat shaft tapering posteriorly; apex of choanal process positioned at about midlength on palatine, process moderately reduced in height, apex broadly rounded; dorsal surface of parietal roughly triangular; zero to one (usually zero) palatine teeth, seven to ten pterygoid teeth, seven to nine dentary teeth; pterygoid teeth extending to level of articulation of pterygoid with ectopterygoid; maxillary fang relatively short, only slightly longer than height of maxilla; fang at rest extending to level of suture between supralabials 7 and 8; splenial and angular bones fused; haemapophyses in contact distally.

Dorsum with zig-zag pattern; ground colour olive-brown, green, or grey, sometimes orange or yellow pigment present; dorsal scales usually finely mottled or speckled with black.

Natural history of *M. barbouri* and *M. browni*

Similar to *Ophryacus*, *Mixcoatlus* is a pitviper genus endemic to the highlands of southern Mexico. *Mixcoatlus* appears to be found only in the western portion of the Sierra Madre del Sur of Guerrero (*M. barbouri* and *M. browni*) and north-western Oaxaca and south-eastern Puebla (*M. melanurus*), making it the most restricted genus of New World

pitviper (Fig. 6 and map 83 in Campbell & Lamar, 2004, respectively). *Mixcoatlus barbouri* and *M. browni* are probably sympatric throughout much of their ranges, possess the same type locality (Omilteme, Guerrero), and have been found near each other in the western part of their ranges. Additionally, *Crotalus intermedius omiltemanus*, *C. ravus*, and *O. undulatus* have also been found near Omilteme and other highland areas in Guerrero, making their sympatry with *M. barbouri* and *M. browni* likely (Davis & Dixon, 1959; Campbell & Lamar, 2004). The highest confirmed elevation records for *M. barbouri* and *M. browni* are 2608 m (MZFC 21432) and 3296 m (KU 182762), respectively.

Campbell (1988) provides a detailed description of *M. barbouri* and *M. browni* habitat in the Sierra Madre del Sur and states that the higher elevations are dominated by pine-oak forest and cloud forest. Although it has been suggested that *M. barbouri* and *M. browni* inhabited cloud forest almost exclusively, observations of this species at lower elevations (Davis & Dixon, 1959; Campbell, 1988; this study) suggest that these species also occurs in upper pine-oak forest where it interdigitates with cloud forest. Additionally, two individuals of *M. browni*, one found in fir-pine-oak forest (UTA R-4450) and the other 'in bunchgrass on the sparsely wooded southern slope of Cerro Teotepec (KU R-182762)', suggests that this species 'at least inhabits several recognizable vegetation associations' (Campbell, 1988: 8). Campbell (1988) also provides an investigation of the locality records for most of the specimens of *M. barbouri* and *M. browni*.

Both *M. barbouri* and *M. browni* are diurnal and are usually found basking, under cover, or moving during the day. While photographing a live *M. browni*, R. C. J. and E. N. S. observed an individual wrapping its tail around the hook to prevent falling (e.g. Fig. 4). This same behaviour was also observed in the field in two other individuals. Although its tail is more prehensile than such terrestrial genera as *Cerrophidion* and *Atropoides*, we have no compelling evidence that *M. browni* is highly arboreal. It does ascend into low vegetation; J. A. C. observed one specimen coiled on top of a stump about 1.5 m above the ground and another in a low, woody shrub about 1.0 m high.

Few *M. barbouri* and *M. browni* have been kept in captivity. One specimen of *M. barbouri* (UTA R-15558) was kept for more than ten years (Campbell, 1988). An adult *M. barbouri* (MZFC 21432) and two *M. browni* (MZFC 21431 & UTA R-56265) were kept in captivity for several months. The *M. barbouri* was quite active and readily ate domestic white mice, whereas one adult (MZFC 21431) and one juvenile (UTA R-56265) *M. browni* required force feeding (A. Carbajal, pers. comm.). Specimens and scats of both *M. barbouri* and *M. browni* have contained rodent hair as well as the lizard *Mesaspis gadovii* (Campbell, 1988). *Mesaspis gadovii* probably constitutes a large portion of the diet for these two pitvipers because of the great abundance of this lizard (Campbell, 1988). Two specimens of *M. browni* (MZFC 21431 & UTA R-56264) were captured within a few metres of *Me. gadovii*. Although only a few diet items have been identified, the diet of *M. barbouri* and *M. browni* probably includes lizards, orthopterans, and mammals, similar to that of *Cerrophidion* species, with less of their diet consisting of birds and amphibians (Campbell, 1988; Campbell & Solórzano, 1992; Campbell & Lamar, 2004; Jadin, 2007, 2010). *Sceloporus adleri* is abundant at these high elevations, representing another potential prey item. Many specimens of *Thorius* and *Pseudoeurycea* were collected in the vicinity of *M. browni*. A plethodontid salamander was found in the stomach of *Cerrophidion petlalcalensis* (López-Luna, Vogt & de la Torre-Loranca, 1999) and *M. barbouri* and *M. browni* may also consume them.

Of the 32 specimens of *M.* [*Agkistrodon*] *browni* and *M.* [*Cerrophidion*] *barbouri* examined in this study, 15 were *M. barbouri* and the other 17 specimens allocatable to *M. browni* (see Appendix S1 for details). We are aware of only three museum specimens not examined by us: MZFC 2880 and 2882 and a recently collected *M. browni* (field number JAC 27714).

Future of pitviper discoveries

Pitvipers have received abundant attention from many scientists involved in molecular and morphological phylogenetics and represent one of the more studied reptile clades. Nonetheless, many new species have been discovered in recent decades and more is continually being revealed about their intriguing evolutionary and natural histories. Pitviper research has been conducted in Mexico by many individuals over the past century, with much of the early groundwork laid by scientists such as Dugès (1896), Cuesta-Terrón (1921), and Martín del Campo (1935). The recognition of this new genus and continual endemic pitviper discoveries like *Porthidium hespere* (Campbell, 1976), *C. tzotzilorum* (Campbell, 1985), *C. petlalcalensis* López-Luna *et al*. (1999), and *Crotalus ericsmithi* Campbell & Flores-Villela (2008) in Mexico underscore the importance of natural history collections already established and additional collecting needed in biotically rich regions of the world. In our current age of biodiversity decline, it is paramount that systematics, ecology, and natural history research in these regions proceed rapidly. Current rates of extinctions, habitat loss, and other anthropogenic changes

undoubtedly will make investigations much less rewarding to future generations.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Taxa, vouchers, locality data, and GenBank accession numbers for sequences used in this study. Sequences newly added specifically for this study are in bold.
Table S2. Pairwise sequence divergences among (below diagonal) and within (diagonal) all New World genera as defined in this study using only the cytochrome *b* gene.
Appendix S1. Specimens examined.

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