

Cryptic diversity in disjunct populations of Middle American Montane Pitvipers: a systematic reassessment of *Cerrophidion godmani*

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The discovery and taxonomic recognition of cryptic species has become increasingly frequent with the application of molecular phylogenetic analyses, particularly for species with broad geographic distributions. In this study we focus on the venomous pitviper species *Cerrophidion godmani* that is widely distributed throughout the highlands of Central America. We provide evidence based on both molecular phylogenetic analyses and morphological data that *C. godmani* represents three deeply divergent lineages and is possibly non-monophyletic. These three lineages are relatively conserved in their morphology and tend to be highly variable among individuals, but we do find sufficient morphological characters to diagnose them as evolutionarily distinct. We apply these data, together with known geographic distributions of populations, to infer boundaries of these three divergent evolutionary lineages. Based on the body of evidence, we formally name and describe two new species of *Cerrophidion* and redescribe *C. godmani sensu stricto*.

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Introduction

Neotropical pitvipers have been the subject of extensive taxonomic and phylogenetic review during the last several decades (see review in Campbell & Lamar 1992; Gutberlet & Harvey 2004; Fenwick *et al.* 2009; Jadin *et al.* 2011). One particular group that has received a notable amount of attention has been what Burger (1971) recognized as *Porthidium*, a group at that time considered to consist of eight species. Although Burger's (1971) dissertation was never formally published, his generic arrangements were adopted by Perez-Higareda *et al.* (1985), Campbell & Lamar (1989) and subsequent workers. Campbell & Lamar (1989) recognized three distinct groups within the genus *Porthidium*: hognosed pitvipers, jumping pitvipers

and montane pitvipers. Werman (1992) removed the jumping pitvipers (*P. nummifer*, *P. olmec* and *P. picadoi*) from *Porthidium*, placing them in a new genus, *Atropoides*, while Campbell & Lamar (1992) removed the montane pitvipers (*P. barbouri*, *P. godmani* and *P. tzotzilorum*) and allocated them to a new genus *Cerrophidion*. *Cerrophidion petlalcalensis* was later described by López-Luna *et al.* (1999), and most recently Jadin *et al.* (2011) found that the taxon *C. barbouri* was composed of two species and allocated both, along with *Ophryacus melanurus*, to a new genus *Mixcoatlus*. Therefore, as currently defined, the genus *Cerrophidion* contains three recognized species: *Cerrophidion godmani*, *C. petlalcalensis* and *C. tzotzilorum*.

Species of *Cerrophidion* occur in Neotropical montane habitats between ca. 1200 and 3500 m in elevation (Campbell & Lamar 2004; Köhler *et al.* 2006). Two of these species, *C. petlalcalensis* and *C. tzotzilorum*, are endemic to Mexico and are restricted to geographically small ranges (Campbell 1985; López-Luna *et al.* 1999; Campbell & Lamar 2004). The third putative species, *C. godmani*, is considered widely distributed and occurs throughout highland regions from southern Mexico to western Panama (Campbell & Solórzano 1992; Campbell & Lamar 2004). Populations of this species occur in disjunct highland habitats (pine-oak forest, cloud forest and alpine meadow) both north and south of Nicaragua, with the lowland Nicaraguan Depression representing a major hiatus in their distribution (Campbell & Lamar 1989; Campbell & Solórzano 1992).

Campbell & Solórzano (1992) provided evidence for ecological distinction between populations from Nuclear Central America and Lower Central America. They, as well as Jadin (2010), further identified morphological characters that differentiate between the populations in these two main geographical areas. Jadin (2010) found little or no support for a monophyletic *C. godmani* and in at least one analysis found the species to be paraphyletic with respect to *C. tzotzilorum*. Also during the last several years, a number of molecular phylogenetic and phylogeographic studies have included sampling of multiple *C. godmani* populations from throughout their range, and have shown evidence that deeply divergent and discrete evolutionary lineages exist within this wide-ranging montane species (Castoe *et al.* 2003, 2005, 2009; Daza *et al.* 2010). Therefore, based on an increasing body of evidence, it has become clear that systematic revision of *Cerrophidion* was necessary to establish a species-level taxonomy that reflects the evolutionary history of these lineages.

In this study, we combine previous molecular evidence with new molecular sampling and morphological analyses that provide conclusive evidence that there are indeed three distinct and diagnosable lineages within *C. godmani*. Based on the allopatric distribution of these lineages, the deep molecular divergences within *C. godmani* dating back nearly 10 million years and the diagnostic characters separating these lineages, we revise the species-level taxonomy of this complex to include two new species of *Cerrophidion*.

Materials and methods

Molecular sampling

For generation of new DNA sequence data, genomic DNA was isolated from muscle tissue of twelve specimens of *Cerrophidion* using a Qiagen DNeasy extraction kit and protocol. Four mitochondrial gene fragments (NADH dehydrogenase subunit 4 (ND4), cytochrome *b* (cyt *b*), 12S

rRNA and 16S rRNA) were independently PCR amplified as described in multiple studies (Knight & Mindell 1993; Arévalo *et al.* 1994; Parkinson *et al.* 1997, 2002) using GoTaq[®] Green master mix by Promega, Madison, WI, USA, the primer pairs: ND4 + LEU, Gludg + AtrCB3, L1091 + 12E and 16SF + 16SR, and annealing temperatures 48, 48, 50 and 45 °C, respectively. Sequencing was performed in both forward and reverse directions using the PCR primers on a Beckman Coulter automated capillary sequencer, and sequence chromatographs were edited using SEQUENCHER 4.2, Gene Codes Corporation, Ann Arbor, MI, USA. Sequences for each gene were aligned separately, first automatically using the program MUSCLE (Edgar 2004) and, then manually rechecked using SE-AL v2.0a11. Gaps in alignments were treated as missing data and no internal stop codons were found in the two protein-coding gene fragments. Novel sequences from this study were deposited in GenBank (JQ627129–42 and JQ724143–81).

All previously published sequences of *Cerrophidion* were downloaded from GenBank and were combined with new sequence data generated in this study (Table S1). Sequences for the corresponding four gene fragments were also downloaded for *Atropoides nummifer*, *A. olmec* and *Porthidium nasutum*, which were used as outgroups to root our *Cerrophidion* phylogeny.

Phylogenetic analyses

Bayesian Markov chain Monte Carlo (BMCMC) methods were used to reconstruct phylogenies. To identify appropriate models of nucleotide substitution for BMCMC analyses, we used the program MRMODELTEST v2.2 (Nylander 2004), run in PAUP* v4.0b10 (Swofford 2002). We used Akaike information criterion (AIC) to select the best-fit models, as estimated by MrModeltest (Table 1). The four gene fragments were concatenated (2307 total bp), and this combined dataset was partitioned by gene and codon position (for cyt-*b* and ND4), resulting in a

Table 1 Results from a priori model selections based on Akaike information criterion (AIC) conducted in MrModeltest 2.2 (Nylander 2004) for partitions of the dataset

Cerrophidion phylogeny	Total characters	Parsimony-informative characters	AIC Model
ND4 1st pos	231	27	GTR+ Γ
ND4 2nd pos	231	11	HKY
ND4 3rd pos	231	117	GTR+ Γ
Cyt b 1st pos	237	23	HKY+I
Cyt b 2nd pos	237	10	HKY+I
Cyt b 3rd pos	237	111	GTR+ Γ
16S	496	14	GTR+I+ Γ
12S	407	23	HKY+ Γ

total of eight partitions as was shown to be justified in analysis of a similar dataset that included these four fragments from these species (Castoe & Parkinson 2006). Stems and loops were not partitioned separately due to a lack of informative characters.

BMCMC phylogenetic analyses were conducted using MRBAYES v3.0b4 (Ronquist & Huelsenbeck 2003). Two simultaneous BMCMC runs were conducted (with the default MCMC settings), and run for a total of 5.0×10^6 generations per run, sampling trees and parameters every 100 generations. We confirmed stationarity using TRACER v1.5.4 (Rambaut & Drummond 2009). Based on this evaluation, the first 1.5×10^5 generations from each run were discarded as burn-in.

Morphological analysis

We examined 201 preserved specimens of *C. godmani sensu lato*, for this study (Appendix S1). Museum acronyms follow Leviton *et al.* (1985). Definitions of scale counts and morphological features follow Campbell & Lamar (2004), and bilateral characters are reported right/left.

We dissected and examined the left hemipenes from specimens deposited at the Amphibians and Reptile Diversity Research Center at the University of Texas at Arlington (UTA R-51399 and UTA R-59478). Hemipenes were dissected and removed 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 petroleum jelly with blue wax-dye until maximum expansion was achieved. Finally, we tied the hemipenes and stored them in 70% ethanol. This procedure is modified from that of Myers & Cadle (2003) and Zaher & Prudente (2003) and is further described and illustrated in Smith & Ferrari-Castro (2008) and Jadin & Smith (2010). Hemipenial terminology follows Dowling & Savage (1960), Keogh (1999) and Savage (2002).

Results

Our four mitochondrial gene fragment BMCMC phylogenetic tree suggests that *C. godmani sensu lato* is composed of three distinct, well-supported putative species-level clades (posterior probability = 100; PP hereafter), which may be paraphyletic with respect to the *C. petlalcalensis*–*C. tzotzilorum* clade (Fig. 1). We find moderately strong support (PP = 0.87) for a sister relationship between *C. godmani* populations from Guatemala and Mexico (Clade 1) and a *C. petlalcalensis*–*C. tzotzilorum* clade. Together, these two lineages are inferred to be the sister group to a clade comprised of two additional distinct lineages of *C. godmani* populations (PP = 1.0), one comprising samples from Costa Rica populations (Clade 2) and a second comprised of Honduras/El Salvador populations

(Clade 3). *Cerrophidion godmani* from Guatemala and Mexico (Clade 1), as well as *C. godmani* from Honduras/El Salvador (Clade 3) exhibits considerable genetic structure across the range of specimens sampled, compared to the relatively low haplotype diversity found in *C. godmani* populations from Costa Rica. In addition to the clear separation, and possible paraphyletic relationships of these three clades currently allocated to *C. godmani*, the divergences separating these clades are substantial. For example, based on a large dataset of pitviper taxa, Castoe *et al.* (2009) estimated the divergence between these lineages of *C. godmani* to have taken place 3–7 million years ago.

Distinguishing features among the three clades of *C. godmani s.l.* include: Individuals from Clade 1 average more scales in the frontal region than populations from Clade 2; Individuals from Clade 2 have fewer prefoveals than individuals from Clades 1 and 3; the dorsal scales are typically disposed in 23–21–17 rows in Clades 1 and 3 and 23–21–19 in Clade 2; individuals from Clades 1 and 2 usually have more scales contacting the supraoculars and a larger median frontal scale than individuals from Clade 3. Additionally, Jadin (2010) found that individuals from Clade 1 have fewer suboculars than Clades 2 and 3 while individuals from Clade 3 have a greater number of interrials than Clades 1 and 2.

Taxonomic conclusions

Based on the results of our molecular phylogenetic analyses (Fig. 1), *C. godmani* appears to be a composite of three evolutionarily distinct, deeply divergent lineages that each merit recognition as distinct species. Additionally, we find morphological distinctions between these three allopatric lineages, further supporting this conclusion. Consequently, we describe two of these lineages as new species and redescribe *C. godmani s.s.*

The history of discovery of this species was reviewed by Campbell & Solórzano (1992). *Cerrophidion godmani* was described by Günther (1863) from Guatemalan specimens (presumed type-locality: Totonicapán: Departamento de Sacatepéquez: Guatemala; Boulenger 1896; Campbell & Solórzano 1992; Campbell & Lamar 2004) and we therefore retain the name *C. godmani* for populations in Guatemala and Mexico (Clade 1) and describe populations from Costa Rica and Panama (Clade 2) and Honduras and El Salvador (Clade 3) as new species.

Genus Cerrophidion Campbell & Lamar 1992

Cerrophidion godmani (Günther 1863)

Bothriechis Godmanni: Günther 1863

Bothrops Brammianus: Bocourt 1868

Bothriopsis godmani: Cope 1871

Bothriopsis godmani: Cope 1871, 1887

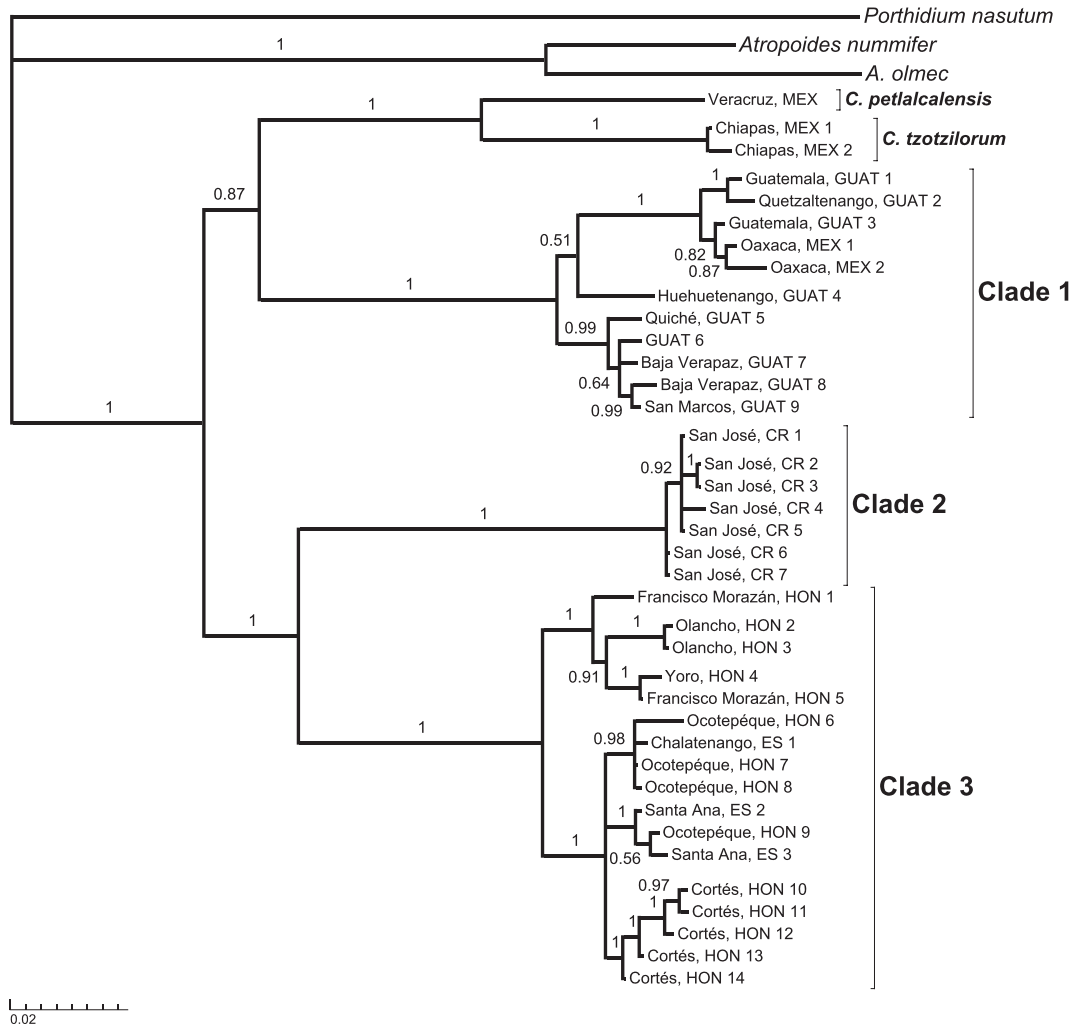


Fig. 1 Bayesian phylogenetic estimate of relationships within *Cerrophiidion* showing three distinct species-level clades within the non-monophyletic *Cerrophiidion godmani*. The tree represents the Bayesian 50% majority-rule consensus phylogram from a partitioned analysis of sequences from four mitochondrial gene fragments (ND4, cyt *b*, 12S and 16S; total of 2307 bp). Sample names follow those given in Table S1.

- Bothrops (Bothriopsis) Godmanii*: Müller 1877
- Bothriopsis Godmanii*: Müller 1877
- Bothrops Godmani*: Müller 1878
- Bothriechis scutigera*: Fischer 1880
- Bothriechis trianguligera*: Fischer 1883
- Bothriopsis scutigera*: Cope 1887
- Bothriopsis trianguligera*: Cope 1887
- Bothriechis godmani*: Günther 1895
- Lachesis godmani*: Boulenger 1896
- Trimeresurus godmani*: Mocquard 1909 [dated 1908]
- Bothrops godmani*: Barbour & Loveridge 1929
- Bothrops godmani*: Amaral 1944; Hoge 1966 [dated 1965]
- Porthidium godmani*: Campbell & Lamar 1989
- Cerrophiidion godmani*: Campbell & Lamar 1992
- English common name*. Godman's Montane Pitviper.

Definition and diagnosis. Similar to other *Cerrophiidion* species, *C. godmani* s.s. is a medium-sized, blotched, terrestrial pitviper. The head is relatively long, the canthal ridge is distinct and raised and two canthals are usually present. There are 3–7 scales (two in one specimen) across the top of the head between the supraoculars. The scales in the frontal region between supraoculars vary from being a single median scale to small, keeled scales approximately of the same size. The population in southeastern Oaxaca is characterized by having a large median frontal scale occupying more than two-thirds of the distance between the supraoculars; in all other populations individuals have median frontals that are undifferentiated from adjacent scales or that occupy less than half the distance between supraoculars. The supraoculars are broad and the nasal is divided. There are 2–5 prefoveals,

a single prelacunal, no lacunolabials, a single loreal, 0–2 subfoveals, 3 preoculars, 8–11 supralabials (usually 9), 9–13 infralabials (usually 10 or 11), 132–150 ventrals ($\bar{x} = 140.57$), 22–36 ($\bar{x} = 28.69$) undivided subcaudals with no significant sexual dimorphism, and usually 21 middorsal scale rows. The cloacal scute is undivided. The tail is relatively short and non-prehensile.

Similar species. The distribution of *C. godmani* is parapatric with that of *C. tzotzilorum*, occurring on the Meseta Central of Chiapas, Mexico. *Cerrophidion godmani* is often sympatric with species of *Atropoides* and *Bothriechis* in parts of Mexico and Guatemala at the lower elevational limits of *C. godmani* (Campbell 1985; Campbell & Lamar 1989, 2004). *Cerrophidion tzotzilorum* is distinguished from *C. godmani* by possessing smaller lateral blotches, a postorbital stripe that has a distinct pale border above and below and usually fewer ventrals. Species of *Atropoides* are extremely stout snakes with tuberculate dorsal scales, typically possessing nasorostrals (although often absent in *A. occiduus*), 23–25 midbody dorsal scale rows, a greater number of intersupraculars and usually fewer ventrals than *C. godmani* (Campbell & Lamar 1989, 2004; Jadin et al. 2010). The palm-pitvipers of the arboreal genus *Bothriechis* have prehensile tails, are typically slender and usually are mostly green.

Cerrophidion sasai sp. n. (Figs 2 and 3).

Holotype. Amphibian and Reptile Diversity Research Center, University of Texas at Arlington (UTA R-51399); an adult male (Fig. 2) from San Ramos de Tres Ríos, Departamento de San José, Costa Rica, collected on 8 November 2001 by M. Sasa.

Paratypes (9). Departamento de Alajuela, Costa Rica: UTA R-35039, male, from Cariblanco, collected in August 1975 by B. Rojas; Departamento de San José, Costa Rica: UTA R-44463–66, all juveniles, from Las Nubes de Coronado, collected in the spring of 1995 by A. Solórzano; UTA R-51400, adult female, from Hacienda la Holanda, Nubes de Coronado, collected on 9 November 2001 by M. Sequeira; UTA R-51401–02, adult female and male, respectively, from Vista del Mar, Guadalupe, Goicochea, collected on 9 November 2001 by J. Aguilar; UTA R-51403, adult male, from Rancho Redondo, Nubes de Coronado, collected on 30 November 2001 by M. Sequeira.

Etymology. The specific epithet is a patronym recognizing Mahmood Sasa Marín, an accomplished Costa Rican herpetologist. Among his many accolades, he is a recent recipient of Award for Young Scientists in 2009 given by the Academy of Sciences for the Developing World (TWAS) and the Consejo Nacional de Investigaciones

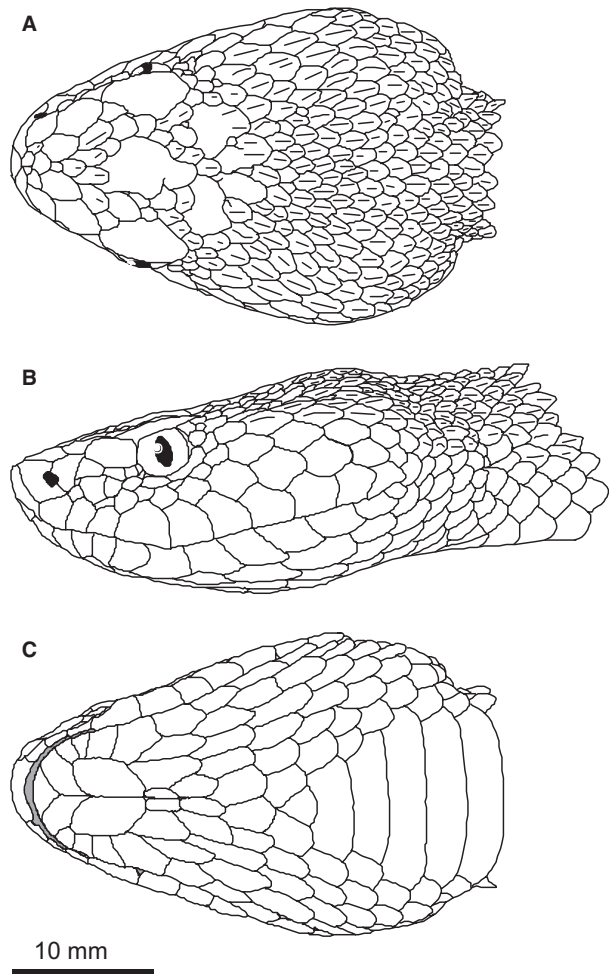


Fig. 2 Head of *Cerrophidion sasai*, male holotype, UTA R-51399, showing arrangement of scales. —A. Dorsal, —B. lateral and —C. ventral views.

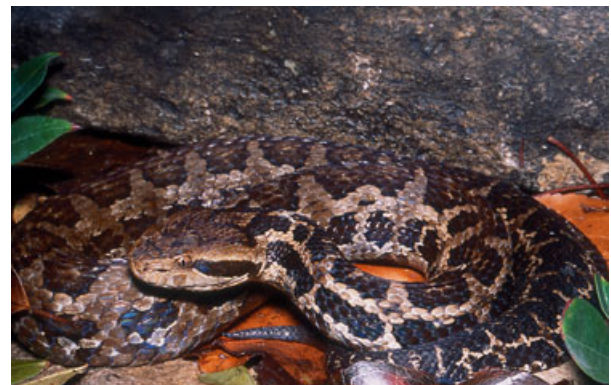


Fig. 3 *Cerrophidion sasai*, male paratype in life, UTA R-51403. UTA slide no. 27134. Photo by Eric N. Smith.

Científicas y Tecnológicas, Costa Rica (CONICIT). He has also published numerous works on what was then *C. godmani* in Costa Rica such as variation in allozymes across Costa Rican populations (Sasa 1997), comparative phylogenetic and biogeographic studies of pitvipers in the region (Castoe *et al.* 2005, 2009) and characterizations of the venom (Durban *et al.* 2011; Lomonte *et al.* 2012).

Suggested English common name. Costa Rica Montane Pitviper.

Definition and diagnosis. Similar to all other *Cerrophidion* species, *C. sasai* is a medium-sized, blotched terrestrial pitviper; head relatively long; distinct and raised canthal ridge, typically two canthals; a large, median frontal plate occupying between 53% and 90% ($\bar{x} = 63.53\%$) of the distance between the supraoculars; broad supraoculars; nasal divided; prefoveals 0–3; prelacunal single; lacunolabials absent; loreal single; subfoveals 0–2; three preoculars; supralabials 8–10, typically 9 or 10; infralabials 9–12, typically 10 or 11; ventrals 134–146 ($\bar{x} = 138.89$), undivided subcaudals 25–34 ($\bar{x} = 29.62$) with no significant sexual dimorphism; cloacal scute undivided; tail relatively short and non-prehensile and typically 21 middorsal scale rows. *Cerrophidion sasai* averages fewer scales in the frontal region, has fewer prefoveals and tends to have a larger median scale in between the oculars than other Central American *Cerrophidion* species (Campbell & Solórzano 1992; Jadin 2010).

Similar species. Several other species of pitvipers may occur sympatrically with *C. sasai* in certain portions of their range. *Bothriechis nigroviridis* has been reported to co-occur with *C. sasai*, but likely only in more humid riparian areas (Campbell & Solórzano 1992). At lower portions of its elevational range, *C. sasai* may occur sympatrically with *Atropoides mexicanus* and *B. lateralis*. These species are easily distinguished from *C. sasai* by the following characteristics: *Bothriechis* species have prehensile tails, are typically slender and are predominately green. The species most closely resembling *C. sasai* are certain populations of *A. mexicanus* and *A. picadoi*, but they have broader heads with more numerous and tuberculate supracephalic scales, a narrower and longer dark postocular, nasorostrals (except *A. picadoi*) and are substantially more heavily bodied than *Cerrophidion*.

Description of holotype. Rostral broader than high (5.33×4.70 mm); four internasals anteriorly; 2/2 canthals, four posterior intercanthals; supraoculars more than twice as long as broad; four intersupraoculars, large median frontal scale occupying 54% of intersupraocular distance; large, flat scales in parietal area; interrials 23; single loreal

bounded by upper preocular and postnasal above, prelacunal, supralacunal, and prefoveals below; prefoveals 1/2 (0/1 tiny, granular), subfoveals 1/1, postfoveals 0/0; prelacunal contacting second supralabial; 3/3 preoculars, upper largest, middle small with vertical suture separating it from supralacunal; suboculars 1/1, elongate, crescent-shaped; 2/3 postoculars; supralabials 10/10; mental broader than long (5.19×2.92 mm); infralabials 11/10; chin shields contacting first five pairs of infralabials; three pairs of gulars between chin shields and first preventral; dorsal scale rows 21-21-19; preventrals 3; ventrals 135; cloacal scute undivided; 33 undivided subcaudals; tail spine as long as preceding three subcaudals, straight, tip rounded (Figs 2 and 3).

Measurements of the holotype. Total length 71.3 cm; tail length 7.8 cm, comprising 10.9% of total; head 33.84 mm from front face of rostral to posterior end of mandible; head 21.7 mm at the broadest point near the rictus; neck 14.1 mm directly behind jaws.

Hemipenis description of holotype. Everted left hemipenis ca. 19 mm in total length and 9.5 mm in maximum width at level of the crotch; on sulcate side base with several rows of small spines (<0.4 mm) for ca. 2 mm, then rows of larger spines and hooks extending for 4 mm, largest protruding ca. 3.5 mm; asulcate side with naked base up to 5 mm before level of bilobation, then with 4–5 mm section of small spines (<1.2 mm) arranged in rows followed by 4.5 mm section of larger spines; each lobe with ca. 60 spines and hooks; ca. 14 spines and hooks around each lobe at the lower rim of calyces; calyces follow spines and hooks distally; calyces scalloped, ca. 19 rows extending 10 mm to apex of the hemipenis on the asulcate side; sulcus spermaticus is deep and bifurcating ca. 3 mm before site of bilobation, extending upwards through spines and calyces to tip of each lobe; border of sulcus spermaticus naked to point of bifurcation where small spines occur on outer border until bilobation at which point both the inner and outer borders of the sulcus spermaticus with small spines for 5 mm to beginning of calyces, which then form border to apex of lobe.

Colour pattern in life. Dorsal ground colour mostly mauve to greyish brown, usually paler on anterior third of body; 28–39 ($\bar{x} = 32.4$) dorsal body blotches, these frequently fused, forming zig-zag dorsal stripe; dorsal blotches dark brown to blackish with dark chestnut brown centres, darker on anterior and posterior portions of the body and uniformly dark; dorsum of tail mostly black; lateral blotches mostly opposite of lateral extensions of dorsal blotches, blackish brown to black, most longer than high,

sometimes considerably so, especially on anterior of body where they may extend 10–20 scale lengths; lateral blotches at about midbody cover areas equal to 5–8 scales; dorsolateral interspaces between dorsal and lateral blotches inconspicuously paler than adjacent ground colour; top of head medium brown, not as dark as body blotches; side of head pale brown with dark brown to black postocular stripe extending from lower posterior edge of eye to past angle of jaw; postocular stripe usually bordered with a narrow pale line ventrally; anterior portion of venter heavily pigmented with gray black or black mottling, particularly toward lateral part of ventrals, becoming even darker posteriorly; subcaudals, including distal most, uniformly dark. Rust-coloured or orange specimens are known, but do not appear to be common (Campbell & Solórzano 1992; Campbell & Lamar 2004).

Distribution and habitat. The presence of *C. godmani* s.l. in the highlands of lower Central America was not reported by Picado (1931) in his early review of the venomous snakes of Costa Rica. Later, however, Picado (1936) reported collecting this species northeast of the city of San José, Costa Rica in the Cordillera Central. Since then, its presence has been well documented in both the Costa Rican and western Panamanian highlands, where it appears to be locally abundant (Campbell & Solórzano 1992; Savage 2002; Solórzano 2004).

The known range of *C. sasai* includes part of two mountain ranges which together cover portions of Costa Rica and Panama. These highland masses, the Cordillera Central and the Cordillera de Talamanca, continue unbroken below the 1500 m contour from the vicinity of San José, Costa Rica, down the primary axis of Isthmian Central America into western Panama. The Cordillera Central of Costa Rica includes four major volcanoes exceeding 2500 m that are connected by highland ridges above 1500 m. The Cordillera Central is connected to the Cordillera de Talamanca by the Ochomogo Pass, at ca. 1500 m (Campbell & Solórzano 1992). Following its abutment with the Cordillera Central, the Cordillera de Talamanca maintains a ridgeline of >2500 m west-southwestward through the Panamanian border into the province of Chiriquí, Panama. Together, these cordilleras form the most extensive highlands in lower Central America and include the highest peaks in the region. Cerro Chirripó in the Costa Rican extension of Cordillera de Talamanca exceeds 3800 m in elevation.

The Cordillera de Talamanca highland complex receives its moisture via the Northeastern Tradewinds conveying moisture laden winds off the Caribbean Sea onto the Atlantic versant of Costa Rica and western Panama, thus forming tracts of wet and moist lower montane forest on

these eastern slopes (Campbell 1999). The leeward sides of these highlands experience a moderate rain shadow creating drier highland habitats, including evergreen and semi-evergreen seasonal forest, which appear to be favoured by *C. sasai* (Campbell & Solórzano 1992). Scott (1969) reported the vertical distribution of *C. godmani* in Costa Rica (*C. sasai*) as being between 1420 and 2450 m.

In addition to lower montane and montane forest habitats inhabited by *C. sasai*, this species also inhabits disturbed highland habitats, at least those contiguous with forest fragments. The holotype and one of the paratypes were found on the edge of a forest fragment that was bordered by grassland pasture, and three other paratypes were collected in grassland. All were found under logs during the day.

Natural history. Gravid females of *C. sasai* range from 341 to 512 mm in SVL and the number of young produced by a female varies between 2 and 8 ($\bar{x} = 5.5 \pm 1.37$) (Campbell & Solórzano 1992). The snout-to-vent length, total length and weight of neonates were all found to be higher in *C. sasai* than in *C. godmani* (Campbell & Solórzano 1992). Reproduction in *C. sasai* is seasonal with females giving birth between April and June (mode = May), during the latter portion of the dry season and onset of the rainy season (Campbell & Solórzano 1992). Contrary to our knowledge of reproductive cycles in many Neotropical pitvipers, *C. sasai* appears to follow a biennial cycle, which may be the result of the constraints posed by its highland distribution (Campbell & Solórzano 1992).

Venom. Reported instances of snakebite by this species are rare (Bolaños 1984). Of a survey including 477 reported cases of snakebite in Costa Rica in 1979, only two were from *C. sasai* (Bolaños 1982). Bolaños (1972) estimated the average venom yield per individual to be 15 mg, with a relatively high LD₅₀ (intravenous) of $76.0 \pm 10 \mu\text{g}$ for 16–18 g mice. Gutiérrez & Chavez (1980) showed that the haemorrhagic effects of venom of *C. sasai* were the second most severe of the 10 Costa Rican species examined, requiring only 0.8 μg of venom to result in a haemorrhagic area of 10 mm in diameter in laboratory rats.

Cerrophidion wilsoni sp. n. (Figs 4 and 5).

Holotype. Amphibian and Reptile Diversity Research Center, University of Texas at Arlington (UTA R-52953); an adult female (Figs 4 and 5), from Cerro Azul, Parque Nacional Montaña de Botaderos, Departamento de Olancho, Honduras, 15.37831°N/86.14200°W, elevation 1420 m, collected on 1 February 2005 at 1530 h by E. N. Smith (original number ENS 10860). The field party

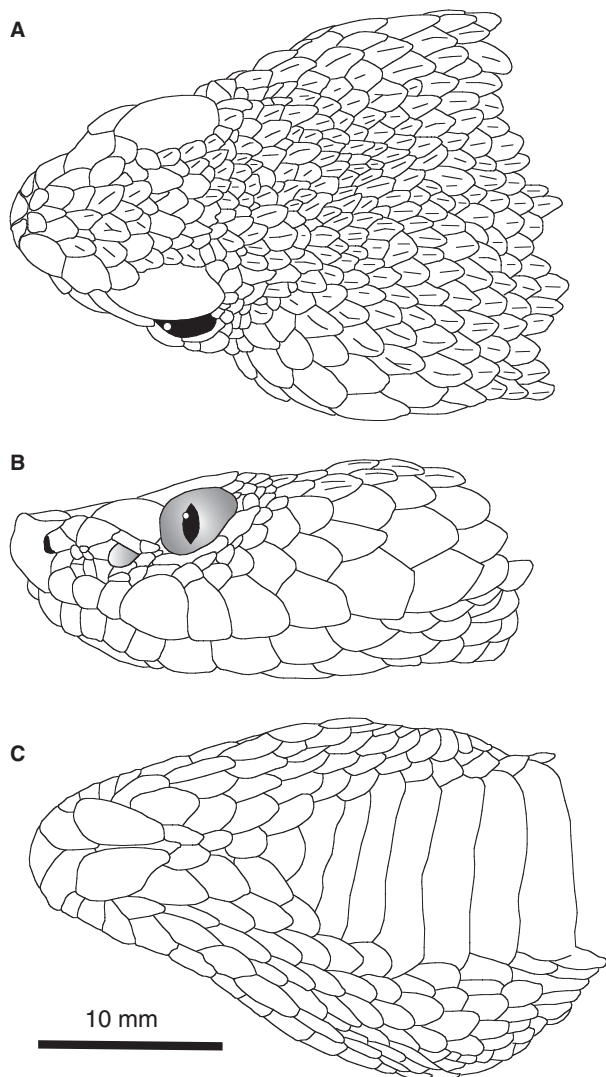


Fig. 4 Head of *Cerrophidion wilsoni*, holotype, UTA R-52953, showing arrangement of scales. —A. Dorsal, —B. lateral and —C. ventral views.

included E. N. Smith, C. Chavez, J. Ferrari-Castro, J. H. Malone, J. L. Murillo, S. Solis and A. Sosa. Type-locality (Fig. 6A,B).

Paratypes (8). UTA R-59478, adult male, from Reserva Biológica Güisayote, Departamento de Ocotepeque, Honduras, 14.4386°N/89.0624°W, 2195 m, collected on 18 June 2008 by I. R. Luque-Montes, M. Medina-Flores, J. H. Townsend and L. D. Wilson. UTA R-59479, subadult female, from Cataguana, Parque Nacional Montaña de Yoro, Departamento de Francisco Morazán, Honduras, 15.0198°N/87.1221°W, 1910 m, collected on 11 March 2007 by J. M. Butler, L. Ketzler, J. H. Townsend,



Fig. 5 *Cerrophidion wilsoni*, female holotype in life, UTA R-52953, 648 mm in total length. Photo by Eric N. Smith.

S. L. Travers and L. D. Wilson. UTA R-59480, a juvenile, from 3.45 km NNE of La Fortuna, Reserva de Vida Silvestre Texíguat, Departamento de Yoro, Honduras, 15.4451°N/87.3046°W, 1885 m, collected on 10 April 2008 by J. M. Butler, L. Ketzler, J. Slapcinsky, N. M. Stewart, J. H. Townsend and L. D. Wilson. USNM 578908, adult female from Los Tres Cerritos, Parque Nacional Sierra de Agalta, Departamento de Olancho, Honduras, 14.9106°N/86.0126°W, 1690 m, collected 2 January 2011 by M. Medina-Flores and O. Reyes-Calderón. UF 147610–11, adult males from Quebrada Cantiles, Parque Nacional Cusuco, Departamento de Cortés, Honduras, 15.5111°N/88.2448°W, 1760 m, collected 13 and 14 March 2006 by J. H. Townsend and L. D. Wilson. KU 291242, adult female from Cerro El Pital, Departamento de Chalatenango, El Salvador, 14.3878°N/89.1167°W, 2485 m, collected on 16 May 2001 by R. Gregorio, R. Bolanos, O. Komar and K. Gomez. KU 289801, adult gravid female from Cerro Montecristo, Parque Nacional Montecristo, Departamento de Santa Ana, El Salvador, 14.4011°N/89.3617°W, 2200 m, collected on 15 July 2000 by E. Greenbaum and J. Porras.

Etymology. The specific epithet is a patronym honouring Larry David Wilson. We are pleased to name this species in honour of him, in recognition of his career-long contributions to Mesoamerican herpetology. His work in Central America began with his first trip to Mexico in 1966 and has resulted in numerous contributions, including two volumes of the book *Snakes of Honduras* (Wilson & Meyer 1982, 1985), numerous other books on regional herpetology (e.g., McCranie & Wilson 2002; Townsend & Wilson 2008), and most recently leading efforts to compile the seminal volume *Conservation of Mesoamerican*

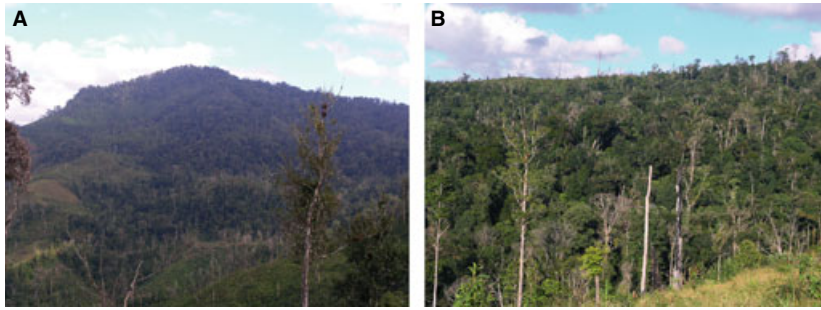


Fig. 6 —A. Distant habitat photograph showing Cerro Azul in Parque Nacional Montaña de Botaderos—the type-locality of *Cerrophidion wilsoni*. —B. Closer photograph of the mountain where the holotype was collected, showing cloud forest that is occupied with *Liquidambar*. Photographs by Eric N. Smith.

Amphibians and Reptiles (Wilson *et al.* 2010). He has described over 70 species of Middle American amphibians and reptiles and worked on a broad range of questions in snake systematics.

Suggested English common name. Honduras Montane Pitviper.

Definition and diagnosis. Similar to other *Cerrophidion* species, *C. wilsoni* is a medium-sized, blotched terrestrial pitviper; head relatively long; distinct and raised canthal ridge, typically two canthals; a frontal plate; broad supraoculars; nasal divided; prefoveals 1–4; prelacunal single; lacunolabials absent; loreal single; subfoveals 0–2; three preoculars; supralabials 7–11, typically 9; infralabials 10–12, typically 11; ventrals 137–151 (\bar{x} = 142.21), undivided subcaudals 23–36 (\bar{x} = 30.12) with no significant sexual dimorphism; cloacal scute undivided; tail relatively short and non-prehensile; and typically 21 middorsal scale rows. *Cerrophidion wilsoni* differs from other *Cerrophidion* species in normally having fewer scales that contact the supraoculars, averaging 9 compared to 10–12 in other *Cerrophidion* species (Jadin 2010). Additionally, the median frontal scale of *C. wilsoni* is often quite small, distinguishing it from other *Cerrophidion* species, and in many individuals the frontal scale is greatly reduced to the size of a normal head scale (Campbell & Solórzano 1992). In those individuals that contain a small frontal plate, this plate occupies an average of 36% of the distance between the supraoculars (Campbell & Solórzano 1992).

Similar species. Several other species of pitviper are sympatric with *C. wilsoni* at various localities in Honduras. The palm-pitviper *Bothriechis marchi* is sympatric with *C. wilsoni* in Parque Nacional Cusuco in the Sierra de Omoa in northwestern Honduras, and *C. wilsoni* is sympatric or nearly sympatric with *Atropoides mexicanus* and

Bothrops asper along the lower edges of the cloud forest (Townsend & Wilson 2008), a situation mirrored with the same four species in Reserva de Vida Silvestre Texíguat in north-central Honduras (Townsend *et al.* 2012). There may also be sympatric or near-sympatric populations of *Bothriechis thalassinus* and *C. wilsoni* in the vicinity of the shared border between Guatemala, Honduras and El Salvador. These species are easily distinguished from *C. wilsoni* by the following characteristics: *Bothriechis* species have prehensile tails, are typically slender and are mostly green, except certain colour variations in *B. schlegelii*. The species most closely resembling *C. wilsoni* are certain populations of *A. indomitus* and *A. mexicanus* but they have broader heads, more numerous distinctly tuberculate supracephalic scales, a narrower and longer dark postocular stripe, nasorostrals present and a much stockier body shape.

Description of holotype. Rostral broader than high (4.73 × 4.10 mm); two internasals anteriorly; 2/2 canthals, four posterior intercanthals; supraoculars more than twice as long as broad; seven intersupraoculars; scales on head of medium size, lacking the large median frontal scale and large flat scales in parietal area usually associated with *Cerrophidion* species; interrials 24; single loreal bounded by canthal above, prelacunal and prefoveals below; prefoveals 5/5 (2/2 tiny, granular), subfoveals 1/1, postfoveals 4/4; prelacunal contacting second and third supralabials; 3/3 preoculars, upper largest, middle small with vertical suture separating it from supralacunal; suboculars 2/2, elongate, crescent-shaped; 3/3 postoculars; supralabials 10/10; mental broader than long (4.68 × 2.72 mm); infralabials 12/12; chin shields contacting first four pairs of infralabials; three pairs of gulars between chin shields and first preventral; dorsal scale rows 23–21–17; preventrals 3; ventrals 148; cloacal scute undivided; 33 subcaudals numbers 1, 22–26, 31–33 are divided the rest undivided; tail spine as long as

preceding three subcaudals, straight, tip rounded (Figs 4 and 5).

Measurement of holotype. Total length 64.8 cm; tail length 7.3 cm, comprising 11.3% of total; head 31.95 mm from front face of rostral to posterior end of mandible near the rictus; head 23.0 mm at broadest point; neck 12.3 mm directly behind jaws.

Hemipenis description of paratype. The holotype for *C. wilsoni* is a female and therefore we describe the everted left hemipenis of a male paratype, UTA R-59478 (SVL 477 mm, TAL 61 mm, subcaudals 32). Hemipenis ca. 17.5 mm in total length and 10 mm in maximum width at level of crotch; on sulcate side base with several rows of small spines (<0.5 mm) followed by rows of larger spines and hooks extending for 4 mm, largest protruding ca. 3 mm; asulcate side with naked base up to 5 mm before level of bilobation, then with 4 mm section of small spines (<0.5 mm) arranged in rows followed by 4 mm section of larger spines; each lobe with ca. 70 spines and hooks; ca. 15 spines and hooks around each lobe at lower rim of calyces; calyces follow spines and hooks distally; calyces scalloped, ca. 17 rows extending 10 mm to apex of hemipenis on asulcate side; sulcus spermaticus deep and bifurcating ca. 2.5 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 bifurcation where small spines occur on outer border until bilobation at which point both the inner and outer borders of the sulcus spermaticus have small spines for 3 mm to the beginning of calyces which form border to apex of lobe.

Colour pattern in life. Dorsal ground colour mostly medium or coffee brown, usually paler on anterior third of body; 32–44 (\bar{x} = 34.8) dorsal body blotches, these usually fused, forming zig-zag dorsal stripe; dorsal blotches very dark brown or chestnut brown with centres that may be only slightly and inconspicuously paler in centres; dorsum of tail mostly dark brown; lateral blotches mostly opposite of lateral extensions of dorsal blotches, blackish brown to almost black, most subcircular, but may be horizontally elongate on anterior of body where they may extend 5–10 scale lengths; most lateral blotches at about midbody cover areas equal to 5–7 scales; dorsolateral interspaces between dorsal and lateral blotches paler than adjacent ground colour; top of head medium to dark brown, not as dark as body blotches; side of head below postocular stripe pale brown with dark brown to black postocular stripe extending from lower posterior edge of eye to past angle of jaw, sometimes coalescing with first lateral blotch on neck; postocular stripe with a conspicuous narrow pale border

ventrally; labial scales without mottling; gular area pale yellow or orange; anterior ventrals pale with little dark stippling or mottling, venter becoming darker posteriorly; proximal subcaudals dark, distalmost subcaudals invariably pale. No distinct colour phases have been reported but considerable variation has been described (Campbell & Solórzano 1992; Campbell & Lamar 2004). An additional description of *C. wilsoni* (as *C. godmani*) in life and in alcohol is found in McCranie (2011:505–506).

Distribution and habitat. *Cerrophidion wilsoni* occurs primarily in lower montane rainforest between 1400 and 3491 m (Campbell & Solórzano 1992; Campbell & Lamar 2004), and may occur peripherally in premontane rainforest and pine-oak forest as low as 1220 m (Wilson & McCranie 2004; Köhler *et al.* 2006; McCranie 2011). Within lower montane rainforest, this species has been observed in undisturbed forest, in areas of disturbed forest, in and around coffee and other agricultural clearings and in mountaintop elfin forest and wind scrub habitat. This species occurs in at least 13 isolated highland forest areas across Eastern Nuclear Central America (as defined by Campbell 1999), and all known populations of *C. wilsoni* are found within the borders of Honduras and El Salvador (Wilson & Meyer 1985; Köhler *et al.* 2006; McCranie 2011). Highland areas that support populations of *C. wilsoni* in Honduras and El Salvador also extend into eastern Guatemala and the species very likely occurs in that country.

Although Villa (1962, 1984) reported *C. godmani s.l.* from northern Nicaragua, neither voucher specimens nor precise locality data exist to verify its presence in this country (Campbell & Solórzano 1992; Campbell & Lamar 2004; Jadin 2010), and repeated visits to highland sites that would be the most likely to support *Cerrophidion* have not produced any physical evidence of its occurrence in Nicaragua (J. Sunyer and S. Travers, pers. comm.). However, J. Sunyer (pers. comm.) shared with us that residents of the Nicaraguan highlands are familiar with a snake they call ‘toboba de altura’, a highland version of the ‘toboba’ (*Porthidium opbryomegas*), which they also clearly differentiate from the ‘mano de piedra’ (*Atropoides mexicanus*). Furthermore, *Mesaspis moreletii* – a member of a genus of lizard which is sympatric throughout most of the range of *Cerrophidion sensu stricto* and *Mixcoatlus barbouri* and *M. browni* with likely similar fundamental environmental niche space – has recently been collected at Reserva Natural Cerro Kilambé in the northern Nicaraguan highlands (Sunyer & Köhler 2007), indicating there is still a possibility that Nicaraguan populations of *Cerrophidion* may yet be discovered. Furthermore, the highest mountain range in Nicaragua, which includes the country’s tallest peak at

Cerro Mogotón (2107 m), straddles the border with Honduras and biological sampling has been limited by the presence of land mines left over from the Contra-Sandinista War of the 1980s.

Natural history. This terrestrial species is active during the day. In Parque Nacional Cusuco, this species has been repeatedly observed while coiled in direct sunlight at the entrances to small holes in root-masses of fallen trees, as well as basking in and around a large landslide. In the same forest, *C. wilsoni* has been observed both coiled and active around foot trails during the day and night, under ground cover during the day, and a large female was active at night crawling along a large log. A male paratype (UTA R-59478) was collected at dusk as it crawled along a road through disturbed cloud forest accessing a mountaintop communications tower in Reserva Biológica Güisayote. In Reserva de Vida Silvestre Texiguat in north-central Honduras, a juvenile paratype (UTA R-59480) was found coiled in direct sunlight on top of a large log at the edge of a recently cleared forest patch. In Parque Nacional Montaña de Yoro, one juvenile was collected while active in leaf litter near a log during the day, and a subadult female paratype (UTA R-59479) was coiled at the edge of a small agricultural clearing near a house. Additional ecological notes for populations herein assigned to *C. wilsoni* are summarized in Wilson & Meyer (1985), Leenders & Watkins-Colwell (2004), Köhler *et al.* (2006) and McCranie (2011).

Reproductive data that can be attributed to *C. wilsoni* is limited. However, Mertens (1952) reported one gravid female from Cerro Montecristo, El Salvador, that contained nine embryos, and McCranie (2011) reported a female collected in May near the Honduran side of Cerro El Pital contained 14 embryos and another collected in August from the same locality was gravid.

Discussion

Systematics and evolutionary morphology

Previous phylogenetic studies (Castoe *et al.* 2005, 2009; Daza *et al.* 2010) have found weak support for the monophyly of *C. godmani sensu lato* to the exclusion of a *C. petlalcalensis*–*C. tzotzilorum* clade. This relationship is not supported in our analyses, and we recovered moderately strong support (PP = 87) for *C. godmani s.s.* as the sister lineage of a *C. petlalcalensis*–*C. tzotzilorum* clade; this cluster is the sister group to a *C. sasai*–*C. wilsoni* clade. Our phylogenetic analysis does, however, agree with these previous studies in recovering strong support for three distinct and deeply divergent species-level clades of *C. godmani s.l.*, which are allopatrically distributed in the highlands of Middle America (see Fig. 7). Previous estimates of the divergence time of these clades of *C. godmani s.l.* infer a shared common ancestor 7.7–11.5 mya, with the most recent divergence between *C. sasai* and *C. wilsoni* occurring 3.1–6.0 mya (Castoe *et al.* 2009; Daza *et al.* 2010).

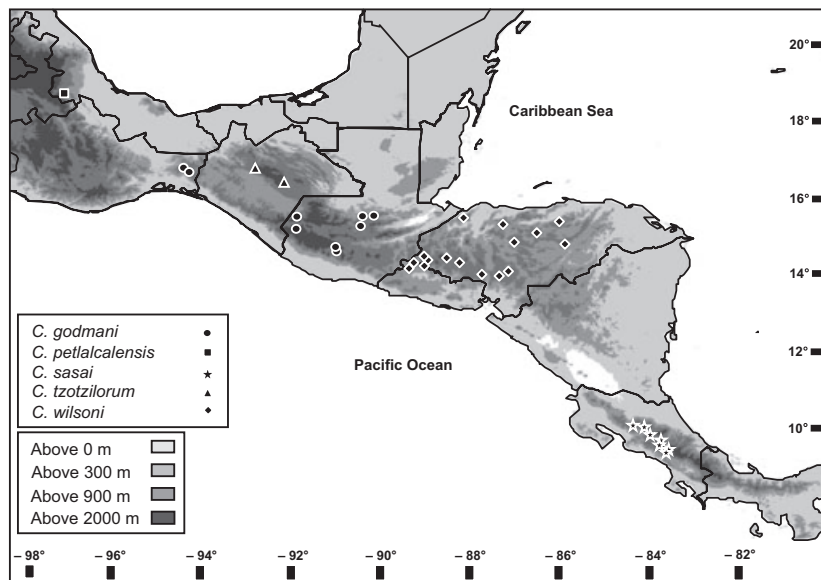


Fig. 7 Locality map of *Cerrobidion* samples throughout Middle America used for the molecular analyses. Symbols correspond to the origin of the samples (taken from Castoe *et al.* 2009 and new localities).

Based on phylogenetic analysis of morphological characters, Jadin (2010) also inferred that Guatemalan populations formed a clade, as did El Salvador and Honduras populations, similar to findings in this study. However, based on this morphological dataset, Jadin (2010) inferred a sister relationship between *C. godmani s.l.* populations from Costa Rica and Mexico. Campbell & Solórzano (1992: 236), on the other hand, did not suggest any close relationship between these populations but were the first to point out that populations at the northern and southern extremes of the range retained certain characters that might be considered pleisiomorphic. Thus, the morphology of *C. godmani s.l.* apparently is more similar between individuals at the periphery of its range than populations in the center. While this relationship has never been supported by molecular data (Castoe *et al.* 2005, 2009; this study), it is intriguing as it suggests that either the geographically peripheral lineages are morphologically convergent, or that these peripheral lineages (*C. godmani* and *C. sasai*) have retained ancestral morphological features that the centrally distributed clade (*C. wilsoni*) has lost (Campbell & Solórzano 1992).

It is reasonable to wonder why such deeply divergent lineages of obviously allopatric snake populations have not been previously recognized as distinct species. One unique characteristic associated with snakes of the genus *Cerrophidion* is a tremendous amount of morphological variation among individuals within a single population, including numbers of particular scales, as well as substantial asymmetry of scales within individuals, and extensive scale fusion that occurs frequently, particularly on the head (Campbell & Solórzano 1992; Jadin 2010). Thus, despite a tremendous amount of morphological variation observed, the extensive variation among individuals within populations has left few obviously diagnostic characters by which populations could be readily distinguished. Often when external morphology presents such challenges, hemipenial characters may be useful to differentiate and diagnose species, but in *Cerrophidion* there is only slight distinctions in hemipenes among the five species (see Fig. 8A–D in this study and Campbell & Lamar 2004: Figs 148–150). Thus, only with the added perspective of molecular data, together with morphological evidence and better-defined geographical distributions of populations based on many years of field collections, have distinctions of the various lineages become obvious, leading to the present systematic revision.

Conservation

The recognition of *Cerrophidion sasai* and *C. wilsoni* adds to the rapidly growing list of new and cryptic species being discovered and described from highland forests in Central America (e.g. Smith & Ferrari-Castro 2008; Campbell

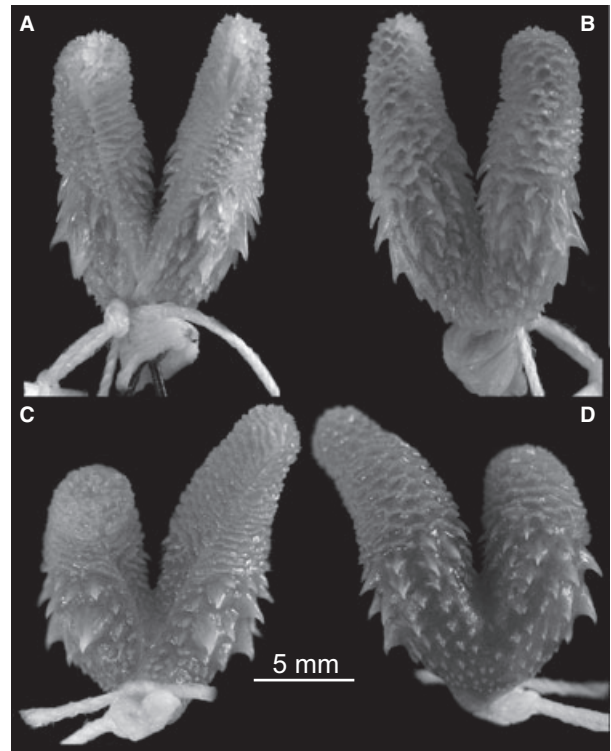


Fig. 8 Sulcate (A, C) and asulcate (B, D) views of the left hemipenes of *Cerrophidion sasai* (holotype, UTA R-51399) and *C. wilsoni* (paratype, UTA R-59478), respectively.

et al. 2010; Townsend *et al.* 2011). In Honduras, much of the known and potential habitat for *C. wilsoni* is found within protected areas, a fortuitous result of the 1987 law that established the Honduran Protected Areas System (SINAPH), which ostensibly protects the sources of potable water (i.e. highland forests above 1800 m) for people living in the lowlands (Townsend & Wilson 2010). In 2011, the newest protected area in Honduras was established, Parque Nacional Montaña de Botaderos, which includes within its limits the type locality of *C. wilsoni*. Including Parque Nacional Montaña de Botaderos, *C. wilsoni* is known to occur within the boundaries of at least 15 protected areas in Honduras, encompassing the entire known range of the species in that country: Parque Nacional Celaque, Parque Nacional Cusuco, Parque Nacional La Tigra, Parque Nacional Montaña de Botaderos, Parque Nacional Montaña de Yoro, Parque Nacional Sierra de Agalta, Parque Nacional Trifinio Montecristo, Refugio de Vida Silvestre La Muralla, Refugio de Vida Silvestre Mixicure, Refugio de Vida Silvestre Texíguat, Reserva Biológica Cordillera de Opalaca, Reserva Biológica Guajiquiro, Reserva Biológica Güisayote, Reserva Biológica El Pital and Reserva Biológica Yerba Buena. Honduras has the

highest rate of herpetofaunal endemism of any Central American country (Wilson & Johnson 2010), due largely to having over 30 isolated highland forest areas that each support their own unique suite of endemic reptiles and amphibians (Wilson & McCranie 2004). A similar situation is found in El Salvador, where known populations of *C. wilsoni* occur within the boundaries of Parque Nacional Montecristo (the Salvadoran side of Parque Nacional Trifinio Montecristo), the largest area of intact montane forest remaining in that country. Given that *C. wilsoni* occurs in at least half of the declared highland forest protected areas in Honduras and El Salvador, and likely is simply undocumented as occurring in others, we would propose this emblematic representative of highland biodiversity as a potential ‘flagship’ species for cloud forest conservation.

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Supporting Information

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

Appendix S1. Specimens examined. Museum acronyms follow Leviton *et al.* (1985).

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.

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