Hidden in the highs: Two new species of the enigmatic toadheaded pitvipers of the genus *Bothrocophias*

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**Abstract**

*Bothrocophias microphthalmus* (Cope, 1875) currently comprises most mid- to highland populations of the genus *Bothrocophias* in the eastern versant of the Andes. We describe two new species of *Bothrocophias* from the highlands of the Colombian Andes previously referred to as *B. microphthalmus* based on morphological and genetic evidence. Our phylogenetic analysis revealed that *B. microphthalmus* as currently recognized is paraphyletic with respect to *B. hyoprora*, and the two new taxa are sister lineages. These new toadheaded pitvipers can be morphologically distinguished from their congeners based on the presence of contact between the internasal scales, the number of prefoveal scales, the presence of a lacunolabial scale, the arrangement of supralabial scales, ventral scale counts, the color pattern of the dorsal and ventral surfaces of the body and tail, and hemipenial morphology. We discuss some possible taxonomic scenarios for the lineages found within the *B. microphthalmus* species complex but refrain from making additional taxonomic changes given our reduced sampling of the southern lineages.

**Keywords**

Cryptic species, high Andean snakes, integrative taxonomy, medically important snakes, mtDNA, South America, Viperidae

**Introduction**

Some of the most enigmatic and poorly known South American vipers are the toadheaded pitvipers of the genus *Bothrocophias* (Gutberlet and Campbell 2001). These snakes inhabit isolated and difficult-to-access ecosystems of South America, such as the hyperhumid Chocooan rainforest, the western lowlands of the Amazonian rainforest, and the highlands of the Pacific and eastern versant of the Andes mountains, and habitats including wet montane forest, cloud montane forest, and high Andean Forest (Rangel-Ch 1995; Warrell 2004). The scarcity of *Bothrocophias* material in biological collections, coupled with the tendency of *Bothrocophias* species to be mistaken for the more common and widespread members of the genus *Bothrops* (namely *B. asper* and *B. atrox*), has greatly impeded taxonomic assessment of this group.

A few taxonomic studies of *Bothrocophias* species have been conducted since the generic recognition of *Bothrocophias* and the description of *B. myersi* by Gutberlet...

*Bothrocophias microphthalmus* has the broadest distribution in the Andes among toadheaded viper species; it ranges from the eastern versant of the Cordillera Oriental of Colombia in the departments of Boyacá, Casanare, and Cundinamarca (1,700–2,400 m asl); the Amazonian slopes of the Ecuadorian Andes in the provinces Morona-Santiago, Pastaza, Tungurahua, and Zamora-Chinchipe (850–1,650 m asl); the Amazonian slopes of Brazil in the state of Rondônia (ca. 150 m asl); the eastern versant of the Peruvian Andes in the departments of Cuzco, San Martín, Huancaco, and Sira (800–2,100 m asl); and across the Bolivian Andean highlands in the departments of Beni, Cochabamba, La Paz, Pando, and Santa Cruz (600–1,600 m asl) (Nicéforo-María 1975; Campbell and Lamar 2004; Harvey et al. 2005; Bernarde et al. 2012; Carrasco et al. 2012; Angarita-Sierra et al. 2013; Valencia et al. 2016; Torres-Carvajal et al. 2019). Schätti and Kramer (1993), when studying Ecuadorian vipers, noticed that Colombian populations of *B. microphthalmus* (referred to as *Porthidium microphthalmum*) had higher scale counts than the Ecuadorian ones. Similarly, Campbell and Lamar (2004) noted that there is considerable variation in morphology among snakes currently assigned to *B. microphthalmus* and emphasized the need for a comprehensive taxonomic assessment of the northern and southern Andean populations. However, the number of records of this species across its broad range is low (~138 according to Gbif. Org 2021), especially in Colombia, where most localities are represented by few specimens (Mo=2).

Given the high diversity of medically important snakes in Colombia, the National Health Institute (INS, Spanish acronym) of Colombia has put much effort over the last decade into making collections of venomous snakes to enhance the production and neutralization capacity of their polyvalent viperid snake antivenom for the treatment of serious snakebite accidents caused by viperid snake species such as *Bothrocophias microphthalmus* (Campbell and Lamar 2004; Chippaux 2017). New *B. microphthalmus* material from the departments of Boyacá, Casanare, Cundinamarca, and Meta has been deposited in the biological collections of the INS because of this initiative, and this has made a comprehensive assessment of the Andean *Bothrocophias* populations possible. These newly collected specimens, as well as the previous specimens reported by Nicéforo-María (1942) and Campbell and Lamar (1989, 2004) from other biological collections in Colombia exhibit unique dissimilarities. Additionally, various characteristics of Colombian specimens do not match those of the *B. microphthalmus* holotype specimen (Cope, 1875) from Peru; available descriptions of *B. microphthalmus* populations from Ecuador, Peru, and Bolivia; nor with descriptions of other recognized *Bothrocophias* species (Gutberlet and Campbell 2001; Campbell and Lamar 2004; Harvey et al. 2005; Carrasco et al. 2012; Hamdan et al. 2019). Here, we show that these new specimens as well as previous specimens reported represent two undescribed evolutionary lineages of *Bothrocophias*. We describe these new taxa based on molecular and morphological data.

**Methods**

**Ethics statement**

Live *Bothrocophias* specimens were obtained as donations from Colombia’s Environmental Regional Autonomous Corporations (CARs, Spanish acronym). When snake specimens died in captivity, they were fixed and preserved in 10% formalin and 70% ethanol, respectively, and deposited in the INS zoological collection (INSZ). The procedures used and environmental conditions during the captivity period and fixing process were approved by the Animal Ethical Use Committees of the Instituto Nacional de Salud de Colombia (protocol INT-R04.0000–001), abided by the Colombian animal welfare law (Congreso de Colombia 2016), and were compliant with the Universal Declaration on Animal Welfare (UDAW) endorsed by Colombia in 2007.

**Species concept and delimitation approach**

There remains much debate over the utility of different species concepts in light of the recognition that no single concept can simultaneously account for the diverse ways in which life evolves (De Queiroz 2007, 2011; Sites Jr et al. 2021). In addition, there is no single property or character that can be considered crucial in species delimitation because every single criterion is likely to fail or yield ambiguous results (Markolf et al. 2011). Nevertheless, all species concepts have one feature in common: all consider species as hypotheses (Hillis et al. 2021; Sites Jr et al. 2021). Thus, the species status of taxa can be tested using different datasets (= lines of evidence) (Mayr 1996).

We believe the various populations of *B. microphthalmus* to be a species complex based on previously discussed taxonomic challenges (Schätti and Kramer 1993; Campbell and Lamar 2004). Given that the original description of *B. microphthalmus* (Cope, 1875) was based
on a specimen from Peru, the Peruvian populations merit the original epithet *microphthalmus*. We hypothesized that the populations of *B. microphthalmus* from Colombia, Ecuador, and Bolivia represent candidate species. Herein, we evaluate the species status of two proposed Colombian candidate species, one with less than 28 dark-brown bands dorsally and lacking a lacunolabial scale (*B. microphthalmus* – Colombia 1) and another with more than 28 dark-brown bands dorsally and with a lacunolabial scale (*B. microphthalmus* – Colombia 2).

We followed the species delimitation approaches proposed by Padial et al. (2010), Hillis et al. (2021), and Sites Jr et al. (2021), and the congruence principle as a method for testing the hypothesis that the Colombian populations of *B. microphthalmus* represent two undescribed species (Crisci 1984; Lienau et al. 2006; Leigh et al. 2011). Thus, we used the hemipenial morphology, color pattern, meristic characters, and molecular data as different lines of evidence with equivalent weight.

### Molecular data collection and laboratory procedures

To evaluate the genetic distinctiveness and phylogenetic position of Colombian populations historically assigned to *B. microphthalmus*, we obtained genetic sequences from five individuals deposited in the Venomous Animal Tissue Bank of the National Institute of Health (INSBT), Bogotá, Colombia (Table 1).

Genomic DNA was extracted using the phenol-chloroform method (Sambrook et al. 1989). We used the primers CYB Pook-F and CYB Pook-R (Pook et al. 2000) and ND4-F and LEU-R (Arévalo et al. 1994) to amplify partial regions of 758 and 890 bp of the mitochondrial genes cytochrome *b* (MT-CYB) and NADH dehydrogenase subunit 4 (MT-ND4). PCR reactions were run in a total volume of 30 μL, including one-unit of Taq polymerase (Bioline; Randolph, MA), 1X buffer (Bioline), 1.5 mM MgCl$_2$ (Bioline), 0.5 μM of each primer, 0.2 mM of each dNTP (Bioline), 0.2 μg of bovine serum albumin (BSA), and approximately 50 ng of DNA. Thermocycling conditions were as follows: 95°C for 5 min; 35 cycles of 94°C for 30 s, 48°C for 45 s, and 72°C for 45 s; and a final extension at 72°C for 5 min. The sequencing processes were carried out at the Instituto de Genética, Universidad Nacional de Colombia, Bogotá-Colombia. Sequence editing and consensus sequence generation were conducted using Geneious 9.1.8 (Kearse et al. 2012). The GenBank accession numbers for all sequences (newly generated sequences included) are presented in Table 1.

### Phylogenetic analyses and genetic distances

The obtained sequences were aligned with sequences of the genus *Bothrocophias* deposited in Genbank. We used *Aezmiops feae*, *Aegistrodon piscivorus*, and *Bothrops pubescens* as outgroups (Table 1). We omitted the Cytochrome *b* sequence available from the Peruvian sample of *B. microphthalmus* LSUMZ-H 9372 (Genbank accession AY223594) because BLAST analysis revealed that it was more similar to *Bothrops bilineatus* than to any *Bothrocophias*. The alignment was conducted using the MAFFT 7.39 algorithm (Katoh and Standley 2013) with the au-
tomatic algorithm selection in Geneious 9.1.8 (Kearse et al., 2012). The two fragment alignments were concatenated using Sequence Matrix 1.8 (Vaidya et al. 2011).

The best partitioning scheme and evolutionary model were obtained using ModelFinder (Kalyaanamoorthy et al. 2017) in IQTree 2 (Minh et al. 2020) using the –m MFP+MERGE option and the “greedy” algorithm (Lanfear et al. 2012). We ran the phylogenetic analyses using maximum likelihood (ML) and Bayesian inference (BI). Obtained partitions and models are described in Table S1. ML analyses were run in IQTree 2 using the models and partitions obtained with ModelFinder. Node support obtained from 5000 ultrafast bootstrap replicates was considered high when values were greater than 95%. BI was run in BEAST 2.6.1 (Bouckaert et al. 2019). We used the best partitioning scheme obtained in ModelFinder, but evolutionary models were inferred using bModelTest 1.2 (Bouckaert and Drummond 2017); all available models were explored (Table S1). All obtained partitions were linked for the tree model, and the Birth Death Model was used as the prior tree. Two independent chains of 10 million generations, with sampling every 1000\(^{a}\) generation, were run in BEAST. Chain convergence and the burn-in value (10\%) were assessed using TRACER 1.7.1 (Rambaut et al. 2018). A consensus tree was summarized using TREANNOTATOR in BEAST 2.6.1 (Bouckaert et al. 2019). Tree annotation and plotting were conducted using the packages ‘ape’ (Paradis and Schliep 2019) and ‘phangorn’ (Schliep 2011) in R. We calculated between-group mean Kimura 2-parameter distances for the concatenated alignment using MEGA X (Kumar et al. 2018).

**Morphological analyses**

We examined specimens of Bothrocophias microphthalmus housed in the following biological collections in Colombia: Museo de la Universidad La Salle (MLS, Bogotá), Pontificia Universidad Javeriana (MUJ, Bogotá), Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH-R, Villa de Leyva), Colección de Animales Venenosos del Instituto Nacional de Salud (INSVSR, Bojacá), and Colección Zoológica del Instituto Nacional de Salud (INSZ, Bogotá). We compared meristic characters of the Colombian populations with those of the B. microphthalmus holotype specimen (housed in The Academy of Natural Sciences of Drexel University, ANSP 11515), as well as published descriptions of B. microphthalmus populations from Ecuador, Peru, and Bolivia. In addition, we compared head pholidosis and the color pattern of the body of Colombian B. microphthalmus with Ecuadorian populations using pictures available from Bioweb Ecuador (Torres-Carvajal et al. 2019) and a species account (Arteaga 2020), and with Peruvian populations using pictures provided by Juan Timms (Appendix). Hemipenial eversion procedures from fixed specimens followed Pesantes (1994), with modifications to Pesantes’ method described by Myers and Cadle (2003) and Zaher and Prudente (2003). Hemipenial eversion procedures from fresh-euthanized specimens followed Manzani and Abe (1988). The staining procedure followed Jadin et al. (2010) and Angarita-Sierra (2014). Terminology for hemipenial morphology followed Campbell and Lamar (2004), and Myers and McDowell (2014).

Terminology used in the diagnosis, comparisons, and description sections followed Guthberlet and Campbell (2001), Campbell and Lamar (2004), Harvey et al. (2005), and Valencia et al. (2016). Sex was determined by checking for the presence of hemipenes using caudal incisions. We measured 19 head characters described by Hoyos et al. (2003) and Matias et al. (2011) from digital pictures of specimens using ImageJ software version 1.52 (Schneider et al. 2012): head length (HL), head width (HW), nostril width (NW), internasal width (IW), cantal width (CW), loreal width (LW), lacunal width (LCW), eye width (EW), intrasupraocular width (ISW), supraocular length (SL), supraocular width (SW), snout-eye length (SEL), snout-lacunal length (SLL), snout-loreal length (SLRL), nostril-eye length (NEL), nostril-lacunal length (NLL), nostril-loreal length (NLLR), maximum length of the 3\(^{a}\) supralabial scale (ML3S), and sinusial length (SL) (Table S2). Snout-vent length (SVL) and caudal length (TL) were measured using measuring tape (± 1 mm).

**Multivariate morphometrics**

We carried out a multidimensional scaling (MDS) test to assess whether the meristic characters and color pattern allow two candidate species to be distinguished among Colombian populations currently assigned to B. microphthalmus. This statistical technique does not require a priori grouping of specimens or linearity assumptions of the dataset (Kruskal and Wish 1978; Guisande-Gonzáles et al. 2014). The goodness of fit between the fitted and observed distances was measured using a stress test (“Kruskal’s stress”, S) (Kruskal and Wish 1978), which is an average of the deviations between the end and the initial spatial distances and normalized so that values range from 0 and 1. Values near 1 indicate the worst fit, and values near 0 indicate the best fit. However, values between 0.025 and 0.05 are considered good values, values less than 0.025 are considered excellent, and values equal to 0 are perfect (Guisande-Gonzáles et al. 2014). The MDS was carried out using the software Rwizard version 4.0 (Guisande-Gonzáles et al. 2014) and the MDS function of the “vegan” package (Oksanen and Simpson 2013). The function “scatterplot” in the “car” package was used to generate the biplot graphs (Fox et al. 2014).

**Envenomation assessment**

Over the last decade (2010–2020), the Colombian surveillance health system (SIVIGILA Spanish acronym) has reported a total of 336 bothropic-like envenomations across known and new localities within Colombia, and 240 of these snakebites have been clearly caused by Bothrops species. Determining the incidence of snakebites of
the two new species is a major challenge because *Bothrocophias* might be misidentified as *Bothrops asper* and *B. atrox*. In addition, *Bothrocophias* is not listed in the list of possible genera that cause snakebites in Colombia on the SIVIGILÁ notification form for reporting snakebite accidents. Thus, there are either no official reports of *Bothrocophias microphthalmus* envenomation, or snakebites attributed to *Bothrops* species might have been caused by *Bothrocophias* species. We reviewed all snakebite records (Instituto Nacional de Salud 2020) attributed to *Bothrocophias* and retained those most likely caused by the two new species described herein using elevation and the presence of *B. atrox* records at the site of reported snakebites as exclusion criteria and the match of reported symptoms of snakebite victims with those described in the literature as inclusion criteria (Campbell and Lamar, 2004).

## Results

### Species delimitation assessment

Our final concatenated alignment of the two mitochondrial fragments comprised 1616 bp. The topology of the BI and ML trees was concordant, and clade support was similar among the two trees (Fig. 1). Our trees indicated that *Bothrocophias microphthalmus*, as traditionally recognized, was paraphyletic with respect to *B. hyoprora* and included three independent clades: one with Bolivian specimens with maximal support; one with Peruvian and Ecuadorian specimens with low support; and one highly supported clade of Colombian specimens. This last clade was sister to *B. hyoprora* in a highly supported clade. The Colombian clade also comprised two highly supported subclades: a northeastern clade and a southwestern clade with high support.

Kimura 2-p genetic distances between the *Bothrocophias* clades ranged from 14.4% to 1.76% (Table 2). The greatest distance was between *B. hyoprora* and *B. loja-nus*, whereas the lowest was between the two Colombian clades. Distances between the *B. microphthalmus* clades from Ecuadorian, Peruvian, and Bolivian populations ranged from 7 to 9.5%.

Our MDS results showed conspicuous distance or dissimilarity between the two Colombian candidate species of the *B. microphthalmus* complex. Both candidate species can be distinguished by quantitative differences in traits, such as the presence/absence of lacunolabial scale, postfoveal scale, presence/absence of internasal scales separated by one small scale, prelacunal scales, subnasal scales and preventral scale counts, and number of dorsal bands. The MDS goodness-of-fit between the fitted and observed distances shows an excellent stress value ($S = 0.019$), and the candidate species showed low overlap in the morphospace, indicating that there is an underlying structure based on the set of the meristic and color pattern characters used that allow the two groups to be clearly differentiated (Fig. 2).

In general, the independent lines of evidence assessed (Table 3) suggest that the Colombian populations of the *B. microphthalmus* complex should be recognized as two undescribed species. Likewise, the three additional lineages obtained can be recognized as undescribed species.
and indicate that the populations from Peru probably can be assigned to *B. microphthalmus*. However, Kimura 2-p genetic distances between the two Colombian lineages are low (1.76%) compared with the distances between the other lineages in the *B. microphthalmus* species group (Table 2), but there are clear differences between them and the remaining lineages within the group, supporting their genetic distinctiveness from the southwestern forms, including the Peruvian sample. Despite the low genetic differentiation, the two Colombian lineages were recovered as reciprocally monophyletic with high support (Fig. 1).

Hence, the combination of independent lines of evidence showed a congruent pattern of divergence among

### Table 2. Estimates of the evolutionary divergence between sequences of *Bothrocophias microphthalmus* populations expressed as percentages (averages). Values below the diagonal represent between-lineage divergences using 1270 bp of the Cytochrome b (MT-CYB) and NADH subunit 4 (MT-ND4) genes.

<table>
<thead>
<tr>
<th>Bothrocophias species/clade</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 <em>B. lojanus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 <em>B. microphthalmus</em>-Bolivia</td>
<td>13.06%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 <em>B. microphthalmus</em>-Ecuador</td>
<td>13.51%</td>
<td>9.52%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 <em>B. microphthalmus</em>-Peru</td>
<td>13.26%</td>
<td>8.64%</td>
<td>7.02%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 <em>B. hyoprora</em></td>
<td>14.44%</td>
<td>9.72%</td>
<td>8.21%</td>
<td>9.31%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 <em>B. myrringae sp. nov.</em></td>
<td>13.88%</td>
<td>9.96%</td>
<td>7.94%</td>
<td>8.04%</td>
<td>6.49%</td>
<td></td>
</tr>
<tr>
<td>7 <em>B. tulitoi sp. nov.</em></td>
<td>12.78%</td>
<td>8.91%</td>
<td>7.30%</td>
<td>7.88%</td>
<td>6.00%</td>
<td>1.76%</td>
</tr>
</tbody>
</table>

the several taxonomic characters assessed, indicating lineage separation between candidate species from Colombia, Ecuador, and Bolivia. Although only four of the five lines of evidence assessed were congruent within Colombian populations, there was clear evidence of lineage separation, including in hemipenial morphology, which is a proxy of reproductive isolation. Therefore, the available data of the present study, including the observed phylogenetic positions and morphological distinctiveness, support the recognition of the two Colombian clades as new taxa, which are described as follows.

**New species descriptions**

**Generic placement.** The new species are recognized as members of the genus Bothrocophias based on their placement in phylogenetic trees and according to the following combination of morphological characters defined by Gutberlet and Campbell (2001) and Campbell and Lamar (2004): (1) moderate length; (2) relatively stout-bodied and terrestrial; (3) slender tail not prehensile; (4) white spots with dark borders on some gulars and infralabials (tricolored ocellus in males and some neonate females); (5) 21–25 middorsal scale rows; (6) 2–9 slightly keeled intersupraoculars scales; (8) absence or presence of a lacunolabial scale; (9) 7–9 supralabial scales; (9) 8–10 infralabial scales; (10) 2–6 prefoveal scales; (11) hemipenial lobes calyculate distally; and (12) hemipenes lacking basal hooks.

**Bothrocophias tulitoi** sp. nov.

http://zoobank.org/A3036137-DDB6-47BA-B61D-923C745D-34F0

Figs 1–6


**Holotype.** (Fig. 3) INSZ 073, an adult male from vereda Ciénaga La Valvanera, municipality of Garagoa, department of Boyacá, Colombia. Coordinates: N 5.106535941, W –73.25888414; elevation 1,894 m asl. The specimen was collected by staff of Corporación Autónoma Regional de Chivor (CORPOCHIVOR, Spanish acronym) on 17th October 2017.

**Paratypes.** COLOMBIA (n=20; Fig. 4): Boyacá: municipality of Garagoa. Locality: unknown, IAvH-R 5742, 6392, 6396, coordinates N 5.08236, W –73.4714222; complete sequence for mitochondrial COI, ND3 and 16S; elevation 1,894 m asl. The specimen was collected by staff of Corporación Autónoma Regional de Chivor (CORPOCHIVOR, Spanish acronym).

**Table 3.** Species delimitation for Bothrocophias microphthalmus populations hypothesized as candidate species using the integrative taxonomic approach of Sites Jr et al. (2021). Bp = posterior probability values from Bayesian inference tree, UFB, UltraFast Bootstrap support obtained in the ML tree. * = Moderate diagnosable means that there was some overlap in the scutellation characters. Values for genetic distinctiveness correspond to the genetic distance to the closest clade (see Table 2).

<table>
<thead>
<tr>
<th>Species hypotheses for Bothrocophias microphthalmus complex</th>
<th>mtDNA tree (UFB/Bp)</th>
<th>mtDNA genetic distinctiveness</th>
<th>Color pattern</th>
<th>Meristic characters</th>
<th>Hemipenial morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. microphthalmus – Bolivia</td>
<td>Distinguishable lineage highly supported (100/1)</td>
<td>Highly distinct (8.64%)</td>
<td>Clearly diagnosable</td>
<td>Clearly diagnosable</td>
<td>Unknown</td>
</tr>
<tr>
<td>B. microphthalmus – Ecuador</td>
<td>Distinguishable lineage highly supported (97/1)</td>
<td>Highly distinct (7.02%)</td>
<td>Clearly diagnosable</td>
<td>Clearly diagnosable</td>
<td>Clearly diagnosable</td>
</tr>
<tr>
<td>B. microphthalmus – Peru</td>
<td>Distinguishable lineage poorly supported (&gt;95/0.95)</td>
<td>Highly distinct (7.02%)</td>
<td>Clearly diagnosable</td>
<td>Clearly diagnosable</td>
<td>Unknown</td>
</tr>
<tr>
<td>B. tulitoi sp. nov.</td>
<td>Distinguishable lineage highly supported (95/1)</td>
<td>Highly distinct (6–12.78%) from non-Colombian populations, but short distance from B. microphthalmus Colombia 1 (&lt;2%)</td>
<td>Clearly diagnosable</td>
<td>Clearly diagnosable</td>
<td>Clearly diagnosable</td>
</tr>
<tr>
<td>B. myrringae sp. nov.</td>
<td>Distinguishable lineage highly supported (99/1)</td>
<td>Highly distinct (6.49–13.88%) from non-Colombian populations, but short distance from B. microphthalmus Colombia 2 (&lt;2%)</td>
<td>Clearly diagnosable</td>
<td>Clearly diagnosable</td>
<td>Between non-Colombian populations, and moderate within Colombian populations*</td>
</tr>
</tbody>
</table>

**Species hypotheses for Bothrocophias microphthalmus complex**

- **B. microphthalmus – Bolivia**: The new species are recognized as members of the genus Bothrocophias based on their placement in phylogenetic trees and according to the following combination of morphological characters defined by Gutberlet and Campbell (2001) and Campbell and Lamar (2004): (1) moderate length; (2) relatively stout-bodied and terrestrial; (3) slender tail not prehensile; (4) white spots with dark borders on some gulars and infralabials (tricolored ocellus in males and some neonate females); (5) 21–25 middorsal scale rows; (6) 2–9 slightly keeled intersupraoculars scales; (8) absence or presence of a lacunolabial scale; (9) 7–9 supralabial scales; (9) 8–10 infralabial scales; (10) 2–6 prefoveal scales; (11) hemipenial lobes calyculate distally; and (12) hemipenes lacking basal hooks.
Table 4. Comparisons of meristic and hemipenial characters between *B. myrringae* sp. nov., *B. tulitoi* sp. nov. and all its congeners of toadheaded pitvipers.

<table>
<thead>
<tr>
<th>Trait</th>
<th><em>B. andianus</em></th>
<th><em>B. campbelli</em></th>
<th><em>B. colombianus</em></th>
<th><em>B. hyopora</em></th>
<th><em>B. lojanus</em></th>
<th><em>B. myersi</em></th>
<th><em>B. myrringae</em> sp. nov.</th>
<th><em>B. tulitoi</em> sp. nov.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snout</td>
<td>No upturned</td>
<td>No upturned</td>
<td>Upturned</td>
<td>No upturned</td>
<td>No upturned</td>
<td>No upturned</td>
<td>No upturned</td>
<td>No upturned</td>
</tr>
<tr>
<td>Intersupraoculars</td>
<td>3–10</td>
<td>3–8 keeled</td>
<td>6–10 Tuberculate</td>
<td>2–9 smooth</td>
<td>3–5 slightly keeled</td>
<td>3–6 smooth</td>
<td>7–9 slightly keeled</td>
<td>5–9 slightly keeled</td>
</tr>
<tr>
<td>Lacunolabial</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Often present</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Dorsal scale surface</td>
<td>Keeled</td>
<td>Keeled</td>
<td>Keeled</td>
<td>Keeled</td>
<td>Keeled</td>
<td>Keeled</td>
<td>Keeled</td>
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</tr>
<tr>
<td>Wall of the hemipenial lobes</td>
<td>Robust and cylindrical</td>
<td>Robust and cylindrical</td>
<td>Conical</td>
<td>Slim and cylindrical</td>
<td>Conical</td>
<td></td>
<td></td>
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<tr>
<td>Walls of the Sulcus spermaticus</td>
<td>Robust and well defined</td>
<td>Robust and well defined</td>
<td>Robust and well defined</td>
<td>Weakly defined</td>
<td>Robust and well defined</td>
<td>Robust and well defined</td>
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<tr>
<td>Size of the lateral and mesial spines of the hemipenis</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Invariable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ornamentation of the hemipenial lobes apex</td>
<td>Large and dense calyces</td>
<td>Large and dense calyces</td>
<td>Large and dense calyces</td>
<td>Large and scarce calyces</td>
<td>Large and dense calyces</td>
<td></td>
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</table>

Comparisons. *Bothrocophias tulitoi* sp. nov. can be distinguished from all *Bothrocophias* species by having a creamy white or pale yellow ventral ground color with ventral scales heavily marked with black pigment towards the edges contacting the paraventral scales, and the presence of spots without a regular pattern on the mesial surface turning heavily mottled with dark brown pigment toward the tail (versus homogeneously dark brown to black in *B. campbelli*; mottled heavily with dark brown pigment, with the pale interspaces between the ventrolateral blotches encroaching on the lateral edges of ventral scales in *B. colombianus*; greyish brown medially, becoming paler laterally, with or without alternating dark brown spots in *B. hyopora*; yellow mottled with pale to dark brown, darker posteriorly in *B. lojanus*; and pale pink to almost white in *B. myersi*); ventral surface of the tail with bright reddish or orange-reddish speckles with black spots without a regular pattern, and heavily marked with dark pigment towards the base (versus proximally dark brown and distally yellow or yellow-green in *B. andianus*; bright yellow to tan with diffuse grayish or brown pigment in *B. campbelli*; cream or pale yellow with a sparse peppering of brown in *B. colombianus*; and whitish with a moderate suffusion of grey in *B. myersi*).

Additionally, *Bothrocophias tulitoi* sp. nov. can be distinguished from Ecuadorian, Peruvian, and Bolivian populations of toadheaded pitvipers currently classified as *B. microphthalmus* by having ventral surface of tail with bright reddish or orange-reddish speckles with black spots without a regular pattern and heavily marked towards the base (versus heavily marked with black or dark brown pigment proximally, mottled medially with pale...
Table 5. Meristic variation in *Bothrocophias tulitoi* sp. nov. and *B. myrringae* sp. nov. Bold numbers represent mode or mean. Numbers within parenthesis represent minimum and maximum values observed. * = meristic characters undifferenced by sex.

<table>
<thead>
<tr>
<th>Trait</th>
<th><em>B. tulitoi</em> sp. nov.</th>
<th><em>B. myrringae</em> sp. nov.</th>
<th><em>B. cf. microphthalmus</em> – Bolivia</th>
<th><em>B. cf. microphthalmus</em> – Ecuador</th>
<th><em>B. microphthalmus</em> – Peru</th>
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<tr>
<td>Sample size</td>
<td>Male (n=10), Female (n=17)</td>
<td>Male (n=3), Female (n=5)</td>
<td>Harvey et al. (2005)</td>
<td>Male (n=10), Female (n=12)</td>
<td>Male (n=2), Female (n=1)</td>
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<td>Upturned snout</td>
<td>Male Absent</td>
<td>Female Absent</td>
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<tr>
<td></td>
<td>Female Absent</td>
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<td>Moderately</td>
<td>Moderately</td>
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<tr>
<td>Snout</td>
<td>Male Prognathous</td>
<td>Female Prognathous</td>
<td>Prognathous</td>
<td>Prognathous</td>
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<td></td>
<td>16–18 *</td>
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<tr>
<td>Dorsal bands</td>
<td>Male 24 (20–29)</td>
<td>Female 26 (19–29)</td>
<td>21 (19–23)</td>
<td>18</td>
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<td>30 (28–34)</td>
<td>30 (25–34)</td>
<td>16 (15–17)</td>
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<td>Preventral scales</td>
<td>Male 4 (3–6)</td>
<td>Female 5 (3–5)</td>
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<td>4 (3–6)</td>
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<td>5 (3–5)</td>
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<td>Ventral scales</td>
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<td>Female 162.2 (150–172)</td>
<td>142–152</td>
<td>148 (139–155)</td>
<td>155–159</td>
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<td></td>
<td>153.7 (152–155)</td>
<td>159.3 (157–161)</td>
<td>143–147</td>
<td>147.3 (138–151)</td>
<td>51–53</td>
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<tr>
<td>Subcaudal scale</td>
<td>Male 51.5 (46–58)</td>
<td>Female 50.2 (45–55)</td>
<td>50</td>
<td>49.7 (46–53)</td>
<td>52–55</td>
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<td>52.7 (52–54)</td>
<td>48 (41–52)</td>
<td>42–45</td>
<td>55.3 (50–60)</td>
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<tr>
<td>Anal scale</td>
<td>Male Single</td>
<td>Female Single</td>
<td>Single</td>
<td>Single</td>
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<td>Male 1</td>
<td>Female 1</td>
<td>1</td>
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<td></td>
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<td>Female Absent</td>
<td>Present</td>
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<td>Present</td>
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<td></td>
<td></td>
<td></td>
<td>Present</td>
<td>Present</td>
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<tr>
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<td>Male 0 (0–1)</td>
<td>Female 0 (0–1)</td>
<td>1</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
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<tr>
<td>Prefoveal</td>
<td>Male 4–8 *</td>
<td>Female 2 *</td>
<td>2</td>
<td>2</td>
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<td>2 (2–4)</td>
<td>4 (5–4)</td>
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<tr>
<td>Subfoveal</td>
<td>Male 1–2 *</td>
<td>Female 2 *</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 (2–4)</td>
<td>4 (5–4)</td>
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<tr>
<td>Postfoveal</td>
<td>Male 1 (0–1)</td>
<td>Female 0 (0–1)</td>
<td>1 (0–2)</td>
<td>1 (0–2)</td>
<td></td>
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<tr>
<td></td>
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<td>1 (0–2)</td>
<td>1 (0–2)</td>
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</tr>
<tr>
<td>Prelacunal</td>
<td>Male 1</td>
<td>Female 1</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
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<td></td>
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<td></td>
<td>1</td>
<td>1</td>
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<tr>
<td>Lacunolabial</td>
<td>Male Absent</td>
<td>Female Absent</td>
<td>Present</td>
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<td>Absent</td>
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<td></td>
<td></td>
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<td>Absent</td>
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<tr>
<td>Supralacunal</td>
<td>Male Usually absent</td>
<td>Female Absent</td>
<td>Absent</td>
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<td></td>
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<td>Present</td>
<td>Absent</td>
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</table>
Two new species of the enigmatic genus of toadheaded pitvipers of the genus *Bothrocophias* were described, *B. tulitoi* sp. nov. and *B. myrringa* sp. nov., from endemic populations in the Bolivian Tarija Department and a previously undescribed population in the Peruvian Amazon. The new species were distinguished by unique morphological characteristics, such as distinct color patterns and hemipenial ornamentation, and were found to be distinct from all previously known species of *Bothrocophias*. The description includes a detailed comparison of meristic and hemipenial characters, with particular emphasis on the morphological features that differentiate the new species from their congeners. The data presented in Table 5 summarize these comparisons, highlighting the unique diagnostic features of each species. The presence of these new species expands the understanding of the diversity within the *Bothrocophias* genus and contributes to the knowledge of the biogeography and systematics of enigmatic pitvipers in the region. The species are named in honor of the scientists and their contributions to the study of these enigmatic taxa.
slopes of Brazil (Rondônia state) were not available for this study.

**Description of holotype.** (Figs 3–5; Table 3) Male, small body size (SVL = 517 mm, TL = 96, ratio 18.5%), head and body strongly differentiated by nuchal constriction; head longer than wide (HW/HL 61.7%); snout prognathous and not upturned; absence of nasorostral and canthorostral scales; two internasal scales separated by a single small cone-shaped scale; rostral visible from dorsal view and in contact with internasals, as well as with the small cone-shaped scale; two canthal scales in broad contact with internasal scales and separating the first preocular and loreal scales in lateral view; four intercanthal scales; two supraocular scales wider than long (SW/SL = 60.9%); seven intersupraocular scales; 26 intertercal scales; two nasal scales: anterior nasal scale in broad contact with rostral, internasal, and first supralabial scale, and posterior nasal scale in contact with loreal, the first two prefoveal and subnasal scales; a single subnasal scale; two to three prefoveal scales on right and left lateral side of the head; a single prelacunal scale in broad contact with second supralabial and supralacunal scales and in narrow contact with prefoveal, sublacunal, and loreal scales; a single supralacunal scale in broad contact with first preocular scale and in narrow contact with loreal, canthal, and second preocular scales; a single sublacunal in broad contact with third supralabial and preocular scales and in narrow contact with prelacunal, supralacunal, and second preocular scales; absence of a lacunolabial scale; a single subocular scale, five intercoulolabial scales; two postocular scales; seven supralabial scales, third supralabial slightly higher and wider than fourth to seventh supralabial scales, and notably higher and wider than first and second supralabial scales; eight infralabial scales, first infralabial scales separated by mental scale and in broad contact with the first pair of gular scales, second and third infralabial scales in contact with first pair of gular scales; two pairs of gular scales; four prefrontal scales; dorsal scale rows 23–23–19; 153 ventral scales; anal scale single; 52 subcaudal scales; slender tail not prehensile.

**Color of the holotype in life.** Ground color of the dorsal surface of the head is pale brown to brown with diffuse marks dark-brown or grey without a distinctive pattern. Ground color of the lateral surfaces of the head is scattered grey-brown from the snout to anterior edge of the eye; a conspicuous dark-brown postocular stripe running obliquely from the posterior edge of the eye to the angle formed by the quadrate and jaw bone joint encompasses the temporal scales, the last two supralabial scales, the last infralabial scale, and the mesial scales located between the prefrontal and infralabial scales; conspicuous tricolored ocelli in the third to fifth supralabial scales and third to seventh infralabial scales with center white or white-cream, followed by an internal edge dark-brown or black, and external broad circle or edge yellow or yellow-reddish. Ventral surfaces of the head are dark orange-gold and peppered with brown with conspicuous

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**Figure 3.** Holotype *Bothrocophias tulitoi* sp. nov. in preservative (INSZ 073. Male). A Dorsal view of the body. B Ventral view of the body. C Dorsal view of the head. D–E Lateral views (right/left) of the head. F Ventral view of the head. Black bar length = 1 cm.
tricolored ocelli as described above in the first, third to seventh infralabial scales and first pair of gular scales. Ground color of the dorsal body surfaces is yellow-tan to brown mottling with dark brown-reddish pigment and weak orange speckles; 27 dark-brown bands and/or opposite or juxtaposed trapezoid-shaped blotches with pale center ornamented with or without brown spots. Ground color of body ventral surfaces is creamy white or yellow with ventral scales heavily marked with black pigment towards the edges contacting paraventral scales and spotted without a regular pattern on the mesial surface turning heavily mottled with dark brown pigment toward the tail; edges of the spots of the mesial surfaces yellow reddish turning dark brown toward the tail. Dorsal surfaces of the tail covered by nine broad dark brown bands separated by four narrow pale bands that fuse toward the distal end of the tail; tail ventral surface with bright reddish or orange-reddish speckles with black spots without a regular pattern, and heavily marked with dark pigment towards the base.

**Color of the holotype in ethanol.** (Fig. 3) After five years in ethanol, the ground color of the dorsal surfaces of the head and body changed from pale brown or grey-brown to greyish blue. Dark brown and chocolate coloration was maintained, and surfaces with brown-reddish, yellow-reddish, or yellow coloration turned pale grey or creamish white.

**Color pattern variation.** (Fig. 4) Adults of *Bothrocophias tulitoi* sp. nov. exhibit sexual dimorphism. The dorsal, lateral, and ventral surfaces of the head and body of males are melanized with conspicuously tricolored ocelli in the third to fifth supralabial scales, third to seventh infralabial scales, and first pair of gular scales, and the lateral and ventral surfaces of the head in females exhibit a homogenous creamish yellow coloration without ocelli in the labial or gular scales; some specimens exhibit weakly visible ocelli (e.g., INSZ148). Neonates and juveniles of both sexes can possess ocelli in some labials or gular scales, but only males retain them until adulthood. In addition, in neonates and juveniles, the dorsal body ground color is pale yellow, and the dorsal bands are conspicuous throughout the body (Fig. 4A); in adults of both sexes, the dorsal ground color is yellow or tan to brown; the bands are inconspicuous in the first third of the body and become more conspicuous towards the medial and posterior body sections.

**Meristic variation.** (Table 4) Female and male adults of *Bothrocophias tulitoi* sp. nov. exhibit sexual dimorphism in ventral scale counts (Table 4). The specimen IAVH 6396, exhibits an unusually low ventral scale count (150), which is an outlier among females in our sample. In four specimens (INSZ 134, 144–46), the supralacunal scale is fused with the third preocular scale and enters the eye orbit. On INSZ 073, the canthal scale separates the
first preocular and loreal scale; in two specimens (MLS 1634, IAvH-R7795), infralabial counts are 10–11 scales; and in INSZ 130, the asymmetrical presence of a single and small canthorostral scale on one side of the head was observed. High counts of interoculolabial scales (range 9–12) were recorded in three neonates born in captivity (INSZ 128, 130, 136).

**Hemipenial morphology.** ($n=7$, Fig. 5A–C) Hemipenes in situ extend to the level of the 8th or 12th subcaudal scale, with the bilobation point ranging between the 3rd or 6th subcaudal scale. The everted organ is deeply bilobed; lobes are conical, bicalyculate and non-capitate; lobe crotch nude; sulcus spermaticus centrolinal and bifurcate, the branches run to the lobe tips, with the bifurcation always below the bilobation point and proximal to the midpoint of the hemipenial body; intrasulcal area densely covered with spines that increase in size distally; towards the distal half each lobe is densely ornamented with calyces; sulcus spermaticus walls robust and well defined. In sulcate view, hemipenial body covered with small spines proximally; ornamented medially by numerous dense, large, and strongly calcified mesial spines arranged in oblique rows, with lateral and mesial spines of the hemipen-
enial body homogenous in size; and distally, the spines in each lobe replaced by dense small calyces arranged in a low-cut front centered in the sulcus spermaticus with two curved edges extending on the side, calyces are spinulated proximally but not distally. In lateral view, hemipenial body nude proximally; lobes ornamented medially by dense, large, and strongly calcified lateral spines equal in size and replaced distally by dense calyces. In asulcate view, hemipenial body nude proximally; hemipenial body ornamented medially by numerous dense, large, and strongly calcified mesial spines arranged in oblique rows which increase in size distally; and in each lobe, the spines replaced by dense calyces through an oblique cut.

**Etymology.** We dedicate this species to the late Colombian educator Tulio Manuel Angarita Serrano (1941–2021, father of the first author), known as Tulito (employing the diminutive Spanish suffix “ito”) by his colleagues, friends, and relatives. The specific epithet *tulitoi* represents the Latin translation of the nickname from the Spanish name Tulito. Professor Angarita-Serrano was a pioneer of the modern Colombian education model that helped catalyze the development of the theoretical and practical tools needed to implement institutional educational projects in Colombian public and private schools (see Angarita-Serrano 1990; Angarita-Serrano 1994; Angarita-Serrano and Chaves 1995; Angarita-Serrano 1996; Angarita-Serrano, 2000). He was also known for being a big thinker, a passionate advocate for the rights to education and free thought, and the development of educational paradigms that have helped Colombians overcome the new social, socioeconomic, and environmental challenges of the third millennium.

**Distribution and natural history.** (Fig. 8) The known localities of *Bothrocophias tulitoi* sp. nov. are distributed between 1,650 and 2,700 m a.s.l. in both the central mountains and eastern slopes of the Cordillera Oriental de Colombia in the municipalities Garagoa, Gauteque, and Miraflorres (Boyacá); Chameza, La Salina, and Yopal (Casanare); and Medina and Ubalá (Cundinamarca). *Bothrocophias tulitoi* sp. nov. appears to be associated with cloud montane, high Andean Forest, and subparamos and is tolerant of disturbed or transformed habitats such as livestock pastures and agricultural fields. Little is known of the natural history of *B. tulitoi* sp. nov. An adult female from the municipality of Garagoa, Boyacá (INSZ 144), gave birth to 15 offspring (two males and 13 females, eight of which are part of the paratype series: INSZ 128, 130, 134–36, 143, 146, 148) after 11 days in captivity at the INS serpentarium.

**Envenomation.** A total of 40 snakebite events over the last decade might have been caused by *B. tulitoi* sp. nov. Both mild and moderate envenomation have been noted in 50% of patients, and no severe cases nor fatalities were reported. Local symptoms reported included oedema (92.5%), pain (87.5%), erythema (47.5%), ecchymosis (20%), paresthesia (17.5%), phlyctens (15%), paresthesia (17.5%), and bruises (7.5%); systemic symptoms included ed sickness (45%), vomiting (15%), vertigo (12.5%), bradycardia (7.5%), gingivorrhea (7.5%), muscular weakness (5%), hematuria (5%), hypotension (5%), abdominal pain (5%), and altered vision (5%).

**Bothrocophias myrringae sp. nov.**

http://zoobank.org/E2AF3B54-1954-43CA-A56E-B525B1C3-169D

Figs 1, 6G–I, 7, 8A–C

**Chresonym.** *Bothrocophias microphthalmus*. Campbell and Lamar (1989): page 255, Figure 229; Campbell and Lamar (2004): Volume 1, Plate 474.

**Holotype.** [Figs 1, 6G–I, 7, 8A–C] INSZ 0268, an adult male from vereda Mundo Nuevo, municipality of La Calera, department of Cundinamarca, Colombia, coordinates: N 4.660602778, W –73.88491667; elevation 2,761 m. a.s.l. The specimen was collected by local people and brought to Francisco Javier Ruiz, staff of the INS, on 29th August 2020.


**Diagnosis.** *Bothrocophias myrringae* sp. nov. can be distinguished from all its congeners by the following combination of characters: (1) 157–161 ventral scales in females, 152–155 ventral scales in males; (2) internasal scales in contact or separated by a single small scale (3) absence of canthorostral scales; (4) lacunolabial scale usually present; (5) hemipenial lobes slim and cylindrical, moderately capitatus distally, weakly ornamented toward the apex with large and scarce calyces with weakly spinulate edges; (8) bifurcation point of the hemipenial lobes about 2–4 sucadual scales; (9) hemipenial body ornamented by numerous mesial spines that increase in size from the center to periphery of the hemipenial body and arranged in oblique lines; (10) in sulcate view, lateral and mesial spines of the hemipenial body variable in size; (11) *sulcus spermaticus* walls weakly defined; (12) usually more than 28 dark-brown bands and/or opposite or juxtaposed trapezoid-shaped blotches with paler centers dorsally; and (13) ventral surfaces of the tail with bright reddish or orange-reddish specles with black spots without a regular pattern and heavily marked with dark pigment towards the base (Fig. 7).
Comparisons. *Bothrocophias myrringae* sp. nov. can be distinguished from all its congeners by its creamy yellow ventral surfaces and ventral scales mottled with dark brown pigment, becoming creamy white toward the edges in contact with the paraventral scales, forming a white-cream paraventral stripe which proximally fuses with the final edges of the postocular stripe, and distally is interrupted by dark spots without a regular pattern; mesial ventral surfaces become heavily mottled with dark brown pigment toward the tail (versus homogeneously dark brown to black in *B. campbelli*; heavily mottled with dark brown pigment, with the pale interspaces between the ventrolateral blotches encroaching on the lateral edges of the ventral scales in *B. colombianus*; greyish brown medially, becoming paler laterally, with or without alternating dark brown spots in *B. hyopora*; yellow mottled with pale to dark brown, darker posteriorly in *B. lojanus*; yellow mottled with pale to dark brown, darker posteriorly in *B. lojanus*; pale pink to almost white in *B. myersi*); ventral tail surface bright reddish or orange-reddish speckles with black spots in an irregular pattern, and the base of tail heavily marked with dark brown pigment (versus base of tail dark brown and distally yellow or yellow-green in *B. andianus*; bright yellow to tan with diffuse grayish or brown pigment in *B. campbelli*; cream or pale yellow with a sparse peppering of brown in *B. colombianus*; whitish with a moderate suffusion of grey in *B. myersi*. Comparisons of meristic and hemipenial characters with its congeners of toadhead pitvipers are summarized in Table 4.

*Bothrocophias myrringae* sp. nov. can be distinguished from Ecuadorian, Peruvian, and Bolivian populations of toadheaded pitvipers currently classified as *B. microphthalmus* by ventral surface of tail with uniformly bright reddish or orange-reddish speckles with black spots without a regular pattern (versus heavily marked proximally with black or dark brown pigment, medially mottled with pale to dark brown, and distally with pale diffuse or pale motting with interspaces in Bolivian and Peruvian populations). Comparisons of meristic and hemipenial characters with the Ecuadorian, Peruvian, and Bolivian populations of toadheaded pitvipers the complex are summarized in Table 5.

Description of holotype. (Figs 5–7, Table 3) Male, small (SVL = 542 mm, TL = 95 mm, ratio 17.5%), head and body strongly differentiated by nuchal constriction; head longer than wide (HW/HL 53.3%); snout prognathous and not upturned; absence of nasorostral and canthorstral scales; two internasal scales in contact; rostral visible from dorsal view and in narrow contact with internasal scales; two canthal scales in broad contact with internasal scales; two prefoveal scales on both sides of the head; a single lacunolabial scale in broad contact with the first and second supralabial scales as well as with prefoveal...
and loreal scales, and in narrow contact with supralacunal scale; a single supralacunal scale in broad contact with first preocular scale and in narrow contact with second preocular and loreal scales; a single sublacunal in broad contact with second supralabial, and in narrow contact with third preocular, lacunolabial, and single posfoveal scale; a single subocular scale; a single posfoveal scale; five interoculolabial scales; two postocular scales; six supralabial scales, second supralabial slightly higher and wider than fourth to seventh supralabial scales, and notably higher and wider than first supralabial scale; eight infralabial scales, first infralabial scales separated by mental scale and in broad contact with first pair of gular scales, second and third infralabial scales in contact with first pair of gular scales; three pairs of gular scales; four preventral scales; dorsal scale rows 21–23–19; 155 ventral scales; anal scale single; 54 subcaudal scales; slender tail not prehensile.

**Color of the holotype in life.** (Fig. 8A–B) The dorsal surface of the head has a dark to reddish-brown ground color with diffuse dark brown marks without a distinctive pattern. The lateral surface of the head is scattered pale yellow from the snout to anterior edge of the eye; a conspicuous dark-brown postocular stripe with white edges running obliquely from the posterior edge of the eye to the angle formed by the quadrate and jaw bone joint encompasses the temporal scales, the last two supralabial scales, the last infralabial scale, and the mesial scales located between the preventral and infralabial scales; conspicuous tricolored ocelli present on fourth to sixth infralabial scales with white or white-cream centers, followed by dark-brown or black edge, and an external broader edge yellow or reddish-yellow. Ventral surfaces of the head are tan with a peppering of brown and dark yellow without ocelli. Ground color of the dorsal body surfaces is pale yellow, tan to brown mottled with dark brown-reddish pigment and weak orange speckles; 34 dark-brown dorsal bands and/or opposite or juxtaposed trapezoid-shaped blotches with pale centre ornamented with or without brown spots. Ventral scales are creamish yellow with ventral scales heavily marked, with brown-reddish and dark yellow pigment towards the edges in contact with the paraventral scales and spotted without a regular pattern on the mesial surface with a conspicuous white-cream paraventral stripe which proximally fuses with the final edges of the postocular stripe, and distally is interrupted by dark spots without a regular pattern. Dorsal surfaces of the tail covered by two broad dark brown bands separated by three narrow pale bands that fuse toward the distal end of the tail; ventral tail surface bright reddish or orange-reddish, darkening heavily towards the base, speckled with black spots in an irregular.

**Color of the holotype in ethanol.** (Fig. 6) After six months in ethanol, dark brown and brown coloration was maintained, and surfaces with brown-reddish, reddish-yellow, and yellow coloration became pale grey or creamy white.

**Color pattern variation.** (Fig. 8C–D) Adults of *Bothrocophias myrringae* sp. nov. exhibit sexual dimorphism. The dorsal, lateral, and ventral surfaces of the head and body of males are melanized with conspicuous tricolored
ocelli on the third to seventh infralabial scales, and the lateral and ventral surfaces of the head in females exhibit a homogenous creamish yellow to pale yellow coloration without ocelli in the labials and gular scales (Fig. 8C–D). In addition, eye color in males is usually homogeneously mottled with dark brown pigment and weak orange speckles around the pupil; in females, it is orange-gold with a peppering of brown. One female (IAvH-R7045: San Juanito, Meta) has 25 dorsal bands, which was an unusual count among our samples.

**Meristic variation.** (Table 4) Female and male adults of *Bothrocophias myrringae* sp. nov. exhibit sexual dimorphism in ventral scale counts (Table 4). One female (IAvH-R6877: Guayabetal, Cundinamarca) has a single canthorostral scale on the right side of the head. One male (INSVSR-00099: El Calvario, Meta) lacks a lacunolabial scale on both sides of the head, and this is the only specimen showing this condition.

**Hemipenial morphology.** (*n*=2, Fig. 5D–F) Hemipenes *in situ* extend to the level of the 8th or 12th subcaudal scale, bilobation point ranges between the 2nd and 4th subcaudal scale. Everted and inflated, the organ is deeply bilobed, bicalyculate and moderately capititate distally; hemipenial lobes slim and cylindrical; in sulcate view, lobe crotch ornamented with scattered spinules; *sulcus spermaticus* cetrolinea, bifurcate and with walls weakly defined, bifurcation occurs below bilobation point and proximal to the midpoint of the hemipenial body; *sulcus spermaticus* branch runs to lobe tips; intrasulcal area barely covered with spines that increase in size distally; second half of each hemipenal lobe scarcely ornamented by large calyces with weakly defined edges; *sulcus spermaticus* walls weakly defined. In sulcate view, hemipenial body barely covered with small spines proximally; hemipenial body ornamented medially by few large and strongly calcified mesial spines arranged in oblique rows, with lateral and mesial spines of the hemipenial body variable in size; the spines in each lobe are replaced distally by large calyces with weakly spinulate edges. In lateral view, hemipenial body barely covered with small spines proximally; lobes ornamented medially by scattered, large, and strongly calcified lateral spines that increase in size distally; and the large spines replaced distally by large, weakly defined calyces. In asulcate view, the hemipenial body is barely covered with small spines proximally; hemipenial body ornamented medially by scattered, large, and strongly calcified lateral spines arranged in oblique rows which increase in size distally; and the spines in each lobe re-

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**Figure 8.** Color in life of *Bothrocophias myrringae* sp. nov., and sexual dimorphism. **A, B** Lateral and dorsal view of the holotype INSZ 0268. **C** Male exhibiting melanic coloration on dorsal body and head surfaces, as well as conspicuous tricolored ocelli on the infralabial scales (paratype INSV-SR009). **D** Female exhibiting creamish-yellow to tan coloration on the dorsal body and head surfaces, without tricolored ocelli on the infralabial scales. Both C and D specimens are from vereda de Cousavistá, municipality of Fómeque, Cundinamarca, coordinates N 4.495001, W –73.852056. Pictures by Ronald A. Díaz-Flores.
placed by large and poorly developed calyces through an oblique cut.

**Etymology.** The specific epithet *myrringae* is the Latin translation of the Spanish nickname “Mirringa,” which means “pinch” or something very small. The word “Mirringa” was popularized by Rafael Pombo (1833–1912), a Colombian poet and writer who wrote a popular fable titled “Mirringa Mirronga.” Given the popularity of the fable, as well as the homophonic similarity of “Mirringa” and the name “Myriam,” the nickname “Myrringa” began to be used as a term of endearment. The name of the new species is in honor of the educator Myriam Sierra Guerrero (mother of the first author). She was the philosophical and conceptual advisor of professor Tulio Manuel Angarita Serrano and contributed to the development of the modern Colombian education model that all schools within Colombia currently employ. Professor Sierra Guerrero also helped develop the theoretical framework for the implementation of institutional educational projects in Colombian public and private schools (see Angarita-Serrano 1990; Angarita-Serrano 1994; Angarita-Serrano and Chaves 1995; Angarita-Serrano 1996; Angarita-Serrano 2000).

**Distribution and natural history.** The known localities of *Bothrocophias myrringae* sp. nov. are from 1754 to 2761 m a.s.l. in both the central mountains and eastern slopes of the Cordillera Oriental of Colombia in the municipalities of La Calera, Choachi, Fómeque, and Guayabetal (Cundinamarca), and El Calvario and San Juanito (Meta, Fig. 9). *Bothrocophias myrringae* sp. nov. appears to be associated with cloud montane, high Andean Forest, and subparamos but is also tolerant of disturbed or transformed habitats such as livestock pastures and agricultural fields. The new species was found in sympatry with *Bothrops atrox* in localities from Fómeque. No natural history data are available.

**Envenomation.** A total of three snakebite events in the last decade might have potentially been caused by *B. myrringae* sp. nov., all of which were from the municipality of El Calvario (Meta). Each case was categorized as mild, moderate, and severe, respectively, and one fatality was reported. Local symptoms reported included oedema (100%), pain (100%), erythema (66%), and phlyctens (33%); systemic symptoms included respiratory failure (33%) and muscular weakness (33%). Symptoms such as paresthesia ecchymosis, bruising, sickness, vomit, vertigo, gingivorrhagia, hematuria, and altered vision were not reported.
Discussion

Our phylogenetic trees returned Bothrocophias microphthalmus as non-monophyletic and support the hypothesis that it is a species complex as suggested by Schätti and Kramer (1993) and Campbell and Lamar (2004). Herein, we were able to recognize at least five lineages that were formerly recognized under the name B. microphthalmus, supported by morphological evidence (meristic characters, hemipenial morphology and color pattern), two of which we name B. tulitoi sp. nov. and B. myrringae sp. nov. (Tables 3–5). The recognized lineages match the country where they are known to occur, except for the two Colombian ones (Fig. 1). Additional studies of the other three lineages comprising populations of toad-headed pitvipers from Bolivia and Ecuador are needed to determine whether these lineages warrant specific recognition (Fig. 1, Table 2).

Bothrocophias hyoprora and B. microphthalmus (sensu lato) are consistently recovered as monophyletic, and the genus Bothrocophias is consistently inferred to be monophyletic according to several molecular and morphological phylogenetic analyses (Fenwick et al. 2009; Carrasco et al. 2012; Fenker et al. 2014; Alencar et al. 2016; Hamdan et al. 2019). Our extended sampling of different populations of B. microphthalmus indicate that B. hyoprora was grouped within the B. microphthalmus species group, and it was a sister group to the Colombian clade comprising B. tulitoi sp. nov. and B. myrringae sp. nov. (Fig. 1). Genetic distances between Colombian samples and those from Bolivia, Ecuador, and Peru were higher than those between B. hyoprora and B. tulitoi sp. nov. or B. myrringae sp. nov. (Colombian clade), which is consistent with the differences observed in morphological characters and the phylogenetic topology of the species complex.

The low genetic distances found between B. tulitoi sp. nov. and B. myrringae sp. nov., could be construed as evidence against the recognition of these lineages as distinctive taxa. However, low genetic divergence is to be expected among recently diverged lineages, which is common among Andean lineages (Gutiérrez-Pinto et al. 2012; Murphy et al. 2017), and with small mtDNA datasets. The latter has been observed in other vertebrates that exhibit low genetic distance, high morphological distinctiveness, and lineage separation according to phylogenetic analyses (Markolf et al. 2011). Most of these cases occur in cryptic and elusive species inferred from mtDNA data from a relatively small number of individuals per location. When mtDNA is sampled from a few individuals per population or locality, it is impossible to know whether the data provide a representative characterization of the intraspecific variation within each species. This is the case in our study; due to the cryptic and elusive behavior of Colombian Bothrocophias populations, the number of samples in our mtDNA dataset was low: two tissues from two different populations and distant localities of B. myrringae sp. nov., and three tissues from a single population and locality of B. tulitoi sp. nov., of which two samples...
(INS099 and 100) were obtained from related individuals (mother and daughter, see Table 1). Therefore, a larger mtDNA dataset for both species could provide more robust insights into levels of intraspecific variation in B. myrringae sp. nov., and B. tulitoi sp. nov.

Nevertheless, according to Mayr (1996), Padial et al. (2010), and Markolf et al. (2011), there is no single property or character that can be considered key in species delimitation because every single criterion is likely to fail or yield ambiguous results. Thus, species should be delimited using different datasets (criteria = lines of evidence). Genetic distance is no exception under this perspective; the recovered phylogenetic structure coupled with the morphological distinctiveness in color pattern, meristic characters and hemipenial morphology support the recognition of the two Colombian lineages as distinctive species. Additional studies with a large mtDNA dataset, including multilocus data, are needed to quantify levels of intraspecific variation within B. myrringae sp. nov., and B. tulitoi sp. nov.

Some meristic abnormalities were observed in captive-born neonate specimens of B. tulitoi sp. nov. (INSZ 130), such as canthorostral scales that were present on one side and absent on the other. Three neonate specimens (INSZ 128, 130, 136) possessed high interocularial counts. The fact that such abnormalities were only observed on captive-born specimens, might suggest an effect of environmental changes during development due to the captive conditions (Fox 1948; Osgood 1978; Sasa 2002; Paterna 2015).

Our data support the speculation of Campbell and Lamar (2004) that speciation within Bothrocophias is driven by the elevational segregation associated with Andean orogeny; the uplift of the Cordillera Oriental thus appears to have mediated the divergence between B. tulitoi sp. nov., B. myrringae sp. nov., and B. hyoprora ancestors. Several lines of evidence (e.g., Paleocurrents, palynological assemblages, and mammal fossils) suggest that the Neogene uplift of the Cordillera Oriental and Mérida Andes has driven the Amazon River capture of the former southern Orinoco drainage system, which resulted in its isolation from the inter-Andean Magdalena drainage system and promoted the formation of the trans-Andean and cis-Andean regions in northern South America (Anderson et al. 2016).

The Cordillera Oriental is a younger divergent contractional belt that reactivated a Mesozoic rift system in a combination of thin-skinned ramp-flat thrust systems and thick-skinned basement-involved structures (Prossl and Grosser 1994; Kellogg et al. 2005; Anderson et al. 2016; Gómez et al. 2020). It spans several physiographic subregions with multiple geological origins and different geological time scales (Gómez et al. 2020), which might have facilitated the vicariant speciation process in the “proto-bothrocophias” lineage in various ways across this mountain belt. In light of the current known distribution, there is no geological evidence or geographical barrier that would provide a basis for speculation on the speciation event that led to the origin of B. tulitoi sp. nov., and B. myrringae sp. nov. Both species occur in areas with the same geological features, such as the Quetame massif, the Cocuy anticlinorium, and tertiary and Late Cretaceous sediments (Branquet et al. 2002). Additional studies will be needed to clarify the speciation event underlying the origin of toadheaded pitvipers of the Colombian Andes.

The estimated diversification time of the genus Bothrocophias (11 Mya) coincides with the most intense peaks of Andean Mountain building following the late middle Miocene (~12 Ma) (Hoorn et al. 2010). The effect of this vicariant speciation event was key for the ecological adaptation and dispersal of various taxa over the Andean zones (Antonelli et al. 2009). Hamdan et al. (2019) highlighted the need for additional biogeographical studies of clades widely distributed throughout forested and open landscapes in the Neotropics. These efforts will contribute to our understanding of the diversification processes of younger clades of South American pitvipers such as Bothrocophias and Bothrops. Available records of Bothrocophias microphthalmus (sensu lato) show marked elevational segregation across its entire distribution (Fig. 9), suggesting that ecological information could also be used for species delimitation in this group (Sites Jr et al. 2021).

**Future work**

An integrative taxonomic approach is still needed to delimit the several lineages within Bothrocophias microphthalmus (sensu lato). The distribution of the Ecuadorian populations is broad (from Pastaza to Zamora Chinchipe provinces), spanning various types of habitats and different Andean regions, and they exhibit pronounced morphological variation, as well as unusual arboreal behaviors not reported in any other population of B. cf. microphthalmus (Cisneros-Heredia et al. 2006; Valencia et al. 2016; Arteaga 2020; Torres-Carvajal et al. 2020); hypotheses regarding the taxonomic status of these populations still require testing. For example, Lachesis pleuroxanthus (Boulenger 1912) is currently assignable to the Ecuadorian lineages identified herein. It was described from the province of Pastaza and has been recognized as a junior synonym of B. microphthalmus (Amaral 1930). However, Schätti and Kramer (1993) proposed that B. pleuroxanthus could be resurrected as a species clearly distinguished from Ecuadorian populations of B. microphthalmus and B. hyoprora by characters such as ventral scale counts and body size. Given that the type specimen of B. pleuroxanthus (BMNH 1946.1.19.88) was not available for this study, additional molecular and morphological sampling will be needed to delimit the Ecuadorian populations, and we refrain from making any taxonomic changes to these lineages here.

To determine the actual distribution of B. microphthalmus (sensu lato), we compared the type specimen (ANSP 11515, Fig. 5D–F, Table 4) with the Bolivian, Ecuadorian and other Peruvian specimens. We found that this specimen shared the most morphological features with ones from Peru, such as the prognathous snout, four prelacrinal scales, two sublacunals, three intercants, only one small
scale separating the internasals and tail coloration (Table 5). This indicates that Peruvian populations merit the nomenotypic name. Nevertheless, we refrain from suggesting additional changes until more data are available for comparison, given that only two Peruvian specimens, aside from the holotype, were available. The taxonomic problems resolved herein suggest that cryptic diversity yet to be described might exist among populations of B. microphthalimus (sensu lato) from Ecuador, Peru, and Bolivia (Campbell and Lamar 2004).

Envenomation by B. tulitoi sp. nov., and B. myrringae sp. nov. appear to have effects on humans like other species in the genus and other bothropoid taxa (Warrell 2004; Pereañez et al. 2020). Snakebite accidents caused by B. tulitoi sp. nov., are apparently more common than those caused by B. myrringae sp. nov. However, our data suggest that the venom of B. myrringae sp. nov. might be more lethal than that of B. tulitoi sp. nov. (one death out of three cases vs. no deaths out of 40 cases). Snakebite accidents caused by snakes in the genus Bothrocophias appear to be uncommon (Warrell 2004), which is probably associated with their low abundances in nature (Campbell and Lamar 2004). Snakebite accidents caused by Bothrocophias are likely underreported because these snakes are commonly confused with Bothrops species, even among trained biologists.

Resolution of the taxonomy of the Bothrocophias microphthalimus species complex has significant implications for the public health of Andean countries that continually face the challenges of snakebite accidents. The first step in treating snakebites is the accurate identification of snakes causing envenomation. Following an accurate identification, medical practitioners can administer an appropriate treatment, including antivenom therapy regimen, as well as anticipate possible clinical complications. Accurate identification of the venomous snakes causing snakebite accidents also facilitates the detection, quantification, and characterization of envenoming events by epidemiological surveillance systems, which aids the development of preventive health strategies and epide- miological efforts to reduce the incidence of snakebite accidents. The new taxonomic insights provided by our study thus significantly contribute to achieving the World Health Organization’s goal of reducing the neglected disease of snakebite envenoming (Minghui et al. 2019).

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References


Angarita-Sierra T et al.: Two new species of the enigmatic genus of toadheaded pitvipers of the genus Bothrocophias


Appendix

Specimens examined or compared with literature descriptions:

**Bothrocophias microphthalmus** (sensu lato)


**Bothrocophias tulitoi** sp. nov.


Supplementary material 1

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<td><strong>Authors:</strong> Anzarita-Sierra T, Cubides-Cubillos SD, Hurtado-Gómez JP (2022) <strong>Data type:</strong> docx <strong>Explanation note:</strong> Partitions and evolutionary models for the concatenated alignment obtained using Model Finder (MF) and bModelTest (bMT). <strong>Copyright notice:</strong> This dataset is made available under the Open Database License (<a href="http://opendatacommons.org/licenses/odbl/1.0">http://opendatacommons.org/licenses/odbl/1.0</a>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited. <strong>Link:</strong> <a href="https://doi.org/10.3897/vz.72.e87313.suppl1">https://doi.org/10.3897/vz.72.e87313.suppl1</a></td>
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Supplementary material 2

Table S2

**Authors:** Angarita-Sierra T, Cubides-Cubillos SD, Hurtado-Gómez JP (2022)

**Data type:** .docx

**Explanation note:** Meristic and mensural (in mm) characters of holotype specimens of *Bothrocophias tulitoi* **sp. nov.**, *B. myrringae** sp. nov., and *B. microphthalmus*.

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