



# 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 viperids 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 Chocó 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

and Campbell (2001). Fenwick et al. (2009) validated the monophyly of *Bothrocophias*, including all the species proposed by Gutberlet and Campbell (2001) and Campbell and Lamar (2004), using morphological and molecular evidence. Carrasco et al. (2012) reallocated *Bothrops andianus* (Amaral, 1923) to *Bothrocophias* based on molecular and morphological evidence, and Hamdan et al. (2019) reallocated *Bothrops lojanus* (Parker, 1930) to *Bothrocophias* based on molecular evidence. The genus *Bothrocophias* thus currently comprises seven nominal species distributed from Colombia to Bolivia at elevations between 50 and 2,400 m above sea level [hereafter asl] (Uetz et al. 2022): *Bothrocophias andianus*, *B. campbelli* (Freire-Lascano, 1991), *B. colombianus* (Rendahl & Vestergren, 1940), *B. hyoprora* (Amaral, 1935), *B. lojanus*, *B. microphthalmus*, and *B. myersi* Gutberlet & Campbell, 2001.

*Bothrocophias microphthalmus* has the broadest distribution in the Andes among toadheaded pitviper 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-Chinchipec (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, Huanuco, 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 *Porthidum 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

**Table 1.** Cytochrome *b* (MT-CYB) and NADH subunit 4 (MT-ND4) sequences of *Bothrocophias* species used in the present study.

| Bothrocophias species         | Locality                          | Genbank accession numbers (CYTB-ND4) | Voucher  | Source                  |
|-------------------------------|-----------------------------------|--------------------------------------|----------|-------------------------|
| <i>B. myrringae</i> sp. nov.  | La Calera, Cundinamarca. Colombia | OP082447–OP082452                    | INS268   | This study              |
| <i>B. myrringae</i> sp. nov.  | El Calvario, Meta. Colombia       | OP082446–OP082451                    | INS099   | This study              |
| <i>B. tulitoi</i> sp. nov.    | Garagoa, Boyacá. Colombia         | OP082448–OP082453                    | INS100   | This study              |
| <i>B. tulitoi</i> sp. nov.    | Garagoa, Boyacá. Colombia         | OP082450–OP082455                    | INS148   | This study              |
| <i>B. tulitoi</i> sp. nov.    | Garagoa, Boyacá. Colombia         | OP082449–OP082454                    | INS169   | This study              |
| <i>B. cf. microphthalmus</i>  | Zamora, Ecuador                   | AF292577.1–AF292615.1                | FHGO2566 | Wüster et al. (2002)    |
| <i>B. cf. microphthalmus</i>  | Zamora, Ecuador                   | FR691570.1–FR691538.1                | QCAZ6016 | Melaun et al. (2010)    |
| <i>B. microphthalmus</i>      | Pasco, Peru                       | AY223594–AY223638.1                  | LS9372   | Parkinson et al. (2003) |
| <i>B. cf. microphthalmus</i>  | Bolivia                           | FR691567.1–FR691540.1                | MNKR4983 | Melaun et al. (2010)    |
| <i>B. cf. microphthalmus</i>  | Bolivia                           | FR691568.1–FR691541.1                | MNKR4988 | Melaun et al. (2010)    |
| <i>B. cf. microphthalmus</i>  | Bolivia                           | FR691569.1–FR691542.1                | MNKR4989 | Melaun et al. (2010)    |
| <i>B. cf. microphthalmus</i>  | Bolivia                           | FR691565.1–FR691539.1                | SMF86334 | Melaun et al. (2010)    |
| <i>B. hyoprora</i>            | Leticia, Amazonas. Colombia       | AY223593.1–FN431781.1                | CLP      | Parkinson et al. (2003) |
| <i>B. hyoprora</i>            | Morona, Ecuador                   | AF292576.1–AF292614.1                | FHGO4005 | Wüster et al. (2002)    |
| <i>B. hyoprora</i>            | Orellana, Ecuador                 | FR691571.1–FR691537.1                | QCAZ5577 | Melaun et al. (2010)    |
| <i>B. lojanus</i>             | Loja, Ecuador                     | FR691566.1–FR691536.1                | QCAZ6018 | Melaun et al. (2010)    |
| <i>B. campbelli</i>           | Chimborazo, Ecuador               | AF292584.1–AF292622.1                | INHMT    | Wüster et al. (2002)    |
| <i>Azemiops feae</i>          | China                             | KJ872487                             |          | Parkinson et al. (2002) |
| <i>Agkistrodon piscivorus</i> | EEUU                              | EF669477                             |          | Zhi et al. (2007)       |
| <i>Bothrops pubescens</i>     | Brazil                            | MN37992                              |          | Ferreira et al. (2020)  |

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 Padiál 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 par-

tial 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  $\mu$ L, including one-unit of Taq polymerase (Bioline; Randolph, MA), 1X buffer (Bioline), 1.5 mM MgCl<sub>2</sub> (Bioline), 0.5  $\mu$ M of each primer, 0.2 mM of each dNTP (Bioline), 0.2  $\mu$ g 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; PCR products were purified using the ammonium acetate protocol (Bensch et al. 2000). 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 *Azemiops feae*, *Agkistrodon 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<sup>th</sup> 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 TREEANNOTATOR 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 pro-

cedures 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).

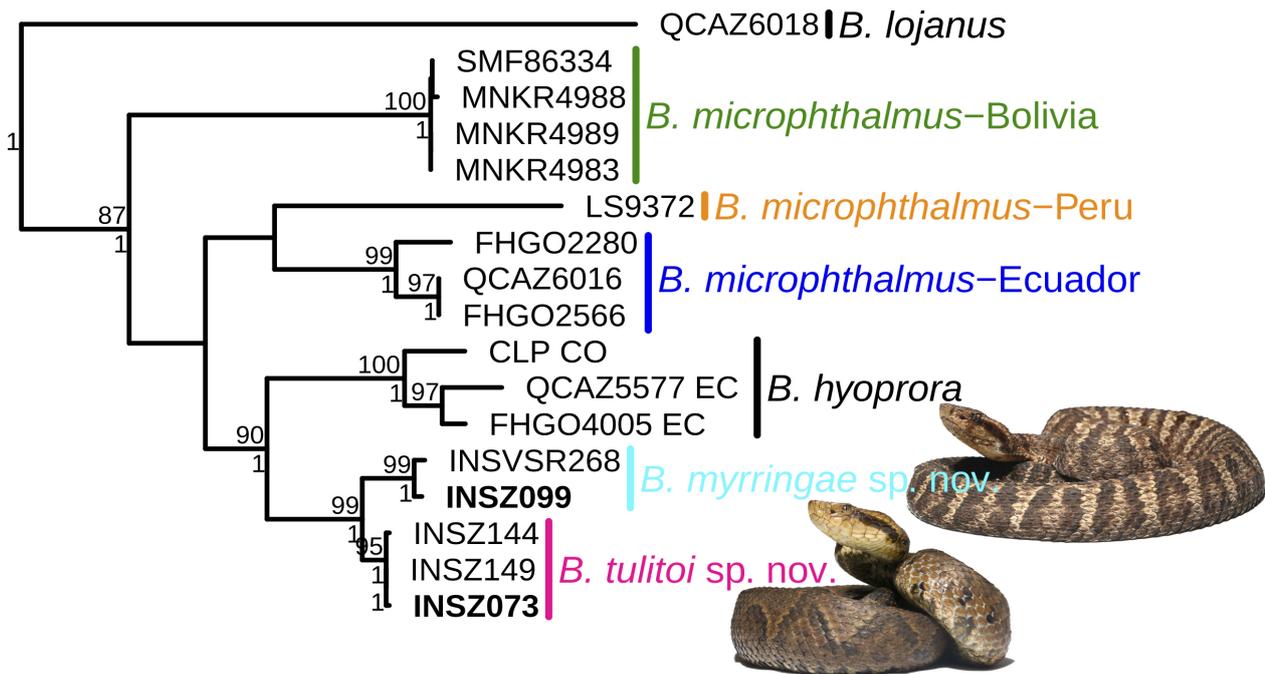
Terminology used in the diagnosis, comparisons, and description sections followed Gutberlet and Campbell (2001), Campbell and Lamar (2004), Harvey et al. (2005), and Valencia et al. (2016). Sex was determined by checking for the presence of hemipenes using caudal incisions. We measured 19 head characters 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 (NLRL), maximum length of the 3<sup>rd</sup> supralabial scale (ML3S), and sinfissial length (SL) (Table S2). Snout-vent length (SVL) and caudal length (TL) were measured using measuring tape ( $\pm 1$  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-González 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-González et al. 2014). The MDS was carried out using the software R wizard version 4.0 (Guisande-González 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



**Figure 1.** Maximum likelihood tree obtained in IQ-tree based on the concatenated alignment of the two mitochondrial fragments (1616 bp), depicting the phylogenetic relationships of the genus *Bothrocophias*. Outgroups were removed for clarity. Values above branches indicate Ultrafast Bootstrap support of the ML tree; values below branches indicate posterior probabilities of the Bayesian tree.

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 SIVIGILA 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.

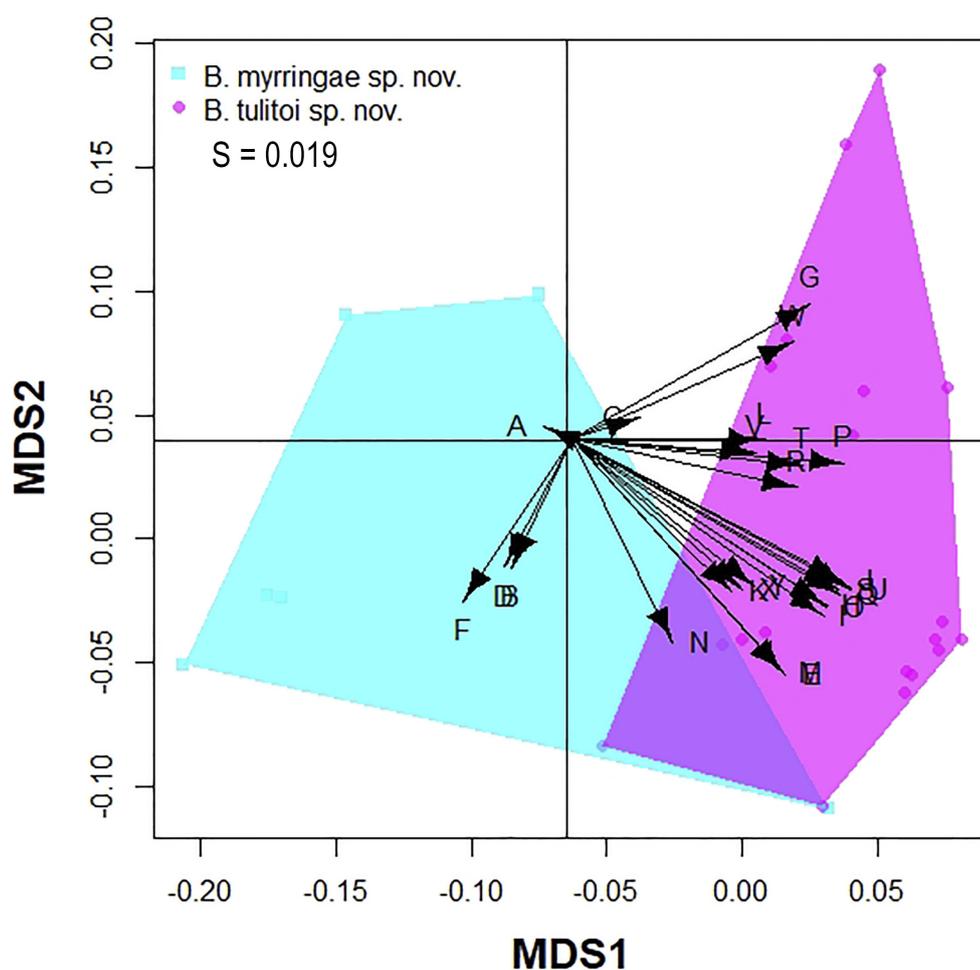
Kimura 2-p genetic distances between the *Bothrocophias* clades ranged from 14.4% to 1.76% (Table 2). The greatest distance was between *B. hyoprora* and *B. lojanus*, whereas the lowest was between the two Colombian clades. Distances between the *B. microphthalmus* clades from Ecuadorian, Peruvian,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

**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.

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



**Figure 2.** Multidimensional scaling test of the merisitic characters between *Bothrocophias tulitoi* sp. nov. (cyan squares) and *Bothrocophias myrringae* sp. nov. (magenta dots). Vectors (black arrows) denote the discrimination capacity of each merisitic variable. **A** Dorsal scale count after head. **B** Dorsal scale count midbody. **C** Dorsal scale count before cloaca. **D** Dorsal bands. **E** Ventral scale counts. **F** Preventral scale counts. **G** Subcaudal scale counts. **H** Subnasal scales. **I** Presence/absence of internasal scales separated by one small scale. **J** Prefoveal scales. **K** Subfoveal scale. **L** Posfoveal scale. **M** Prelacunal scales. **N** Presence or absence of lacunolabial scale. **O** Sublacunal scales. **P** Supralacunal scales. **Q** Interoculabial scales. **R** Intersupraocular scales. **S** Cantal scales. **T** Intercantal scales. **U** Interrictal scales. **V** Sinsfisial scales. **W** Supralabial scales. **X** Infralabial scales. **Y** Preocular scale.

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, support-

ing 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 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).

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

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

**Chresonymy.** *Bothrocophias microphthalmus*. (MLS 1632–34, 1636): Nicéforo-María (1975), Campbell and Lamar (1989): page 255, Figure 230; Campbell and Lamar (2004): Volume 1, Plate 473; (MPUJ 1364); Angarita-Sierra et al (2013).

**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 17<sup>th</sup> October 2017.

**Paratypes.** COLOMBIA ( $n=20$ ; Fig. 4): **Boyacá:** municipality of Garagoa. Locality: unknown, IAvH-R 5742, 6392, 6396, coordinates N 5.08236, W –73.36334 (approximate to the town). Locality: vereda Ciénaga de la Valvanera, INSZ 128, 130, 134–38, 143–48, coordinates N 5.106535941, W –73.25888414. Municipality of Guateque. Locality: unknown, IAvH-R6391, coordinates N 5.006386111, W –73.47142222 (approximate to the town). Municipality of Miraflores. Locality: vereda

**Table 4.** Comparisons of meristic and hemipenial characters between *B. myrringae* sp. nov., *B. tulitoi* sp. nov. and all its congeners of toadheaded pitvipers.

| Trait  | <i>B. andianus</i> | <i>B. campbelli</i>     | <i>B. colombianus</i>   | <i>B. hyoprora</i>      | <i>B. lojanus</i>   | <i>B. myersi</i> | <i>B. myrringae</i> sp. nov. | <i>B. tulitoi</i> sp. nov. |
|--|--------------------|-------------------------|-------------------------|-------------------------|---------------------|------------------|------------------------------|----------------------------|
| Snout  | No upturned        | No upturned             | No upturned             | Upturned                | No upturned         | No upturned      | No upturned                  | No upturned                |
| Intersupraoculars                                      | 3–10               | 3–8 keeled              | 6–10 Tuberculate        | 2–9 smooth              | 3–5 slightly keeled | 3–6 smooth       | 7–9 slightly keeled          | 5–9 slightly keeled        |
| Lacunolabial   | Present            | Absent                  | Absent                  | Often present           | Absent              | Present          | Present                      | Absent                     |
| Dorsal scale surface                                   | Keeled             | Keeled                  | Tuberculate             | Keeled                  | Keeled              | Keeled           | Keeled                       | Keeled                     |
| Ventral scale counts                                   | 154–179            | 152–177                 | 162–173                 | 118–142                 | 144–145             | 139–151          | 152–161                      | 150–168                    |
| Subcaudal scale counts                                 | 49–63              | 48–64                   | 51–54                   | 44–52                   | 37–46               | 44–52            | 41–54                        | 46–58                      |
| Shape of the hemipenial lobes                          |                    | Robust and cylindrical  | Robust and cylindrical  | Conical                 |                     |                  | Slim and cylindrical         | Conical                    |
| Walls of the <i>Sulcus spermaticus</i>                 |                    | Robust and well defined | Robust and well defined | Robust and well defined |                     |                  | Weakly defined               | Robust and well defined    |
| Size of the lateral and mesial spines of the hemipenis |                    | Variable                | Variable                | Variable                |                     |                  | Variable                     | Invariable                 |
| Ornamentation of the hemipenial lobes apex             |                    | Large and dense calyces | Large and dense calyces | Large and dense calyces |                     |                  | Large and scarce calyces     | Large and dense calyces    |

El Tunjito, Finca San Antonio, IAvH-R6387, coordinates N 5.12216, W -73.21212. — **Casanare:** municipality of La Salina. Locality: unknown, MLS 1632-333, 1634, 1636, coordinates N 6.127602778, W -72.33372222 (approximate to the town). Municipality of Chámeza. Locality: vereda Centro-Norte, La Mosquera, MPUJ 1364, coordinates N 5.243917, W -72.89125; locality: vereda Centro Norte, Cerro Pan de Azúcar, IAvH-R7795, coordinates N 5.25, W -72.883333. Municipality of Yopal. Locality: corregimiento El Morro, vereda El Progreso, Highland Forest, IAvH-R8711, coordinates N 5.50775, W -72.428528. — **Cundinamarca:** municipality of Ubalá. Locality: unknown IAvH-R 5956, coordinates: N 4.743647222, W -73.53498889 (approximate to the town).

**Diagnosis.** *Bothrocophias tulitoi* sp. nov. can be distinguished from all its congeners by the following combination of characters: (1) 150–172 ventral scales in females, 153–162 ventral scales in males; (2) internasal scales in contact or separated by a single small scale; (3) absence of canthorostral scales; (4) absence of lacunolabial scale; (5) one prelacunal scale; (6) hemipenial lobes subconical and ornamented toward the apex by large and dense calyces with spinulate edges; (7) bifurcation point of hemipenial lobes about 3–6 subcaudal scales; (8) hemipenial body ornamented by numerous dense, large, and strongly calcified mesial spines arranged in oblique lines; (9) in sulcate view, lateral and mesial spines of the hemipenial body homogeneous in size; (10) body surface with less than 28 dark-brown bands dorsally and/or juxtaposed trapezoid-shaped blotches with paler centers; and (11) ventral surface of the tail uniformly bright reddish or orange-reddish speckles with black spots without a regular pattern (Figs 3, 4).

**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. hyoprora*; 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*). Comparisons of meristic and hemipenial characters with all its congeners of toadheaded pitvipers are summarized in Table 4.

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

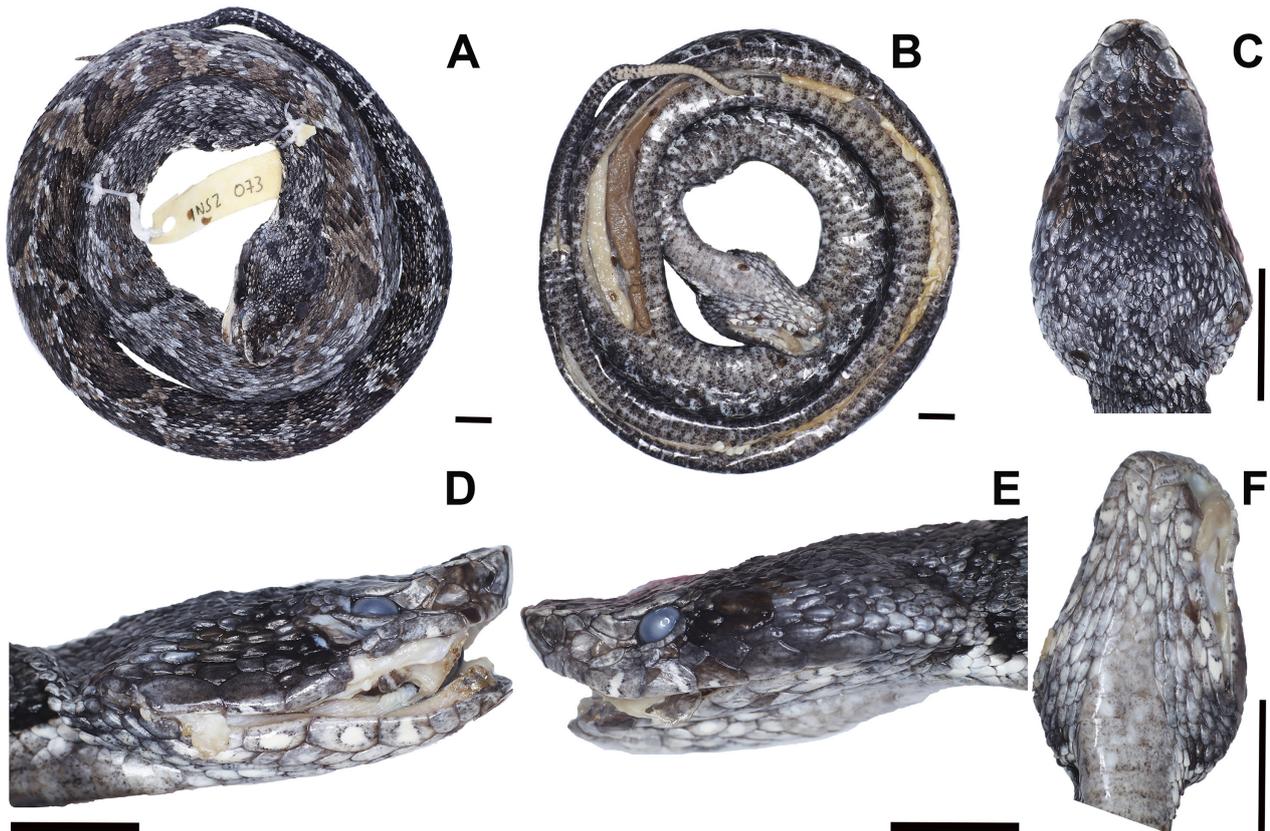
**Table 5.** Meristic variation in *Bothrocophias tulitoi* sp. nov. and *B. myrringae* sp. nov. Bold numbers represent mode or mean. Numbers within parenthesis represent minimum and maximum values observed. \* = meristic characters undifferentiated by sex.

| Trait             | <i>B. tulitoi</i> sp. nov.                                | <i>B. myrringae</i> sp. nov.                              | <i>B. cf. microphthalmus</i> – Bolivia.                   | <i>B. cf. microphthalmus</i> – Ecuador.                   | <i>B. microphthalmus</i> – Peru               |
|-------------------|---|---|---|---|---|
| Sample size       | Male ( <i>n</i> =10),<br>Female ( <i>n</i> =17)           | Male ( <i>n</i> =3),<br>Female ( <i>n</i> =3)             | Harvey et al. (2005)                                      | Male ( <i>n</i> =10),<br>Female ( <i>n</i> =12)           | Male ( <i>n</i> =2),<br>Female ( <i>n</i> =1) |
| Upturned snout    |   |   |   |   |   |
| Male              | Absent  | Absent  | Moderately  | Moderately  | Moderately                                    |
| Female            | Absent  | Absent  | Moderately  | Moderately  | Moderately                                    |
| Snout             |   |   |   |   |   |
| Male              | Prognathous   | Prognathous   | Prognathous   | Prognathous   | Truncate                                      |
| Female            | Prognathous   | Prognathous   | Prognathous   | Prognathous   | Truncate                                      |
| Dorsal scales     |   |   | <b>21</b> (21–23) / <b>23</b> (21–23) / <b>19</b> (17–19) | *   |   |
| Male              | <b>23</b> (22–24) / <b>23</b> (21–23) / <b>19</b> (17–19) | <b>23</b> (21–23) / <b>23</b> / <b>19</b>                 |   | <b>23</b> (21–25) / <b>23</b> (21–25) / <b>18</b> (15–20) |   |
| Female            | <b>23</b> (21–24) / <b>23</b> (22–25) / <b>19</b> (17–19) | <b>23</b> (21–23) / <b>23</b> (21–23) / <b>19</b> (17–19) |   | <b>23</b> (22–24) / <b>23</b> (21–23) / <b>19</b> (17–19) |   |
| Dorsal bands      |   |   | 16–18 *   |   |   |
| Male              | <b>24</b> (20–29)   | <b>30</b> (28–34)   |   | <b>21</b> (19–23)   | 18  |
| Female            | <b>26</b> (19–29)   | <b>30</b> (25–34)   |   | <b>16</b> (15–17)   | 20  |
| Preventral scales |   |   | <b>2</b> (1–3) *  |   |   |
| Male              | <b>4</b> (3–6)  | <b>4</b> (4–5)  |   | <b>2</b> (1–2)  | <b>4</b> (3–6)                                |
| Female            | <b>5</b> (3–5)  | <b>5</b> (3–5)  |   | <b>2</b> (1–2)  | <b>5</b> (3–5)                                |
| Ventral scales    |   |   |   |   |   |
| Male              | <b>157.2</b> (153–162)                                    | <b>153.7</b> (152–155)                                    | 142–152   | <b>148</b> (139–155)                                      | 144–146                                       |
| Female            | <b>162.2</b> (150–172)                                    | <b>159.3</b> (157–161)                                    | 143–147   | <b>147.3</b> (138–151)                                    | 155–159                                       |
| Subcaudal scale   |   |   |   |   |   |
| Male              | <b>51.5</b> (46–58)                                       | <b>52.7</b> (52–54)                                       | 50  | <b>49.7</b> (46–53)                                       | 52–55   |
| Female            | <b>50.2</b> (45–55)                                       | <b>48</b> (41–52)   | 42–45   | <b>55.3</b> (50–60)                                       | 51–53   |
| Anal scale        |   |   |   |   |   |
| Male              | Single  | Single  | Single  | Single  | Single  |
| Female            | Single  | Single  | Single  | Single  | Single  |
| Rostral           |   |   |   |   |   |
| Male              | 1   | 1   | 1   | 1   | 1   |
| Female            | 1   | 1   | 1   | 1   | 1   |
| Cantorostral      |   |   |   |   |   |
| Male              | Absent  | Absent  | Present   | Present   | Present                                       |
| Female            | Absent  | Absent  | Present   | Present   | Present                                       |
| Subnasal          |   |   |   |   | 1   |
| Male              | <b>0</b> (0–1)  | <b>0</b> (0–1)  |   | 1   |   |
| Female            | <b>0</b> (0–1)  | <b>0</b> (0–1)  |   | 1   |   |
| Prefoveal         |   |   | 4–8 *   |   | 2 *   |
| Male              | <b>2</b> (2–4)  | <b>2</b>  |   | <b>2</b> (2–7)  |   |
| Female            | <b>3</b> (2–4)  | <b>2</b> (3–4)  |   | <b>4</b> (5–4)  |   |
| Subfoveal         |   |   | 1–2 *   |   | 2 *   |
| Male              | <b>0</b>  | <b>0</b>  |   | <b>1</b> (0–2)  |   |
| Female            | <b>0</b> (0–1)  | <b>0</b>  |   | <b>1</b> (0–2)  |   |
| Postfoveal        |   |   |   |   | 2   |
| Male              | <b>1</b> (0–1)  | <b>0</b> (0–1)  |   | <b>1</b> (0–2)  |   |
| Female            | <b>1</b> (0–2)  | 1   |   | <b>1</b> (0–2)  |   |
| Prelacunal        |   |   | 1 *   |   | 4 *   |
| Male              | 1   | Absent  |   | <b>1</b>  |   |
| Female            | 1   | Absent  |   | <b>1</b> (1–2)  |   |
| Lacunolabial      |   |   |   |   |   |
| Male              | Absent  | Present   | Absent  | Absent  | Absent  |
| Female            | Usually absent  | Present   | Absent  | Absent  | Absent  |
| Supralacunal      |   |   |   |   |   |

| Trait                                  | <i>B. tulitoi</i> sp. nov.   | <i>B. myrringa</i> sp. nov.   | <i>B. cf. microphthalmus</i> – Bolivia. | <i>B. cf. microphthalmus</i> – Ecuador.  | <i>B. microphthalmus</i> – Peru      |
|--|--|---|---|--|--------------------------------------|
| Male                                   | 1  | 1   | 1                                       | 1  | 1                                    |
| Female                                 | 1 (1–2)  | 1 (1–2)   | 1                                       | 1  | 1                                    |
| Sublacunal                             |  |   |   |  | 2                                    |
| Male                                   | 1  | 1   | 1                                       | 1  |                                      |
| Female                                 | 1 (1–2)  | 1   | 1                                       | 1  |                                      |
| Internasals                            | Usually in contact, rarely separated by one small scale  | Usually in contact, rarely separated by one small scale   | Separated by 2–3 scales                 | Usually separated by 2–3 scales  | Usually separated by one large scale |
| Interoculolabial                       |  |   | 3–4 *                                   |  | 6–9 *                                |
| Male                                   | 5 (5–9)  | 7 (6–7)   |   | 8 (8–10)   |                                      |
| Female                                 | 8 (6–8)  | 7 (6–8)   |   | 8 (8–9)  |                                      |
| Intrasupraoculars                      |  |   | 5–9 *                                   |  |                                      |
| Male                                   | 7 (6–10)   | 8 (7–9)   |   | 8 (8–9)  |                                      |
| Female                                 | 8 (5–12)   | 7 (7–8)   |   | 8 (8–9)  |                                      |
| Intercantals                           |  |   | 5–4 *                                   |  | 3 *                                  |
| Male                                   | 4 (2–4)  | 3   |   | 2  |                                      |
| Female                                 | 4 (3–5)  | 4 (3–4)   |   | 2  |                                      |
| Interrictals                           |  |   | 28 (26–31) *                            | 26 (23–31) *   | 26 *                                 |
| Male                                   | 25 (23–28)   | 27 (27–28)  |   |  |                                      |
| Female                                 | 25 (23–29)   | 25 (25–26)  |   |  |                                      |
| Supralabials                           |  |   | 7–8 *                                   |  | 7 *                                  |
| Male                                   | 7 (7–8)  | 6 (6–7)   |   | 7 (7–8)  |                                      |
| Female                                 | 7 (6–8)  | 6   |   | 7 (7–8)  |                                      |
| Infralabials                           |  |   | 8–10 *                                  |  | 10 *                                 |
| Male                                   | 9 (9–10)   | 8 (9–10)  |   | 8 (8–10)   |                                      |
| Female                                 | 9 (8–11)   | 9 (8–10)  |   | 9 (8–10)   |                                      |
| Preoculars                             |  | 2   |   |  | 2 *                                  |
| Male                                   | 3 (2–3)  | 2 (2–3)   |   | 2 (2–3)  |                                      |
| Female                                 | 3 (2–3)  |   |   | 2 (2–3)  |                                      |
| Postoculars                            |  |   |   |  |                                      |
| Male                                   | 2  | 2   | 2                                       | 2  | 2                                    |
| Female                                 | 2  | 2   | 2                                       | 2 (1–2)  | 2                                    |
| Hemipenial lobes shape                 | Conical  | Cylindrical   |   | Cylindrical  |                                      |
| Hemipenial ornamentation               | Numerous dense, large, and strongly calcified mesial spines; hemipenial lobes distally ornamented by dense and small calyces | Few large and strongly calcified mesial spines arranged in oblique rows, with lateral and mesial spines of the hemipenial body variable in size; hemipenial lobes distally ornamented by large and weakly developed calyces |   | Few large and strongly calcified mesial spines arranged in oblique rows, with lateral and mesial spines of the hemipenial body variable in size; hemipenial lobes distally ornamented by dense and small calyces |                                      |
| Walls of the <i>Sulcus spermaticus</i> | Well defined   | Weakly defined  |   | Well defined   |                                      |
| SVL (mm)                               |  |   | 327–724 *                               |  |                                      |
| Male                                   | 377.4 (157–554)  | 491.7 (388–570)   |   |  |                                      |
| Female                                 | 459.9 (177–820)  | 595 (436–754)   |   |  |                                      |
| TL (mm)                                |  |   | 49–108 *                                |  |                                      |
| Male                                   | 70.7 (32–105)  | 89.0 (68–103)   |   | 780  |                                      |
| Female                                 | 71.4 (27–119)  | 83.7 (67–102)   |   | 908  |                                      |

to dark brown, and pale diffuse mottling or pale with interspaces mottled distally in Bolivian and Peruvian populations). Comparisons of meristic and hemipenial characters with the Ecuadorian, Peruvian, and Bolivian

populations of toadheaded pitvipers currently classified as *B. microphthalmus* are summarized in Table 5. Specimens, taxonomic descriptions, and pictures of snakes classified as *B. microphthalmus* from the Amazonian



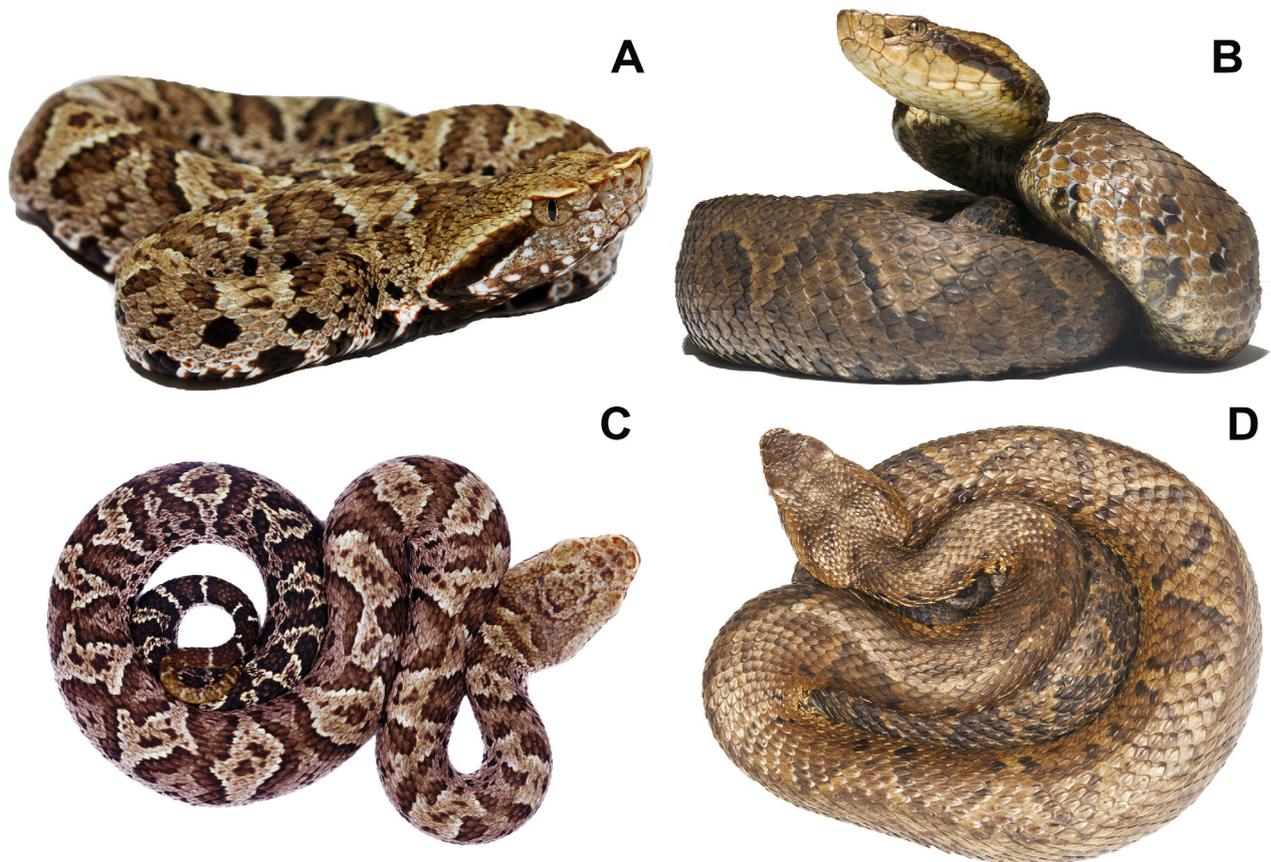
**Figure 3.** Holotype *Bothrocophias tulitoi* sp. nov. in preservative (INSZ 073. Male). **A** Dorsal view of the body. **B** Ventral view of the body. **C** Dorsal view of the head. **D–E** Lateral views (right/left) of the head. **F** Ventral view of the head. Black bar length = 1 cm.

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 interterrestrial 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 lacunola-

bial scale; a single subocular scale, five interoculolabial 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 pre-ventral 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 pre-ventral 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



**Figure 4.** Color in life of *Bothrocophias tulitoi* sp. nov. **A, C** Lateral and dorsal view of a male neonate (paratype INSZ 0128). **B, D** Lateral and dorsal view of an adult female (paratype INSZ 0144). All specimens from vereda Ciénaga La Valvanera, municipality of Garagoa, department of Boyacá, Colombia. Coordinates: N 5.106535941, W -73.25888414.

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



**Figure 5.** Hemipenial architecture. **A, B, C** Sulcate, lateral, and asulcate views of the hemipenis of the holotype of *Bothrocophias tulitoti* sp. nov. (INSZ 073) from vereda Ciénaga La Valvanera, municipality of Garagoa, department of Boyacá, Colombia. Coordinates: N 5.106535941, W -73.25888414; elevation 1894 m. asl. **D, E, F** Sulcate, lateral, and asulcate views of the hemipenis of the holotype of *Bothrocophias myrringae* sp. nov. (INSZ 0268) from vereda Mundo Nuevo, municipality of La Calera, department of Cundinamarca, Colombia. Coordinates: N 4.660602778, W -73.88491667; elevation 2,761 m asl.

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 canthorostrual 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 8<sup>th</sup> or 12<sup>th</sup> subcaudal scale, with the bilobation point ranging between the 3<sup>rd</sup> or 6<sup>th</sup> subcaudal scale. The everted organ is deeply bilobed;

lobes are conical, bicalyculate and non-capitate; lobe crotch nude; *sulcus spermaticus* centrolineal 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; intrasulcar 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 hemip-

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 sulcate 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 of Colombia in the municipalities Garagoa, Gauteque, and Miraflores (Boyacá); Chámeza, 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 includ-

ed sickness (45%), vomiting (15%), vertigo (12.5%), bradycardia (7.5%), gingivorrhoea (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>

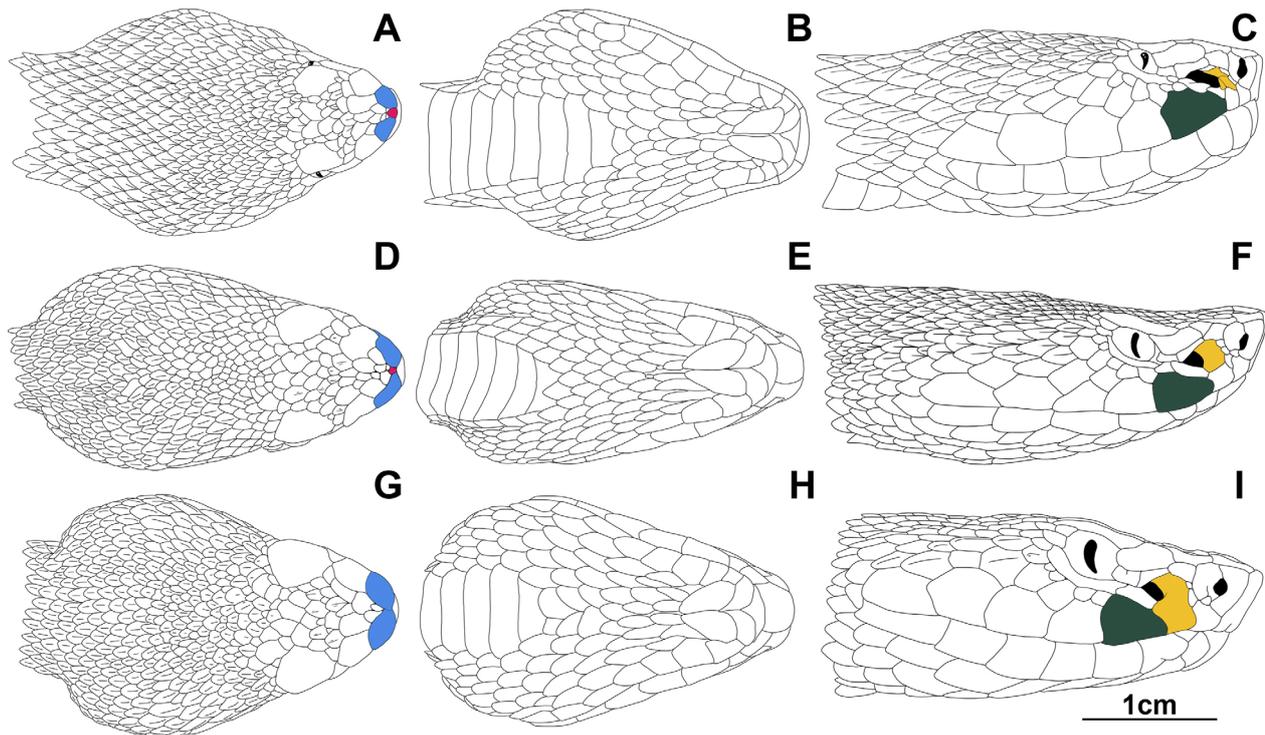
Figs 1, 6G–I, 7, 8A–C

**Chresonymy.** *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 29<sup>th</sup> August 2020.

**Paratypes.** COLOMBIA [ $n=5$ ; Fig. 8C–D]: **Cundinamarca:** Municipality of Guayabetal. Locality: unknown, IAvH-R 6877, coordinates N 4.17508, W -73.88117 (approximate to the town). Municipality of Choachí. Locality: Palo Alto, IAvH-R6840, coordinates N 4.61578, W -73.8904. Municipality of Fómeque. Locality: vereda de Coasavistá, INSV-SR-00365, coordinates N 4.495001, W -73.852056. — **Meta:** Municipality of El Calvario. Locality: unknown, INSVSR-0099, coordinates N 4.358925, W -73.71358889. Municipality of San Juanito. Locality: unknown, IAvH-R7045, coordinates N 4.457913889, W -73.67618889 (approximate to the town).

**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 capitate distally, weakly ornamented toward the apex with large and scarce calyces with weakly spinulate edges; (6) bifurcation point of the hemipenial lobes about 2–4 sudcaudal scales; (7) 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; (8) in sulcate view, lateral and mesial spines of the hemipenial body variable in size; (9) *sulcus spermaticus* walls weakly defined; (10) usually more than 28 dark-brown bands and/or opposite or juxtaposed trapezoid-shaped blotches with paler centers dorsally; and (11) ventral surfaces 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 (Fig. 7).



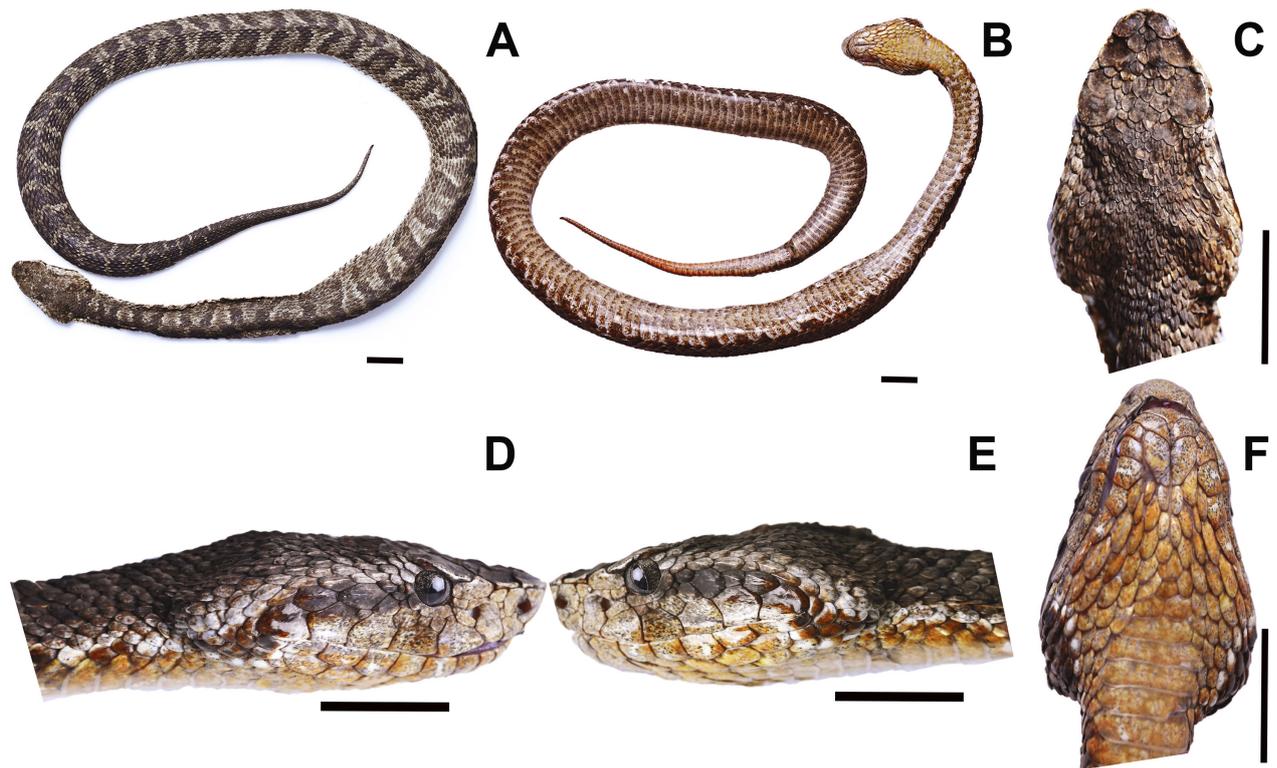
**Figure 6.** Comparison of the head pholidosis among the holotypes of *Bothrocophias microphthalmus*, *Bothrocophias tulitoi* sp. nov. and *Bothrocophias myrringae* sp. nov. **A, B, C** Dorsal, lateral, and ventral view of the head of *B. microphthalmus* ANSP 11515. **D, E, F** Dorsal, lateral, and ventral view of the head of *B. tulitoi* sp. nov. INSZ 073. **G, H, I** Dorsal, lateral, and ventral view of the head of *B. myrringae* sp. nov. Scales: dark green = second/third supralabial scale. yellow = lacunolabial/prelacunal. Blue = internasal scales. Fuchsia = scale separating internasal scales.

**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. hyoprora*; 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 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 all its congeners of toadheaded 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 mottling 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 canthorostral 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 internasals, the first preocular, and loreal scales in lateral view; three intercanthal scales; two supraocular scales wider than long (SW/SL = 66.4%); seven intersupraocular scales; 26 interribral 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, two prefoveal, and subnasal scales; a single subnasal scale; 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



**Figure 7.** Holotype of *Bothrocophias myrringae* sp. nov. in life (INSZ 0268, 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.

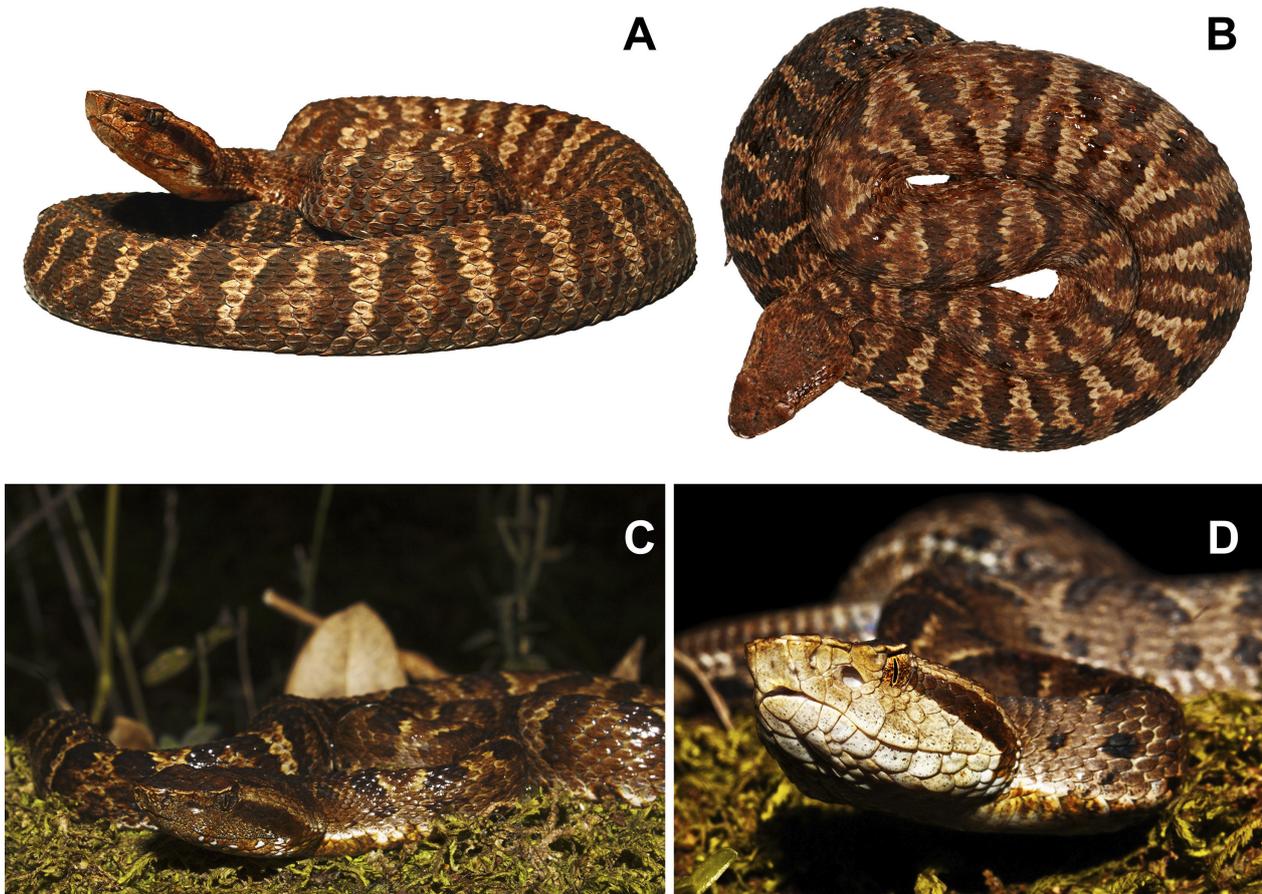
and loreal scales, and in narrow contact with supralacunal scale; a single supralacunal scale in broad contact with first preocular scale and in narrow contact with second preocular and loreal scales; a single sublacunal in broad contact with second supralabial, and in narrow contact with third preocular, 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



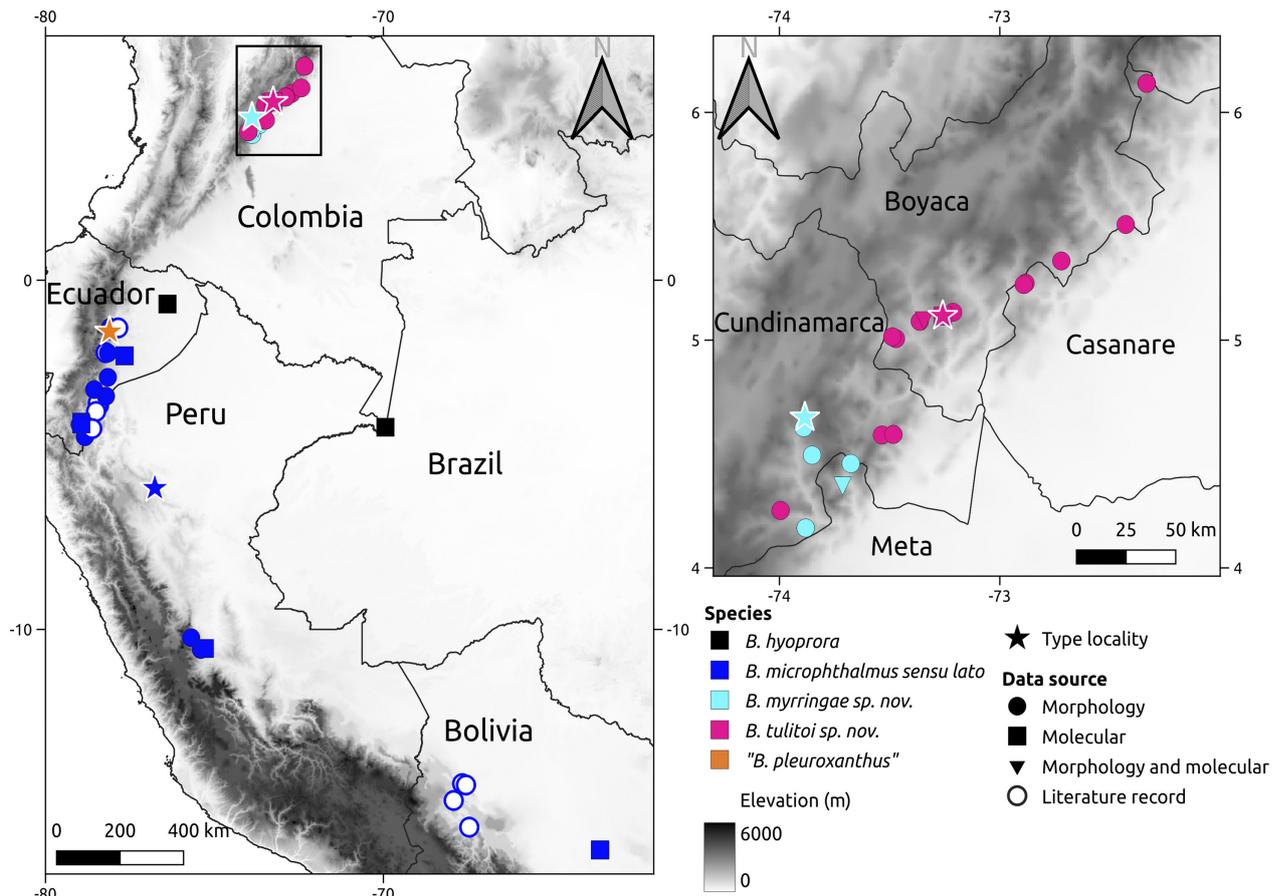
**Figure 8.** Color in life of *Bothrocophias myrringae* sp. nov., and sexual dimorphism. **A, B** Lateral and dorsal view of the holotype INSZ 0268. **C** Male exhibiting melanic coloration on dorsal body and head surfaces, as well as conspicuous tricolored ocelli on the infralabial scales (paratype 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 Coasavistá, municipality of Fómezque, Cundinamarca, coordinates N 4.495001, W -73.852056. Pictures by Ronald A. Díaz-Flores.

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 canthorostrual 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 8<sup>th</sup> or 12<sup>th</sup> subcaudal scale, bilobation point ranges between the 2<sup>nd</sup> and 4<sup>th</sup> subcaudal scale. Everted and inflated, the organ is deeply bilobed, bicalyculate and moderately capitate distally; hemipenial

lobes slim and cylindrical; in sulcate view, lobe crotch ornamented with scattered spinules; *sulcus spermaticus* cetrolineal, 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; intrasulcar area barely covered with spines that increase in size distally; second half of each hemipenial 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-



**Figure 9.** Geographic distribution of *Bothrocophias microphthalmus sensu lato*, *B. tulitoi* sp. nov., and *B. myrringae* sp. nov., including type localities. The background map was obtained from the Esri open database through the following sources: DeLorme. USDS. NPS; USGS. NOAA.

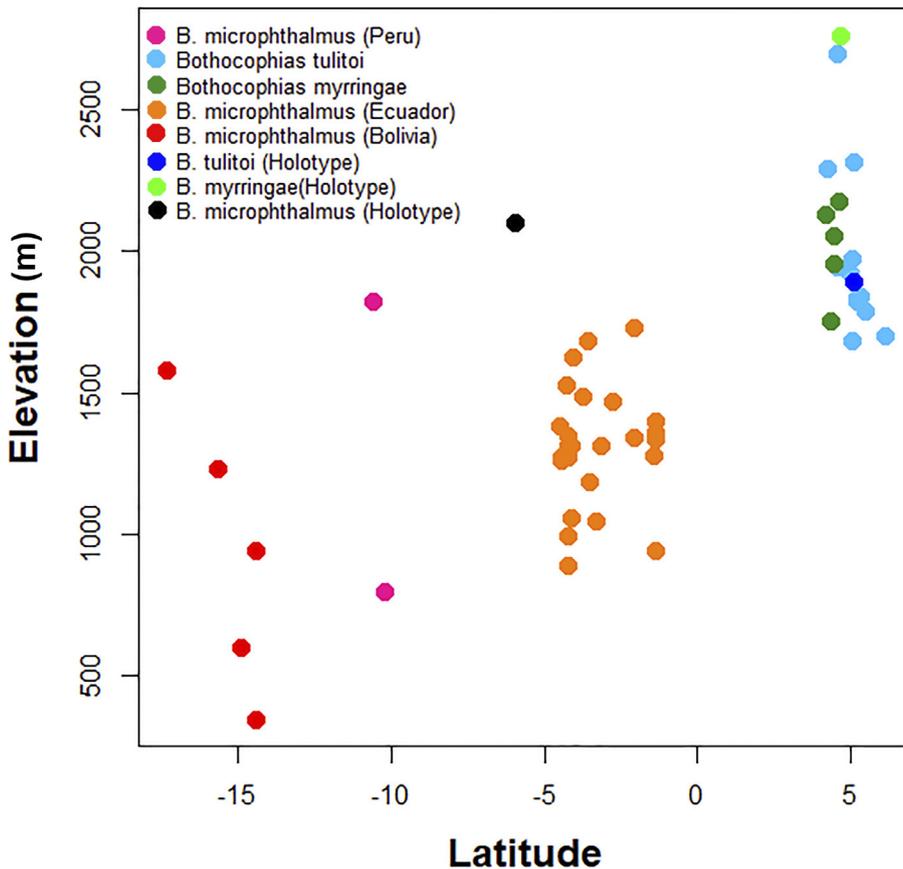
placed by large and poorly developed calyces through an oblique cut.

**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 “Myrringae” 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, Choachí, Fómeque, and Guaya-betal (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.



**Figure 10.** Elevational segregation in the *Bothrocophias microphthalmus* species complex across its entire distribution.

## 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 interocular 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 bivergent 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 prelacunal scales, two sublacunals, three intercantals, only one small

scale separating the internasals and tail coloration (Table 5). This indicates that Peruvian populations merit the nominotypical 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. microphthalmus* (*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 microphthalmus* 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 epidemiological 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

- Alencar LRV, Quental TB, Graziotin FG, Alfaro ML, Martins M, Venzon M, Zaher H (2016) Diversification in vipers: Phylogenetic relationships, time of divergence and shifts in speciation rates. *Molecular Phylogenetics and Evolution* 105: 50–62. <https://doi.org/https://doi.org/10.1016/j.ympev.2016.07.029>
- Amaral A (1923) Estudios sobre Ophidios Neotropicos: XXX Novo genero e especie de Colobrideo na fauna Colombia. *Memorias do Instituto Butantan* 8: 157–159.
- Amaral A (1930) Estudos sobre ophidios neotropicos. XVII. Valor sistematico de varias formas de ophidios neotropicos. *Memórias do Instituto Butantan*: 1–68.
- Amaral A (1935) Estudos sobre ophidios neotropicos XXXIII. Novas especies de ophidios do Colombia. *Memorias do Instituto Butantan*: 219–223.
- Anderson VJ, Horton BK, Saylor JE, Mora A, Tesón E, Breecker DO, Ketcham RA (2016) Andean topographic growth and basement uplift in southern Colombia: Implications for the evolution of the Magdalena, Orinoco, and Amazon river systems. *Geosphere* 12: 1235–1256. <https://doi.org/10.1130/GES01294.1>
- Angarita-Sierra T (2014) Hemipenial morphology in the semifossorial snakes of the genus *Ninia* and a new species from Trinidad, West Indies (Serpentes: Dipsadidae). *South American Journal of Herpetology* 9: 114–130. <https://doi.org/10.2994/SAJH-D-12-00004.1>
- Angarita-Sierra T, Ospina-Sarria JJ, Anganoy-Criollo M, Pedroza-Banda R, Lynch JD (2013) Guía de campo de los Anfibios y Reptiles del departamento de Casanare – Colombia. Universidad Nacional de Colombia, Sede Orinoquia. YOLUKA ONG, Fundación de Investigación en Biodiversidad y Conservación, Bogotá D.C., 117 pp. Available from: [http://yoluka.org.co/dropbox/publicaciones/guias/Guia\\_Casanare\\_alta.pdf](http://yoluka.org.co/dropbox/publicaciones/guias/Guia_Casanare_alta.pdf)
- Antonelli A, Nylander JAA, Persson C, Sanmartín I (2009) Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences* 106: 9749–9754. <https://doi.org/10.1073/pnas.0811421106>
- Arévalo E, Davis SK, Sites Jr. JW (1994) Mitochondrial DNA Sequence Divergence and Phylogenetic Relationships among Eight Chromosome Races of the *Sceloporus Grammicus* Complex (Phrynosomatidae) in Central Mexico. *Systematic Biology* 43: 387–418. <https://doi.org/10.1093/sysbio/43.3.387>
- Arteaga AF (2020) *Bothrocophias microphthalmus* (Small-eyed Toadhead). In: Arteaga AF, Bustamante L, Vieira J, Guayasamin J (Eds), *Reptiles of Ecuador: Life in the middle of the world*. Tropical Herping, Quito, 1–5. Available from: [www.reptilesfocuecuador.com](http://www.reptilesfocuecuador.com)
- Bensch S, Stjernman M, Hasselquist D, Ostman O, Hansson B, Westerdahl H, Pinheiro RT (2000) Host specificity in avian blood par-

- asites: a study of Plasmodium and Haemoproteus mitochondrial DNA amplified from birds. *Proceedings. Biological Sciences* 267: 1583–1589. <https://doi.org/10.1098/rspb.2000.1181>
- Bernarde PS, de Albuquerque S, Barros TO, Turci LCB (2012) Serpentes do estado de Rondônia, Brasil. *Biota Neotropica* 12: 155–182. <https://doi.org/10.1590/s1676-06032012000300018>
- Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, De Maio N, Matschiner M, Mendes FK, Müller NF, Ogilvie HA, du Plessis L, Poppinga A, Rambaut A, Rasmussen D, Siveroni I, Suchard MA, Wu C-H, Xie D, Zhang C, Stadler T, Drummond AJ (2019) BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 15: e1006650. <https://doi.org/10.1371/journal.pcbi.1006650>
- Bouckaert RR, Drummond AJ (2017) bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMC Evolutionary Biology* 17: 42. <https://doi.org/10.1186/s12862-017-0890-6>
- Boulenger GA (1912) Descriptions of new reptiles from the Andes of South America, preserved in the British Museum. *The Annals and magazine of natural history* 8: 420–424.
- Branquet Y, Cheillett A, Cobbold PR, Baby P, Laumonier B, Branquet Y, Cheillett A, Cobbold PR, Baby P, Laumonier B (2002) Andean deformation and rift inversion, eastern edge of Cordillera Oriental (Guatque-Medina area), Colombia. *Journal of South American Earth Sciences* 15: 391–407. [https://doi.org/10.1016/S0895-9811\(02\)00063-9](https://doi.org/10.1016/S0895-9811(02)00063-9)
- Campbell JA, Lamar WW (1989) *The Venomous Reptiles of Latin America*. Campbell JA, Lamar WW (Eds). Comstock Pub. Associates, 425 pp.
- Campbell JA, Lamar WW (2004) *The venomous reptiles of the western hemisphere* (Vol. 1). Comstock Publishing, New York, 475 pp.
- Carrasco PA, Mattoni CI, Leynaud GC, Scrocchi GJ (2012) Morphology, phylogeny and taxonomy of South American bothropoid pitvipers (Serpentes, Viperidae). *Zoologica Scripta* 41: 109–124. <https://doi.org/10.1111/j.1463-6409.2011.00511.x>
- Chippaux JPJ-P (2017) Incidence and mortality due to snakebite in the Americas. Gutiérrez JM (Ed.). *PLoS ONE Neglected Tropical Diseases* 11: 1–39. <https://doi.org/10.1371/journal.pntd.0005662>
- Cisneros-Heredia D, Borja M, Proano D, Touzet J (2006) Distribution and natural history of the Ecuadorian Toad-headed Pitvipers of the genus *Bothrocophias*. *Herpetozoa* 19: 17–26.
- Congreso de Colombia (2016) Ley 1774 de 2016 por medio de la cual se modifican el código civil, la ley 84 de 1989, el código penal, el código de procedimiento penal y se dictan otras disposiciones. Colombia, 1–5 pp. Available from: <http://es.presidencia.gov.co/normativa/normativa/LEY%201774%20DEL%206%20DE%20ENERO%20DE%202016.pdf>
- Cope E (1875) Report on the Reptiles brought by Professor James Orton from the middle and upper Amazon and western Peru. *Journal of the Academy of Natural Sciences of Philadelphia*. *Journal of the Academy of Natural Sciences of Philadelphia* 2: 159–183. Available from: <https://www.biodiversitylibrary.org/item/114076>.
- Crisci JV (1984) Taxonomic Congruence. *Taxon* 33: 233–239. <https://doi.org/10.2307/1221163>
- Fenker J, Tedeschi LG, Pyron RA, Nogueira C de C (2014) Phylogenetic diversity, habitat loss and conservation in South American pitvipers (Crotalinae: Bothrops and Bothrocophias). *Diversity and Distributions* 20: 1108–1119. <https://doi.org/10.1111/ddi.12217>
- Fenwick AM, Gutberlet RL, Evans JA, Parkinson CL (2009) Morphological and molecular evidence for phylogeny and classification of South American pitvipers, genera *Bothrops*, *Bothriopsis*, and *Bothrocophias* (serpentes: Viperidae). *Zoological Journal of the Linnean Society* 156: 617–640. <https://doi.org/10.1111/j.1096-3642.2008.00495.x>
- Ferreira JK, Machado LG, Araújo ML, Alves ML, Junqueira-de-Azevedo I, Vieira GA, Prosdociimi F, Graziotin F, Netto CC, Zingali RB (2020) Brazilian *Bothrops diporus*, in fact a lineage of *Bothrops pubescens*: Mitogenomic, venomomic and ontogenetic studies. *Toxicon* 177: S38. <https://doi.org/10.1016/j.toxicon.2019.12.061>
- Fox W (1948) Effect of Temperature on Development of Scutellation in the Garter Snake, *Thamnophis elegans atratus*. *Copeia* 1948: 252. <https://doi.org/10.2307/1438712>
- Freire-Lascano A (1991) *Dos nuevas especies de Bothrops en el Ecuador*. Publicación Trabajos Científicos del Ecuador, Universidad Técnica de Machala: 11.
- Gbif.Org (2021) Occurrence Download. <https://doi.org/10.15468/dl.tf-hdsb>
- Gómez J, Núñez-Tello A, Mateus-Zabala D, Alcárcel-Gutiérrez F, Lasso-Muñoz R, Marín-Rincón E, Marroquín-Gómez MP (2020) Chapter 1 Physiographic and Geological Setting of the Colombian Territory. In: Gómez J, Mateus-Zabala D (Eds), *An Automated Irrigation System Using Arduino Microcontroller*. Servicio Geológico Colombiano, Bogotá D.C.: 1–16. <https://doi.org/10.32685/pub.esp.35.2019.01>
- Guisande-González C, Vaamonde-Liste B, Barreiro-Felpeto A (2014) *Rwizard*: 1–452. Available from: <http://www.ipez.es/Rwizard>
- Gutberlet J, Campbell JA (2001) Generic Recognition for a Neglected Lineage of South American Pitvipers (Squamata: Viperidae: Crotalinae), with the Description of a New Species from the Colombian Chocó. *American Museum Novitates* 3316: 1–16. [https://doi.org/10.1206/0003-0082\(2001\)316<0001:GRFANL>2.0.CO;2](https://doi.org/10.1206/0003-0082(2001)316<0001:GRFANL>2.0.CO;2)
- Gutiérrez-Pinto N, Cuervo AM, Miranda J, Pérez-Emán JL, Brumfield RT, Cadena CD (2012) Non-monophyly and deep genetic differentiation across low-elevation barriers in a Neotropical montane bird (*Basileuterus tristriatus*; Aves: Parulidae). *Molecular Phylogenetics and Evolution* 64: 156–165. <https://doi.org/10.1016/j.ympev.2012.03.011>
- Hamdan B, Guedes TB, Carrasco PA, Melville J (2019) A complex biogeographic history of diversification in Neotropical lancehead pitvipers (Serpentes, Viperidae). *Zoologica Scripta* 49: 145–158. <https://doi.org/10.1111/zsc.12398>
- Harvey MB, Aparicio JE, Gonzales LA (2005) Revision of the venomous snakes of Bolivia. II: The pitvipers (Serpentes: Viperidae). *Annals of Carnegie Museum* 74: 1–37. [https://doi.org/10.2992/0097-4463\(2005\)74\[1:ROTVSO\]2.0.CO;2](https://doi.org/10.2992/0097-4463(2005)74[1:ROTVSO]2.0.CO;2)
- Hillis DM, Chambers EA, Devitt TJ (2021) Contemporary Methods and Evidence for Species Delimitation. *Ichthyology and Herpetology* 109: 895–903. <https://doi.org/10.1643/h2021082>
- Horn C, Wesselingh FP, ter Steege H, Bermudez MA, Mora A, Seville J, Sanmartín I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, Jaramillo C, Riff D, Negri FR, Hooghiemstra H, Lundberg J, Stadler T, Särkinen T, Antonelli A (2010) Amazonia Through Time: Andean Uplift, Climate Change, Landscape Evolution, and Biodiversity. *Science* 330: 927–931. <https://doi.org/10.1126/science.1194585>
- Hoyos M, Otero R, Saldarriaga M, Jaramillo N (2003) Divergencia morfométrica entre *Bothrops atrox* y *Bothrops asper* (Serpentes: Viperidae). *Actualidades Biológicas* 79: 157–165.
- Jadin RC, Velasco JR, Smith EN (2010) Hemipenes of the long-tailed rattlesnakes (Serpentes: Viperidae) from Mexico. *Phyllomedusa* 9: 69–73. <https://doi.org/10.11606/issn.2316-9079.v9i1p69-73>

- Jiang ZJ, Castoe TA, Austin CC, Burbrink FT, Herron MD, McGuire JA, Parkinson CL, Pollock DD (2007) Comparative mitochondrial genomics of snakes: Extraordinary substitution rate dynamics and functionality of the duplicate control region. *BMC Evolutionary Biology* 7: 114. <https://doi.org/10.1186/1471-2148-7-123>
- Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature methods* 14: 587–589. <https://doi.org/10.1038/nmeth.4285>
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution* 30: 772–780. <https://doi.org/10.1093/molbev/mst010>
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)* 28: 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kellogg J, Ojeda G, Cerón J (2005) Structure of the Eastern Cordillera of Colombia. In: 6th International Symposium on Andean Geodynamics. ISAG, Barcelona, 424–427.
- Kruskal JB, Wish M (1978) *Multidimensional Scaling*. Sage Publications Inc., Newbury Park, 1–98 pp. <https://doi.org/10.4135/9781-412985130>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*: 1547–1549.
- Lanfear R, Calcott B, Ho SY, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701.
- Leigh JW, Lapointe FJ, Lopez P, Baptiste E (2011) Evaluating phylogenetic congruence in the post-genomic era. *Genome Biology and Evolution* 3: 571–587. <https://doi.org/10.1093/gbe/evr050>
- Lienau EK, DeSalle R, Rosenfeld JA, Planet PJ (2006) Reciprocal illumination in the gene content tree of life. *Systematic Biology* 55: 441–453. <https://doi.org/10.1080/10635150600697416>
- Manzani P, Abe A (1988) Sobre dois novos metodos de preparado do hemipenes de serpentes. *Memórias do Instituto Butantan* 50: 15–20.
- Markolf M, Brameier M, Kappeler PM (2011) On species delimitation: Yet another lemur species or just genetic variation? *BMC Evolutionary Biology* 11: 15–19. <https://doi.org/10.1186/1471-2148-11-216>
- Matias NR, Alves MLM, Araujo ML de, Jung DMH (2011) Variação morfológica em *Bothropoides jararaca* (Serpentes, Viperidae) no Rio Grande do Sul. *Iheringia. Série Zoologia* 101: 275–282. <https://doi.org/10.1590/s0073-47212011000300001>
- Mayr E (1996) What is a species, and what is not? *Philosophy of Science* 63: 262–277.
- Minghui R, Malecela MN, Cooke E, Abela-Ridder B (2019) WHO's Snakebite Envenoming Strategy for prevention and control. *The Lancet Global Health* 7: e837–e838. [https://doi.org/10.1016/S2214-109X\(19\)30225-6](https://doi.org/10.1016/S2214-109X(19)30225-6)
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution* 37: 1530–1534. <https://doi.org/10.1093/molbev/msaa015>
- Murphy JC, Angarita-Sierra T, Downie JR, Jowers MJ (2017) Toads, tall mountains and taxonomy: The *Rhinella granulosa* group (Amphibia: Anura: Bufonidae) on both sides of the Andes. *Salamandra* 53: 267–278.
- Myers CW, Cadle JE (2003) On the Snake Hemipenis, with Notes on Psomophis and Techniques of Eversion: A Response to Dowling. *Herpetological Review* 34: 295–302.
- Myers CW, McDowell SB (2014) New Taxa and cryptic species of neotropical snakes (Xenodontinae), with commentary on hemipenes as generic and specific characters. *Bulletin of the American Museum of Natural History* 385: 1–112. <https://doi.org/10.1206/862.1>
- Nicéforo M (1942) Los ofidios de Colombia. *Revista de la Academia Colombiana de Ciencias Exactas Físicas y Naturales*: 89–101.
- Nicéforo M (1975) Contribución al estudio de las serpientes de Colombia II. *Boletín del Instituto de La Salle*: 1–4.
- Oksanen J, Simpson G (2013) Ordination methods, diversity analysis and other functions for community and vegetation ecologists. Available from: <https://cran.r-project.org/web/packages/vegan/index.html>
- Osgood DW (1978) Effects of Temperature on the Development of Meristic Characters in *Natrix fasciata*. *Copeia* 1978: 33. <https://doi.org/10.2307/1443819>
- Padial JM, Miralles A, De la Riva I, Vences M (2010) The integrative future of taxonomy. *Frontiers in Zoology* 7: 1–14. <https://doi.org/10.1186/1742-9994-7-16>
- Paradis E, Schliep K (2019) ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics (Oxford, England)* 35: 526–528. <https://doi.org/10.1093/bioinformatics/bty633>
- Parker H (1930) Two new reptiles from southern Ecuador. *Annals and Magazine of Natural History* 10: 568–571. <https://doi.org/https://doi.org/10.1080/00222933008673167>
- Parkinson CL, Campbell JA, Chippindale P (2002) Multigene phylogenetic analysis of pitvipers, with comments on their biogeography. In: Schueett GW, Höggren ME, Douglas ME, Green WW (Eds), *Biology of the vipers*. Eagle Mountain Publishing, Salt Lake City, 93110.
- Paterna A (2015) Morphological traits of hatchlings of the western whip snake *Hierophis viridiflavus* (Lacépède, 1789) from a central Italian population. *Russian Journal of Herpetology* 22: 179–187.
- Pereañez JA, Preciado LM, Fernández J, Camacho E, Lomonte B, Castro F, Cañas CA, Galvis C, Castaño S (2020) Snake venomomics, experimental toxic activities and clinical characteristics of human envenomation by *Bothrocophias myersi* (Serpentes: Viperidae) from Colombia. *Journal of Proteomics* 220: 103758. <https://doi.org/10.1016/j.jprot.2020.103758>
- Pesantes O (1994) A Method for Preparing the Hemipenis of Preserved Snakes. *Journal of Herpetology* 28: 93–95.
- Pook CE, Wüster W, Thorpe RS (2000) Historical biogeography of the Western Rattlesnake (Serpentes: viperidae: *Crotalus viridis*), inferred from mitochondrial DNA sequence information. *Molecular phylogenetics and evolution* 15: 269–282. <https://doi.org/10.1006/mpev.1999.0756>
- Prossl KF, Grosser JR (1994) Some New Palynomorphs from Silurian of the Quetame Massif, Cordillera Oriental, Colombia, South America. *Boletín Geológico* 34: 11.
- De Queiroz K (2007) Species concepts and species delimitation. *Systematic Biology* 56: 879–886. <https://doi.org/10.1080/10635150701701083>
- De Queiroz K (2011) Branches in the lines of descent: Charles Darwin and the evolution of the species concept. *Biological Journal of the Linnean Society* 103: 19–35. <https://doi.org/10.1111/j.1095-8312.2011.01634.x>
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7.

- Systematic Biology 67: 901–904. <https://doi.org/10.1093/sysbio/syy032>
- Rangel-Ch OJ (1995) Colombia Diversidad Biotica I. Rangel-Ch OJ (Ed.). Instituto de Ciencias Naturales. Universidad Nacional de Colombia, Bogotá, 442 pp. Available from: [http://www.colombiadiversidadbiotica.com/Sitio\\_web/Bienvenida.html](http://www.colombiadiversidadbiotica.com/Sitio_web/Bienvenida.html)
- Rendahl H, Vestergren G (1940) Notes on Colombian snakes. Arkiv för Zoologi 33: 1–16.
- Sambrook J, Fritsch E., Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.
- Sasa M (2002) Morphological variation in the lancehead pitviper *Bothrops asper* (Garman) (Serpentes: Viperidae) from Middle America. Revista de Biología Tropical 50: 259–271.
- Schätti B, Kramer E (1993) Ecuadorianische Grubenottern der Gattung *Bothriechis*, *Bothrops* und *Porthidium* (Serpentes: Viperidae). Revue Suisse de Zoologie 100: 235–278. <https://doi.org/10.5962/bhl.part.79860>
- Schliep KP (2011) Phangorn: phylogenetic analysis in R. Bioinformatics 27: 592–593. <https://doi.org/10.1093/bioinformatics/btq706>
- Schneider C, Rasband W, Eliceiri K (2012) ImageJ. Nature Methods 9: 671–675. [https://doi.org/10.1007/978-1-84882-087-6\\_9](https://doi.org/10.1007/978-1-84882-087-6_9)
- Sites Jr J., Myers E, Ruane S (2021) Species delimitation and integrative taxonomy: Challenges and opportunities in snake systematics. In: da Silva Jr N, Porras L, Aird S, da Costa Prudente AL (Eds), Advances in Coralsnake Biology: with Emphasis on South America. PUC Goiás's, Eagle mountain Publishing, Goiás, 1–36.
- Torres-Carvajal O, Pazmiño-Otamendi G, Salazar-Valenzuela D (2019) Reptiles of Ecuador: A resource-rich online portal, with dynamic checklists and photographic guides. Amphibian and Reptile Conservation 13: 209–229.
- Torres-Carvajal O, Pazmiño-Otamendi G, Ayala-Varela F, Salazar-Valenzuela D (2020) Reptiles del Ecuador Version 2020.1. 15 December 2020: Museo de Zoología, Pontificia Universidad Católica. Available from: <https://bioweb.bio/faunaweb/reptiliaweb> (January 23, 2021).
- Uetz P, Freed P, Hošek J (2022) The Reptile Database. Update 17 Dec 2021. Available from: <https://reptile-database.reptarium.cz> (January 23, 2021).
- Vaidya G, Lohman DJ, Meier R (2011) Cladistics multi-gene datasets with character set and codon information. Cladistics 27: 171–180.
- Valencia J, Garzón-Tello K, Barragán-Paladines M (2016) Serpientes Venenosas del Ecuador. Fundación Herpetológica Gustavo Orcés, Quito, 652 pp.
- Warrell DA (2004) Epidemiology, clinical features, and clinical management. In: Campbell JA, Lamar WW (Eds), The Venomous Reptiles of the Western Hemisphere Vol. 2. Cornell Press, Itaca, 709–761. [https://doi.org/https://doi.org/10.1007/springerreference\\_205004](https://doi.org/https://doi.org/10.1007/springerreference_205004)
- Zaher H, Prudente ALC (2003) Hemipenes of *Siphlophis* (Serpentes, Xenodontinae) and Techniques of Hemipenial Preparation in Snakes: A Response to Dowling. Herpetological Review 34: 302–307.

## Appendix

### Specimens examined or compared with literature descriptions:

#### *Bothrocophias microphthalmus (sensu lato)*

BOLIVIA ( $n=5$ , Harvey et al. 2005): **Beni**: Municipality of José Balivián. Locality: Rurrenabaque, NK96 coordinates N 14.45644167, W -67.55350278. — **Cochabamba**: Municipality of Carrasco. Locality: Carrasco National Park, Cerro Leñe CBF1899 coordinates N 17.35285556, W -65.06338611. — **La Paz**: municipality of Nor Yungas. Locality: Serranía Bellavista, CBFXX coordinates N 15.66472222, W -67.45638889; municipality of Sud Yungas. Locality: Serranía Beu NK1801 coordinates N 14.9, W -67.91666667; Locality: Serranía Chepete NK1814 coordinates N 14.41667, W -67.66667.

ECUADOR ( $n=22$ ): **Morona Santiago**: Cantón Morona: Parroquia Sinai. Locality: Banks of the Jurumbuno River, QCAZR13300, coordinates N 2.08617, W -78.1501. Cantón: Santiago. Parroquia: Patuca. Locality: Puchimi. In a creek near the camp, QCAZR15974, coordinates N 2.78075, W -78.15412. Cantón San Juan Bosco: Parroquia: San Carlos de Limón. Locality: Comunidad Shuar Kunkuk, foothills of the Cordillera del Cóndor, QCAZR16144-5, coordinates N 3.321027, W -78.2121; Parroquia: Pan de Azúcar. Referencia: Sector Siete Iglesias-San Juan Bosco. Locality: upper part of the transverse ravine to the Pan de Azúcar River, QCAZR17021 coordinates N 3.13196, W -78.55739. — **Pastaza**: Cantón: Mera; Parroquia: Mera. Locality: Llanganates National Park, Comunidad Zarentza. Line October 9 of Llanganates National Park, 400m from the Zarentza community school, QCAZR13730-36, coordinates N 1.35935, W -78.05774. — **Zamora Chichipe**: Catón Nangaritzza: Reserva Maycú, QCAZR12637, coordinates N 4.233086, W -78.61119. Cantón Zamora: Parroquia Zamora. Locality: Main trail, gate to Podocarpus National Park, Bombuscaro, QCAZR13858 coordinates N 4.114639, W -78.96702; Locality: Higuerones trail, sector of the Avecillas farm QCAZR13858 coordi-

nates N 4.133359, W -78.98547. Cantón: Nangaritzza. Parroquia: Zurmi. Locality: Maycu Nature Reserve, trail in terra-firme forest, surroundings of creek QCAZR15498 coordinates N 4.24706, W -78.65253; Locality: Trail parallel to the Nangaritzza River and tributary streams; Locality: via Las Orquídeas-Nuevo Paraiso. Tepuy Lookout Trail QCAZR15500, coordinates N 4.25707, W -78.68095; Locality: trail ladera baja QCAZR15501 coordinates N 4.21895, W -78.62946. Parroquia: Nuevo Paraiso. Locality: Camp next to the Nangaritzza River, near the tarabita. Trail from camp upstream QCAZR15770-72 coordinates N 4.45291, W -78.81508.

PERU ( $n=3$ ): **San Martín**: Municipalities of Balsa Puerto and Moyabamba. Locality: unknown, pictures of the holotype provided by Dr. Ned Gilmore ANSP 11515 coordinates N 5.952166667, W -76.76213889 (approximate coordinates taken from midpoint between Puerto and Moyabamba municipalities). — **Pasco**: Oxapampa. Locality: unknown, pictures shared by Juan Timms from a single specimen housed in Oxapampa hospital, coordinates N 10.5775, W -75.40167 (see Supplement material). Pozuzo. Locality: unknown, picture shared by Juan Timms from a single specimen housed in Pozuzo hospital, coordinates N 10.23767, W -75.69016.

#### *Bothrocophias tulitoi sp. nov.*

COLOMBIA ( $n=2$ ): **Cundinamarca**: Municipality of Gutiérrez. Locality: unknown, IAvH-R6879-80, coordinates N 4.25472, W -74.0025 (approximate to the town).

## Supplementary material 1

### Table S1

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

**Data type:** .docx

**Explanation note:** Partitions and evolutionary models for the concatenated alignment obtained using Model Finder (MF) and bModelTest (bMT).

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**Link:** <https://doi.org/10.3897/vz.72.e87313.suppl1>

## 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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