Evolutionary Systematics. 8 2024, 15–64 | DOI 10.3897/evolsyst.8.114527

PENSOFT.

EVOLUTIONARY SYSTEMATICS

Systematic revision of the Eyelash Palm-Pitviper *Bothriechis schlegelii* (Serpentes, Viperidae), with the description of five new species and revalidation of three

Alejandro Arteaga[1,2], R. Alexander Pyron[3,4], Abel Batista[5,6,7], Jose Vieira[2,8], Elson Meneses Pelayo[9,10], Eric N. Smith[11], César L. Barrio Amorós[12], Claudia Koch[13], Stefanie Agne[14], Jorge H. Valencia[15,16], Lucas Bustamante[2,17], Kyle J. Harris[18]

1 *Khamai Foundation, Quito, Ecuador*
2 *Tropical Herping S.A., Quito, Ecuador*
3 *Department of Biological Sciences, The George Washington University, Washington DC, USA*
4 *Division of Amphibians & Reptiles, Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington DC, USA*
5 *Universidad Autónoma de Chiriquí (UNACHI), Instituto Interdisciplinario de Investigación e Innovación, David, Panama*
6 *Fundación Los Naturalistas, Boquete, Chiriquí, Panama*
7 *Sistema Nacional de Investigación (SNI), SENACYT, Clayton Panamá, Panama*
8 *ExSitu, Quito, Ecuador*
9 *Grupo de Estudios en Anfibios y Reptiles de Santander, Universidad Industrial de Santander, Bucaramanga, Colombia*
10 *Grupo de Estudios en Biodiversidad, Universidad Industrial de Santander, Bucaramanga, Colombia*
11 *Amphibian and Reptile Diversity Research Center, Department of Biology, University of Texas at Arlington, Arlington, Texas, USA*
12 *CRWild, Bahía Ballena, Uvita, Costa Rica*
13 *Leibniz Institute for the Analysis of Biodiversity Change (LIB), Museum Koenig, Bonn, Germany*
14 *Evolutionary Adaptive Genomics, Department of Mathematics and Natural Sciences, University of Potsdam, Potsdam, Germany*
15 *Red de Estudios Moleculares Avanzados, Instituto de Ecología, Xalapa, Veracruz, Mexico*
16 *Red de Biogeografía y Ecología Espacial (BioGeo2), Universidad Regional Amazónica Ikiam, Tena, Ecuador*
17 *Savia Fund, Quito, Ecuador*
18 *Department of Biology, Liberty University, Lynchburg, Virginia, USA*

https://zoobank.org/42D6D571-379D-4EB0-BC8D-B3134A4E0912

Corresponding author: Alejandro Arteaga (alejandro@khamai.bio)

Academic editor: Oliver Hawlitschek ♦ **Received** 21 October 2023 ♦ **Accepted** 1 February 2024 ♦ **Published** 8 February 2024

Abstract

We present a taxonomic review and systematic revision of the Eyelash Palm-Pitviper *Bothriechis schlegelii* (Berthold, 1846) based on examination of 400 museum specimens, a phylogeographic analysis of 818 locality records, and 80 individuals sampled for molecular characters. We find morphological and phylogenetic support for five new species of *Bothriechis* Peters, 1859, which we describe here based on their unique combination of molecular, meristic, hemipenial, and color pattern characteristics. They are: *B. klebbai* **sp. nov.**, *B. rasikusumorum* **sp. nov.**, *B. khwargi* **sp. nov.**, *B. rahimi* **sp. nov.**, and *B. hussaini* **sp. nov.** Finally, we revalidate the names *B. nigroadspersus* (Steindachner, 1870), *B. nitidus* (Günther, 1859), and *B. torvus* (Posada Arango, 1889a), and provide a redescription of *B. schlegelii*.

Key Words

Colombia, Costa Rica, Ecuador, Panama, phylogeography, Serpentes, snake, Squamata, venomous, taxonomy

Introduction

The Eyelash Palm-Pitviper *Trigonocephalus schlegelii* Berthold, 1846 was described by Arnold Berthold in 1846 based on a juvenile specimen (ZFMK 32554) collected in Popayán, Colombia (Böhme 2010). The species was diagnosed primarily based on its enlarged supraciliary scales that resemble "eyelashes" and is now placed in the genus *Bothriechis* Peters, 1859. We do not review the history of generic reassignments in depth but refer to the synonyms in their original genus on first mention below.

Other eyelash viper species distinct from Berthold's *Trigonocephalus schlegelii* were described for Latin America in the period between 1859 and 1954. We refer to this group as the *Bothriechis schlegelii* species complex or the "eyelash clade" of the genus *Bothriechis*. The first of these was *Lachesis nitidus* Günther, 1859, described by Albert Günther based on a specimen (BMNH 1946.1.17.73) from the "western Andes of Ecuador," and differing from *B. schlegelii* primarily based on its green coloration with reddish dorsal stripes. The second was *Bothrops nigroadspersus* Steindachner, 1870, described by Franz Steindachner based on a specimen (NMW 18811) from "Central America," and differing from *B. schlegelii* primarily based on its bright yellow coloration and higher number of ventral scales and dorsal scale rows. The third was *Thanatophis torvus* Posada-Arango, 1889a, described by Andrés Posada Arango based on individuals (types not designated but reported as lost by Campbell and Lamar 2004) from the warm regions of Antioquia, and differing from *B. schlegelii* primarily based on the immaculate yellowish white ventral coloration. The fourth was *Thanatophis colgadora* García, 1896, described by Evaristo García based on individuals (specimens not designated) from the mountains of Cauca department, Colombia, and differing from *B. schlegelii* primarily based on its bluish-green dorsal coloration and larger body size. The fifth was named *Teleuraspis birri*, Posada Arango, 1909, a different name given to the reprinted description of *Thanatophis torvus*. The latter is therefore an objective junior synonym.

A sixth taxon, *Bothrops schlegelii supraciliaris* Taylor, 1954, previously considered conspecific or a subspecies of *Bothriechis schlegelii*, has since been revalidated and given full species status (Solórzano et al. 1998). Individuals of this species have been shown to be readily separated from Central American *B. schlegelii* based on scale counts and dorsal coloration (Solórzano et al. 1998). Using a sample of *B. supraciliaris* and one of *B. schlegelii* from Costa Rica, Castoe et al. (2009) presented evidence that both species were also genetically distinct. However, the inclusion of additional DNA sequences of *B. schlegelii* from Ecuador, Honduras, and Nicaragua by Daza et al. (2010) and Townsend et al. (2013) rendered *B. schlegelii* paraphyletic with respect to *B. supraciliaris*.

With the exception of *Bothriechis supraciliaris*, all of the aforementioned names have subsequently been subsumed under *B. schlegelii* (see Boulenger 1896; Daniel 1949; Peters and Orejas-Miranda 1970; Campbell and Lamar 2004; Wallach et al. 2014) into what is now considered a widely distributed species exhibiting remarkable variation in coloration, scale counts, shape of the supraciliary scales (Kuch and Freire 1995; Campbell and Lamar 2004; Hurtado-Gómez 2009), genetics (Wüster et al. 2002; Townsend et al. 2013), and venom composition (Kuch et al. 1996; Pla et al. 2017).

Bothriechis schlegelii is not monophyletic (Townsend et al. 2013) and is probably a species complex (Hurtado-Gómez 2009). In the words of Jay M Savage (cited in Campbell and Lamar 2004), "the great individual variation in coloration has precluded any attempt to fragment the species." To address this issue, we present a comprehensive taxonomic review and systematic revision of *B. schlegelii* based on 400 museum specimens, 818 locality records, and 80 individuals sampled for DNA-sequence data. We resolve the paraphyly of *B. schlegelii*, describe five new species, and revalidate the names *B. nigroadspersus*, *B. nitidus*, and *B. torvus*. Although additional diversity and potential taxonomy complexity exists in the group, to be examined by future researchers, the current proposal offers a robust and stable platform for additional study.

Materials and methods

Morphological data and collection acronyms

Terminology for *Bothriechis* cephalic shields is explained in Fig. 1. Ventral and subcaudal counts follow Dowling (1951). We examined fluid-preserved specimens from the herpetology collections at American Museum of Natural History (**AMNH**), Academy of Natural Sciences of Philadelphia (**ANSP**), Colección de Herpetología de la Universidad del Quindío (**ARUQ**), Círculo Herpetológico de Panama (**CH**), Colección Científica de Referencia Zoológica del Chocó-Herpetología (**COLZOOCH**), Colección de Prácticas Zoológicas de la Universidad del Valle (**CPZ-UV**), Colegio San José De la Salle (**CSJ**), Centro de Rescate de Vida Silvestre en Machala (**CZ**); Colección Zoológica de la Universidad ICESI (**CZI**), Colección Zoológica de la Universidad del Tolima (**CZUT**), División de Herpetología del Instituto Nacional de Biodiversidad (**DHMECN**), Colección Biológica Universidad EAFIT (**EAFIT**), Fundación Herpetológica Gustavo Orcés (**FHGO**), Colección Herpetológica del Instituto Alexander von Humboldt (**IAvH**), Colección de Herpetología del Instituto de Ciencias Naturales (**ICN**), Kansas University Natural

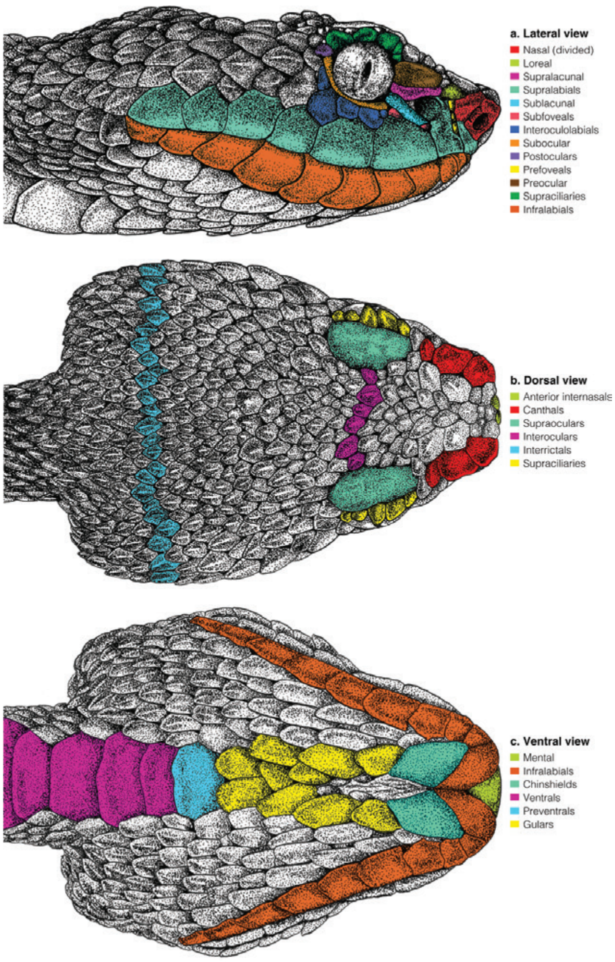

Figure 1. Terminology of *Bothriechis* cephalic lepidosis used in this work. Interrictals are counted across the head between the last supralabials. We consider interoculolabials to be only the scales directly below the subocular and above the supralabials. We do not include perisupraoculars (scales bordering the supraoculars) in the count of canthals. Method for counting ventrals follows Dowling (1951). Illustrations by Valentina Nieto.

History Museum (**KU**), Museum of Natural Science of the Louisiana State University (**LSUMZ**), Museum of Comparative Zoology of Harvard University (**MCZ**), Museo Herpetológico de Chiriquí (**MHCH**), Museo de Historia Natural de la Universidad del Cauca (**MHNUC**), Museo de Herpetología de la Universidad de Antioquia (**MHUA**), Colección de Herpetología Museo de La Salle Bogotá (**MLS**), Muséum National d'Histoire Naturelle (**MNHN**), Museo de Vertebrados de la Universidad de Panamá (**MVUP**), Museo de Zoología de la Universidad del Azuay (**MZUA**), Museo de Zoología de la Universidad Tecnológica Indoamérica (**MZUTI**), Naturhistorisches Museum Wien (**NMW**), Senckenberg Forschungsinstitut Frankfurt (**SMF**), Serpentario de la Universidad de Antioquia (**SUA**), Museo de Zoología de la Universidad de Costa Rica (**UCR**), Florida Museum of Natural History (**UF**), Colección de Herpetología de la Universidad Industrial de Santander (**UIS**), National Museum of Natural History (**USNM**), Amphibian and Reptile Diversity Research Center at the University of Texas at Arlington (**UTA**), Colección de Anfibios y Reptiles de la Universidad del Valle (**UV-C**), Leibniz Institute for the Analysis of Biodiversity Change, Museum Koenig (**ZFMK**), and Museo de Zoología de la Universidad San Francisco de Quito (**ZSFQ**) (Suppl. material 1). Labels AA, ANF, JPH, FP indicate individuals released after sampling; those listed PR are housed at ICN. Abbreviations are as follows: snout-vent length (SVL); tail length (TL).

Preparation of hemipenial morphology

The hemipenes were removed and prepared from museum specimens using the procedures of Pesantes (1994) and Zaher (1999). Hemipenial terminology is based on Dowling and Savage (1960), Zaher (1999), and Myers and MacDowell (2014).

Sampling and laboratory techniques

Genomic DNA was extracted from 96% ethanol-preserved tissue samples (liver, muscle tissue, or scales) using either a guanidinium isothiocyanate extraction protocol (Peñafiel et al. 2020) or a modified salt precipitation method based on the Puregene DNA purification kit (Gentra Systems). The nucleotide sequences of the primers and the PCR conditions applied to each primer pair are detailed in Appendix 1. PCR products were cleaned with either ExoSAP-IT (Affymetrix, Cleveland, OH) or Exonuclease I and Alkaline Phosphatase (Illustra ExoProStar by GE Healthcare) before they were sent to Macrogen Inc (Seoul, South Korea) for Sanger sequencing. All PCR products were sequenced in both forward and reverse directions with the same primers that were used for amplification. The sequences for the holotypes NMW 18811 and ZMFK 32554 were obtained using ancient DNA methods as described in Agne et al. (2022). Raw sequence data underwent removal of adapters (1 bp overlap) and short reads (> 30 bp) using Cutadapt v2.8 (Martin 2011), and of PCR duplicates (-q 30) using Samtools v1.10 (Li et al. 2009). Mitochondrial genes were assembled using the iterative mapper MITObim v. 1.9.1 (Hahn et al. 2013) and default values except for a mismatch parameter of six. For the assembly, the following sequences were downloaded from GenBank as references: 12S gene (MK313305.1), 16S gene (AF057260.1), COI gene (MH140083.1), CYTB (AF039270.1), and ND4 gene (MK313435.1). We generated sequence data for samples marked with an asterisk in Suppl. material 2. The sequences were deposited in GenBank and the accession numbers are listed in Suppl. material 2.

DNA phylogenetic analyses

A total of 279 DNA sequences were used to build a phylogenetic tree of the genus *Bothriechis*, of which 206 were generated during this work and 73 were downloaded from GenBank. Of these, 39 sequences are 398–408 bp long fragments of the 12S gene, 52 are 376–495 bp long fragments of the 16S gene, 20 are 493 bp long fragments of the COI gene, 78 are 143–712 bp long fragments of the CYTB gene, 71 are 378–675 bp long fragments of the ND4 gene, 9 are 185–522 bp long fragments of the NT3 gene, and 10 are 185–522 bp long fragments of the RAG1 gene. New sequences were edited and assembled using the program Geneious Pro™ 2021.1.1 (Drummond et al. 2021) and aligned with those downloaded from GenBank (Suppl. material 2) using MAFFT v.7 (Katoh and Standley 2013) under the default parameters. Gene fragments were concatenated into a single matrix with 20 partitions, one per non-coding gene and three per protein coding gene corresponding to each codon position. The best partition strategies along with the best-fit models of evolution were obtained in PartitionFinder 2.1.1 (Lanfear et al. 2016) under the Bayesian Information Criterion.

Phylogenetic relationships were assessed under a Bayesian inference (BI) approach in MrBayes 3.2.0 (Ronquist and Huelsenbeck 2013). Four independent analyses were performed to reduce the chance of converging on a local optimum. Each analysis consisted of 20,000,000 generations and four Markov chains with default heating settings. Trees were sampled every 1,000 generations and 25% of them were arbitrarily discarded as ‘‘burn-in.” The resulting 15,000 trees saved per analysis were used to calculate the posterior probabilities (PP) for each bipartition in a 50% majority-rule consensus tree. We used Tracer 1.6 (Rambaut et al. 2022) to assess convergence and effective sample sizes (ESS) for all parameters. Additionally, we verified that the average standard deviation of split frequencies between chains and the potential scale reduction factor (PSRF) of all the estimated parameters approached values of ≤ 0.01 and 1, respectively.

Distribution maps and ecological niche models

We present ranges of occurrence for the ten species of *Bothriechis* of the “eyelash clade.” Presence localities are derived from museum vouchers (Suppl. material 1), photographic records (iNaturalist), and the literature (all summarized in Suppl. material 3). For each species, a binary environmental niche model (ENM) is presented. These ENMs estimate potential areas of distribution based on observed presences and a set of environmental predictors (Elith and Leathwick 2009). To delimit the occupancy areas and the potential species distribution, we used the BAM diagram proposal (Soberón and Peterson 2005; Peterson et al. 2011). To create the models, we used presence localities listed in Suppl. material 3, 19 bioclimatic variables from Worldclim 1.4 (Hijmans et al. 2005), and Maxent 3.4.1k, an algorithm based on the principle of maximum entropy (Phillips et al. 2006; Elith et al. 2011; Renner and Warton 2013).

For the first explorative exercise, we used the 19 climate layers from the WorldClim project and assessed which variables were the most important for the model, according to the Jackknife test calculated in MaxEnt (Royle et al. 2012). Correlated environmental variables ($r < 0.8$) were identified using the PEARSON correlation test of PAST 3. In a second modelling exercise, we used the locality records for each species and the variables identified in the first approach to generate the species distribution. 5,000 iterations were specified to the program with clamping and no extrapolation. All other parameters in MaxEnt were maintained at default settings. To create the binary environmental niche models, suitable areas were distinguished from unsuitable areas by setting a “minimum training presence” threshold value. The logistic format was used to obtain the values for habitat suitability (continuous probability from 0 to 1), which were subsequently converted to binary presence-absence values based on the established threshold value, defined herein as “the minimum training presence.” The convergence threshold was set to 10^{-5}, maximum iterations to 500, and the regularization parameter to “auto.”

Rationale for definition of species-level candidate taxa

We here recognize species limits following an integration by congruence approach (Padial et al. 2010) based on the intersection of evidence from three or more independent taxonomic characters. We follow the general species concept of de Queiroz (2007) in defining species as independent evolutionary if two or more independent lines of evidence support their distinctness. We start the species delimitation procedure by seeking mitochondrial clades divergent from other mitochondrial clades by sequence divergences > 4% in a 700 bp fragment of the CYTB gene (Table 1), given that this degree of divergence has been found to correspond to species-level units in other *Bothriechis*, such as the species pairs *B. lateralis*–*B. guifarroi* (Townsend et al. 2013), with some exceptions explained below. We then tested if the individuals belonging to these mitochondrial lineages can be unambiguously diagnosed based on at least 4 of the 21 morphological characters summarized under Table 2. We considered in decreasing order of importance: (1) lepidosis; (2) hemipenial morphology; (3) coloration; and (4) size. We subsequently tested if males belonging to each species-level candidate taxa can easily be diagnosed on hemipenial morphology. We also evaluated if these mitochondrial lineages are congruent geographically and supported by the two nuclear genes studied. Thus, in our species delimitation approach, we consider groups of individuals as species if they (1) form a monophyletic group based on mtDNA; (2) differ from other such groups by > 4% sequence

Table 1. Between-group mean distance in percent (number of base differences per site from averaging over all sequence pairs between groups) for species of *Bothriechis* reviewed in this work, based on 700 bp of the mitochondrial CYTB gene.

	B. klebbai sp. nov.	*B. khwargi* sp. nov.	*B. hussaini* sp. nov.	*B. nitidus*	*B. torvus*	*B. rahimi* sp. nov.	*B. schlegelii*	*B. rasikusumorum* sp. nov.	*B. nigroadspersus*
B. klebbai sp. nov.	–								
B. khwargi sp. nov.	5.4	–							
B. hussaini sp. nov.	4.5	3.7	–						
B. nitidus	5.1	3.9	1.5	–					
B. torvus	3.1	3.6	1.5	1.3	–				
B. rahimi sp. nov.	4.9	5.1	2.5	2.6	3.8	–			
B. schlegelii	5.2	3.9	4.6	4.3	2.8	5.0	–		
B. rasikusumorum sp. nov.	6.6	6.4	6.2	5.5	5.2	6.6	5.8	–	
B. nigroadspersus	10.7	9.0	10.7	10.3	9.1	11.8	9.2	9.9	–
B. supraciliaris	10.8	11.3	11.4	11.7	11.0	11.6	11.4	11.7	10.2

divergence (mean p-distance); (3) are diagnosable based on external characters of coloration and lepidosis; (4) are diagnosable based on hemipenial morphology; and (5) are congruent ecologically and biogeographically.

Results

Molecular phylogeny

Selected partitions and models of evolution are presented in Table 3. We consider strong support for a clade when Bayesian analyses yield posterior probability values > 95%, following Felsenstein (2004). The topology and support (Fig. 2) of our phylogenetic tree is identical to that of Doan et al. (2016) and Mason et al. (2019) for all species of *Bothriechis* other than those within the *B. schlegelii* species complex. The most notable similarity is the relationship of the "eyelash vipers" as sister to the remaining members of the genus. Below, we comment on the topology of the "eyelash" clade.

At a continental scale, we found two strongly supported reciprocally monophyletic clades within the *Bothriechis schlegelii* species complex. The first is a Central American (CA; red in Fig. 3) clade and the second is a South American (SA) clade (Fig. 3). The CA clade includes *B. supraciliaris* and another species for which the name *B. nigroadspersus* is available (see account below). We sequenced the holotype of *B. nigroadspersus* (NMW 18811; Fig. 4). In our analysis, this specimen is deeply nested within this clade and is closely related to specimens from Costa Rica (Fig. 5). We also found two strongly supported subclades within *B. nigroadspersus*: one represents an almost entirely Panamanian population with one record in Cerro Tacarcuna, Colombia (COLZOOCH 581), and the other represents all other populations of *B. nigroadspersus* throughout Central America.

The SA clade includes eight species-level candidate taxa. The most early divergent of these is also the most geographically isolated: a new species endemic to Huila department, Colombia, at the junction between Cordillera Central and Cordillera Oriental (Dark blue in Fig. 3). This is the only species in the *Bothriechis schlegelii* complex known to occur along the Amazonian slopes of the Andes and is sister to all other SA *Bothriechis* species.

A second species occurs along the Chocó-Río Magdalena valley biome in Colombia (turquoise clade in Figs 2, 3). This species marginally enters Darién province in Panama, where it occurs in sympatry (see section on the distribution of *Bothriechis nigroadspersus*) with *B. nigroadspersus* along the Serranía de Pirré. Samples of this species, for which the name *B. torvus* is available, are sister to the remaining SA *Bothriechis*. We restrict the name *B. torvus* to this clade based on this species' type locality ("warm regions of Antioquia") and description of its coloration: belly yellowish white without spots. This description (Posada Arango 1889a) unambiguously rules out the other Antioquian species, *B. schlegelii sensu stricto* (see below for our updated definition), which occurs only in the highlands (Fig. 3) of the department and has a dark ventral coloration.

We restrict the name *Bothriechis schlegelii* to the magenta clade in Figs 2, 3, which includes samples from throughout Colombia's Cordillera Occidental and Cordillera Central, based on the inclusion of DNA sequences from the holotype (ZFMK 32554; Fig. 6) in this group. Consequently, *B. schlegelii sensu stricto* is primarily a highland species, endemic to Colombia, and sister to the remaining species in the SA clade exclusive of the species from Huila, Colombia and *B. torvus*. Its distribution only overlaps slightly with that of the previous species (turquoise clade), although we did not find areas of sympatry. The holotype of *B. schlegelii* is listed (Böhme 2010) as having been collected at Popayán, Cauca province, and our results agree with this view (see Discussion for additional detail on this locality).

A second new species occurs on the lowlands and mid-elevations of Colombia's Cordillera Oriental towards the Río Magdalena valley, in the northeast of the country (cyan clade in Fig. 3). The included samples are sister to the remaining SA species. This taxon occurs nearby, but is not sister to, a third new species that is distributed on the highlands of the western slopes of the Cordillera Oriental. The predicted distribution of both species overlaps (21–29%; darker shade of cyan in Fig. 3) and we found them to occur in sympatry at elevations above 1,700 m on the western slopes of the Serranía de los Yariguíes. This third new species (rose red clade) is sister to the remaining three SA *Bothriechis*, all of which occur south of the aforementioned ones, primarily in Ecuador.

Table 2. Differences in coloration, scale counts, hemipenial architecture, size, and median lethal dose (LD50), between members of the *Bothriechis schlegelii* species complex. The range of each continuous variable is from our own sample (Suppl. material 1), Kuch and Freire (1995), and Solórzano et al. (1998).

Character	*B. klebbai* sp. nov.	*B. rasikusumorum* sp. nov.	*B. khwargi* sp. nov.	*B. hussaini* sp. nov.	*B. nigroadspersus*	*B. nitidus*	*B. rahimi* sp. nov.	*B. schlegelii*	*B. supraciliaris*	*B. torvus*
Condition of supraciliary scales	Low and granular or two raised, but not sharp	Low and granular, or two pointy but not raised	Two, triangular, and moderately raised	Low and granular to triangular	Two raised and spinelike	Low and granular or two pointed but not raised	Two or three raised and triangular or spinelike	Two, triangular, and moderately raised	Two or three raised and spinelike	Three raised, triangular, but rounded and not spinelike
Anterior head scales keeled	No	No	Yes	No, or barely	Yes	No, or barely	Yes	No	No, or barely	Yes
Condition of gular scales	Similar in size to chinshields	Similar in size to chinshields	Much smaller than chinshields	About 1/2 size of chinshields	Much smaller than chinshields	Usually much smaller than chinshields; similar to chienshields in some cloud forest populations	Much smaller than chinshields	Variable	Much smaller than chinshields	Much smaller than chinshields
Interoculolabials	7–11	5–10	7–14	5–8	7–24	3–8	8–13	3–11	6–15	6–18
Canthals	2–4	2–3	3–4	2–3	2–6	2–3	4–5	1–3	4–5	2–8
Condition of canthals	Flat or slightly raised forming a ridge along the canthus	Flat or slightly raised forming a ridge along the canthus	With raised triangular projections	Flat or slightly raised forming a ridge along the canthus	Variable	Raised slightly forming a ridge along the canthus	Raised slightly forming a ridge along the canthus	Flat or slightly raised forming a ridge along the canthus	With raised triangular projections	With raised triangular projections
Loreal in contact with preocular	Yes in ~1/3 of specimens	No	Yes in ~1/3 of specimens	Yes	No	No	No	Yes in ~1/3 of specimens	Yes	Yes in western populations
Yellow morph (*oropel*)	No	No	No	Yes	Yes	No	Yes	No	Extremely rare	No
Color of dorsal bands (when present)	Black	Dark-brown or black	Bands absent, faint, or restricted to top of dorsum in juveniles	Black and pink	Bands absent	Dark reddish brown, red, or pink	Pink, faint	Black or dark brown	Blotches or bands rich reddish brown	Pink or red
Opposing kidney shaped dorsal marks	No	No	No	No	Yes	No	No	No	No	No
Dorsal pattern of snout in contrastingly marked specimens	Many irregular black spots and speckles	Many irregular black spots and speckles	No markings or fine black speckling	Coarse black speckling or spots	Two paramedian blotches that may touch each other or be fused to form a short transverse band, but nevertheless remain discernible	Black speckles, spots, or irregular markings	No markings	Irregular black speckling or a pair of black oval blotches	Single anteriormost dorsal blotch centrally on the snout	Irregular dark speckles or faint marks
Black speckles on dorsal scales	Yes	Yes	No	Yes	Usually no, but yes in some specimens of the golden morph	Usually no, but yes in some cloud forest populations	No	Usually no	No	No
Black speckling on ventral surfaces	Yes	Yes	No	Yes	Usually absent or faint and light brown	Usually no, but yes in some cloud forest populations	No	Usually no, but yes in some populations	Faint or absent	If present, the speckling is minute and faint
Entirely pale white belly	No	No	Yes, in most individuals	No	Yes, in some individuals	Yes, in some individuals	Yes	No	No	Yes, in some individuals
Iris color	Rich dark reddish brown	Light green, yellow, or reddish brown with black reticulations	Pale green or straw yellow	Green to yellow with fine black speckles, spots, or reticulations	Pale straw yellow to golden with black reticulations or spots	Pale golden yellow to light green with or without black speckles or spots	Pale straw yellow with fine black speckles	Pale orange, light green, or golden yellow usually without speckles	Straw yellow with fine black speckles	Pale bluish-green or straw yellow with fine black speckling
Dorsal scale rows at mid-body	21–23	21–23	21–23	21–23	21–25	19–23	21–23	21–25	21–23	21–23
Ventrals in males	144–151	144–152	144–153	140–151	153–169	129–156	137–145	139–156	144–150	148–159
Ventrals in females	141–151	152–153	145–154	147–153	148–167	132–158	146–151	135–153	139–148	141–155
Condition of basal hooks on sulcate view of hemipenial body	Absent	Present, about 2X larger than adjacent spines	–	Present, about 4X larger than adjacent spines	Present, about 3X larger than adjacent spines	Present, about 2X larger than adjacent spines	–	Present, about 3–10X larger than adjacent spines	Absent	Present, about 3X larger than adjacent spines
Maximum total length in males (mm)	671	650	219	608	626	594	336	834	583	378
Maximum total length in females (mm)	874	799	610	656	916	857	494	969	382	657
LD50 (mg/kg)	–	–	–	–	1.7–5.6	6.5	–	10.3	6.0	9.2

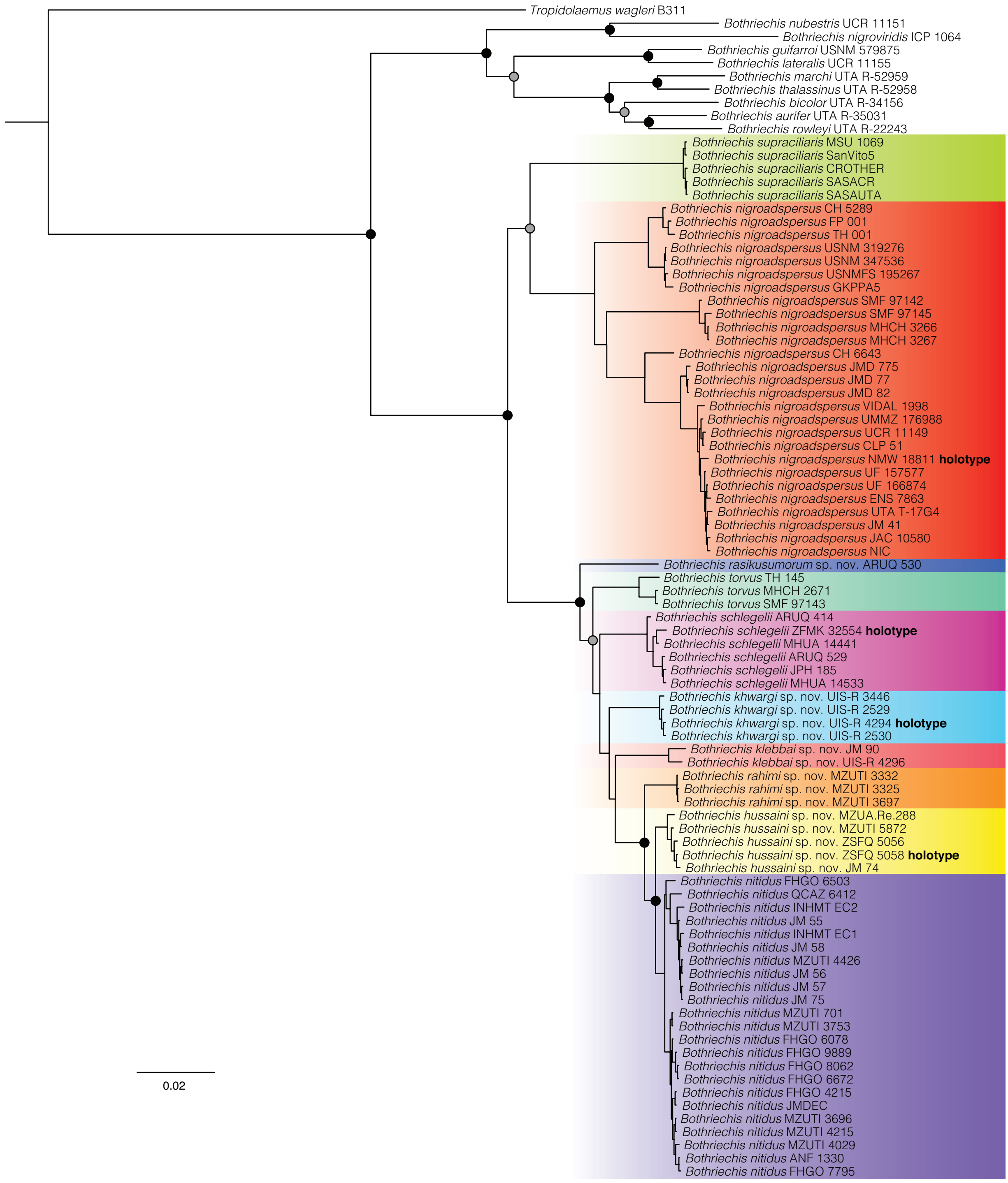

Figure 2. Phylogenetic relationships within *Bothriechis* inferred using a Bayesian inference and derived from analysis of DNA gene fragments 12S, 16S, COI, CYTB, ND4, NT3, and RAG1. Support values on intra-specific branches are not shown for clarity. Voucher numbers for sequences are indicated for each terminal. Black dots indicate clades with posterior probability values from 95–100%. Grey dots indicate values from 70–94%. Values < 50% not shown. Colored clades correspond to the species' distribution presented in the maps.

Table 3. Partition scheme and models of evolution used in phylogenetic analyses. Numbers in parentheses indicate codon position.

Partition	Best model	Gene regions	Number of aligned sites
1	GTR+I+G	12S, 16S, CYTB(2), ND4(1)	1368
2	HKY+I+I	COI(1), COI(2), CYTB(3), ND4(2), NT3(1), RAG1(2)	1264
3	GTR+G	COI(3), CYTB(1), ND4(3)	627
4	JC	NT3(2), NT3(3)	348
5	F81+I	RAG1(1), RAG1(3)	597

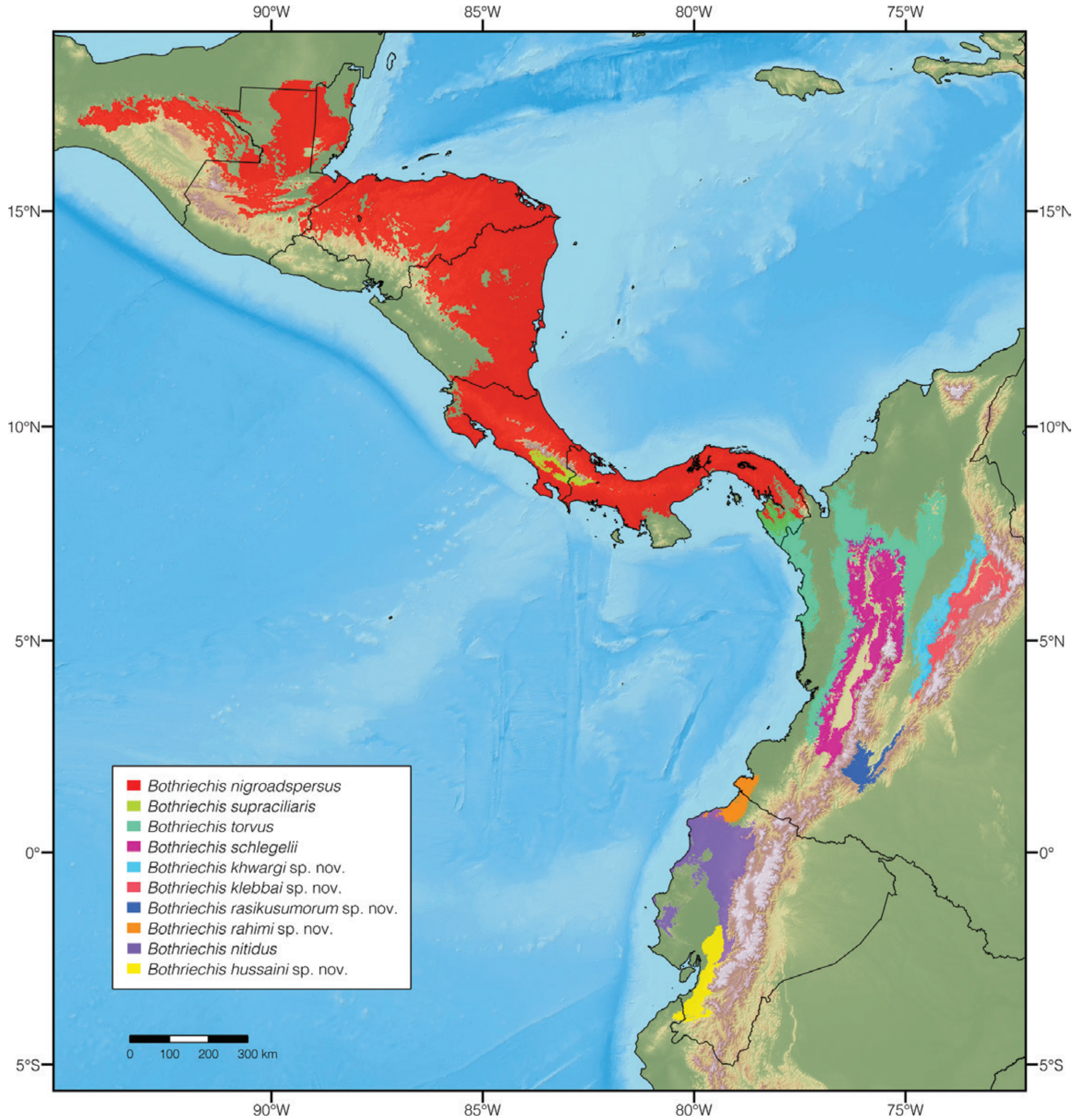

Figure 3. Distribution of species of *Bothriechis* previously subsumed under *B. schlegelii sensu lato*. Each colored area is a geographic representation of the suitable environmental conditions for one of the clades recovered in the phylogeny of Fig. 2. Lime green shade in southeastern Panama represents overlap between between *B. nigroadspersus* and *B. torvus*.

A fourth new species (orange clade; Figs 2, 3) is sister to the remaining southernmost members of the SA group. This species is known only from the Chocoan lowlands of extreme southwestern Colombia and adjacent northwestern Ecuador. We apply the name *Bothriechis nitidus* to a taxon endemic to west-central Ecuador (purple clade) based on three lines of evidence (see Discussion): the number of ventrals in the holotype, the travel logs of the collector Louis Fraser, and the illustration of the holotype by George Henry Ford (Fig. 7), which depicts a specimen that agrees in coloration with members of the purple clade, not those of the yellow clade. Finally, the yellow clade corresponds to the southernmost species, a fifth new species that inhabits dryer ecosystems in the transition area between the Chocó and the Tumbes biogeographic regions, in southwestern Ecuador and extreme northwestern Peru.

Systematic accounts

Species delimitation and the distinction between species-level and intraspecific variation is a complex topic (Carstens et al. 2013; Burbrink et al. 2022). In this instance, we name and provide descriptions only for species that are monophyletic in our molecular phylogeny and share diagnostic features of their coloration pattern, lepidosis, hemipenial morphology, and biogeography. Future studies might elucidate species boundaries based on explicit species-delimitation analyses. Based on these delimitation criteria, which follow the general lineage species concept of de Queiroz (2007), we describe five new species of *Bothriechis* and revalidate *B. nigroadspersus*, *B. nitidus*, and *B. torvus*. While additional genetic variation and potentially undescribed species may exist in the group and in the region, we are confident that these species represent ecologically, geographically, morphologically, and phylogenetically differentiated lineages that are equivalent in their evolutionary distinctiveness as compared to other recognized snake taxa. Consequently, we revise the *B. schlegelii* species complex to include:

Bothriechis nigroadspersus **(Steindachner, 1870)**

Figs 4, 5, 8

Bothrops nigroadspersus Steindachner, 1870: 348. Holotype NMW 18811 (Fig. 4), an adult female from Central America.
Teleuraspis nigroadspersus Garman, 1884: 108.

Referred specimens. All labeled *Bothriechis nigroadspersus* in Suppl. material 1.

Proposed standard English names. Central American Eyelash-Pitviper

Spanish names. Bocaracá, toboba de pestañas, víbora de pestañas, oropel (yellow morph).

Diagnosis. *Bothriechis nigroadspersus* is diagnosed based on the following combination of characters: (1) two raised and spinelike supraciliary scales; (2) anterior dorsal head scales keeled; (3) gular scales much smaller than chinshields; (4) 7–24 interoculolabials; (5) 2–6 canthals which may be nearly flat or with raised triangular projections; (6) loreal not in contact with preocular; (7) yellow morph present and common in some areas; (8) dorsal bands absent; (9) opposing kidney shaped dorsal marks present in the majority of individuals; (10) black speckles on dorsal scales usually absent; (11) black speckling on ventral surfaces usually absent or brown and faint; (12) ventral surfaces entirely white in some individuals; (13) iris pale straw yellow to golden with black reticulations or spots; (14) 21–25 dorsal scale rows at mid-body; (15) 153–169 ventrals in males, 148–167 in females; (16) maximum total length in males 626 mm, in females 916 mm.

Comparisons. *Bothriechis nigroadspersus* is compared to other species of the genus previously subsumed under *B. schlegelii sensu lato* (differences summarized in Table 2). It differs from all of them by having a higher number of ventral scales in both males and females (although there is overlap with some species), two raised and spinelike supraciliary scales (vs low and granular or broad and triangular in the other species), and by lacking dorsal bands. Instead, most individuals of *B. nigroadspersus* have either opposing kidney shaped reddish dorsal marks o are of the golden morph (=oropel) (Fig. 5). *Bothriechis nigroadspersus* differs from *B. supraciliaris* by having a higher number of ventral scales, no broad blotches or dorsal bands, and a different pattern on the dorsal aspect of the snout. *Bothriechis nigroadspersus* differs from *B. torvus* by having opposing kidney-shape dorsal marks, a higher number of ventrals, loreal not in contact with preocular, and comparatively smaller spines on the hemipenial body (Fig. 8).

Description of holotype. An adult female, SVL 532 mm, tail length 96 mm (18.1% SVL); head length 34.4 mm (6.5% SVL) from tip of snout to angle of jaw; head width 28.5 mm (82.9% head length) taken at broadest point; rostral broader than high; nasal not entirely divided, but fused with first supralabial; loreal about 1/2 size of pit, in contact with nasal, canthals, 1 suprafoveal, 2 prefoveals, prelacunal, and supralacunal; prefoveals 4; subfoveals 3/3; postfoveals 0; prelacunal fused with second supralabial; sublacunals 1/1; supralacunal elongated and in contact with orbit; preoculars 1/1 (2/2 if supralacunal is considered a preocular); suboculars 1; postoculars 2; loreal pit large, directed anteriorly, located slightly below line drawn from center of eye to naris; supralabials 9 (including lacunolabial); infralabials 12, first pair meet posteriorly; mental broader than long; 1 pair of chin shields; 5 pairs of gulars between chin shields and preventrals; preventrals 3; anterior internasals 3; canthals 3/3; 2/2 moderately triangular but low supraciliary scales; supraoculars kidney-shaped, 2.2× longer than wide; intersupraoculars 5; anterior dorsal head scales keeled; posterior head scales keeled; dorsal scale rows at mid-body 23; ventrals 160; cloacal plate entire; 55 undivided subcaudals; tail prehensile.

Hemipenial morphology. (n = 2, Fig. 8) Everted and inflated, the organ is deeply bilobed, unicalyculate and slightly capitate; hemipenial lobes thick and cylindrical; in sulcate and asulcate views, lobe crotch ornamented with scattered spinules; sulcus spermaticus centrolineal, bifurcate and with walls weakly defined, bifurcation occurs below bilobation point and proximal to the base of the hemipenial body; sulcus spermaticus branch runs to lobe tips; distal one third of each hemipenal lobe ornamented with calyces with spinulate edges. In sulcate view, hemipenial body surface nude, but with a pair of enlarged and strongly calcified lateral spines (basal hooks), one on each side; each hemipenial lobe ornamented with 1–3 mesial spines and 5–7 lateral spines, all about a third of the size of the basal hooks; the spines in each lobe are replaced distally by calyces with spinulate edges. In lateral view, hemipenial body nude with the exception of two basal spines; lobes also largely nude but with 5–6 smaller spines that are replaced distally by calyces. In asulcate view, the hemipenial body is nude with the exception of the pair of large lateral spines and also a pair of smaller mesial spines; hemipenial lobes largely nude but ornamented with smaller spines that decrease in size towards the lobe crotch.

Natural history. *Bothriechis nigroadspersus* is an arboreal snake that inhabits evergreen lowland/foothill forests, plantations, and rural gardens. In Panama (Sorrell 2007, 2009) and Costa Rica (Solórzano 2004) these vipers were found to be mostly active at night or at dusk and on the base of tree or on low shrubby vegetation, and Leenders (2019) and Witold Lapinski (pers. comm. to AA) reports that they have been found in the canopy at heights of 32–35 m in Costa Rica. Sorrell (2007, 2009) observed that some snakes reside in the same perch for up to 14 days, 70.2% of individuals relocated each night, and only 6.4% remained at the same daytime perch site

Figure 4. Adult female holotype of *Bothriechis nigroadspersus* NMW 18811 in **a.** Dorsal and **b.** Ventral view. Photos by Alice Schumacher.

for more than two days. According to Rand and Myers (1990), Seifert (1983), Savage (2002), and our own observations, *B. nigroadspersus* is primarily nocturnal. During the day, most individuals of *B. nigroadspersus* remain in hunting posture on or close to their night perches, but others hide inside bromeliads, or occasionally remain active and move at ground level or on vegetation. Sorrell (2009) showed that members of this species are primarily ambush predators, but they also forage actively in search for food. In a study by Antonio (1980), captive juveniles fed mostly on frogs and attracted them by means of moving their bright yellow tails as a lure. Campbell and Lamar (2004), Sorrell (2009), Barrio-Amorós (2015), Morgan and Barrio-Amorós (2015), and Entiauspe-Neto et al. (2021) provide details on the dietary preference of adults encountered in the wild. These authors found that this age category also feeds on frogs (primarily treefrogs and rainfrogs), but also on lizards (anoles, whiptails, and geckos), birds (including hummingbirds), and mammals (bats, mice, and mouse opossums). We report on additional specimens found feeding on bats: CH 5651 from Coclé, Panama is preserved with an *Artibeus jamaicensis*

Figure 5. Photographs of some specimens of *Bothriechis nigroadspersus* in life. **a.** Adult female from a private collection in Costa Rica; **b.** MHCH 3269 adult from Guabito, Bocas del Toro province, Panama; **c.** Subadult from Parque Nacional Gandoca Manzanillo, Limón province, Costa Rica; **d.** Adult from a private collection in Costa Rica; **e, f.** Juveniles from Parque Nacional Gandoca Manzanillo, Limón province, Costa Rica; **g.** MHCH 3268 adult female from Portón, Chiriquí province, Panama; **h.** MHCH 3266 adult female from Chucantí Reserve, Darién province, Panama; **i.** Subadult from Parque Nacional Gandoca Manzanillo, Limón province, Costa Rica; **j.** Adult female from a private collection in Costa Rica; **k.** FP 001 from Cerro Gaital, Coclé province, Panama; **l.** MHCH 3267 juvenile male from Chucantí Reserve, Darién province, Panama. Photos by Jose Vieira.

Figure 6. Juvenile holotype of *Bothriechis schlegelii* ZFMK 32554 in lateral view. Photo by Alice Schumacher.

in its mouth. An uncollected specimen photographed by Philipp Hoenle in Chiapas, Mexico was feeding on an unidentified bat, as well as it was another uncollected individual found by Barrio-Amorós near Arenal, Costa Rica, on the ground on a dirt road feeding on a bat, with only one wing out of its mouth. Gerhardt et al. (1993), Laurencio (2005), and Chavarría and Barrio-Amorós (2014) provide accounts of predation on this viper by hawks (*Buteogallus urubitinga* and *Herpetotheres cachinnans*) and snakes (*Clelia clelia*). Solórzano (2004) suggests that in Costa Rica, breeding in *Bothriechis nigroadspersus* coincides with the rainy season. Blody (1983) observed that females become sexually mature at an age of less than three years and can produce more than one litter per year. Antonio (1980) described the courtship and copulatory behavior of captive *B. nigroadspersus* from Honduras and Gómez et al. (2015) recorded a case of a female from Costa Rica that produced a litter after presumably storing sperm for no less than ~35 months (slightly under three years). A specimen from Lago Yojoa, Honduras, kept at Centro El Ocotal, produced a litter of four eggs and one live young after being kept in a terrarium without a male for 18 years (Alejandro Velasquez pers. comm. to AB). Antonio (1980), Blody (1983), Gómez et al. (2015), and Murphy and Mitchell (1984) report a gestation period of 150–166 days (~5 months) and litters of 6–23 neonates that measure 16–22.5 cm in total length at birth. Campbell and Lamar (2004) report that captive *B. nigroadspersus* have lived up to 20 years.

Venom. In a series of 477 snakebite cases in Costa Rica in 1979, 18.9% were caused by *Bothriechis nigroadspersus* (Bolaños 1984). Mekbel and Céspedes (1963) report that four of a series of 27 autopsied snakebite cases in San José were caused by this species. Savage (2002) observed that between 90 and 100 bites by *B. nigroadspersus* are reported in Costa Rica in a typical year, with 3–6 resulting in deaths according to Seifert (1983). An average bite results in the injection of ~0.5 cc of venom. The venom is hemotoxic and strongly myonecrotic when compared to other Central American vipers (Gutiérrez and Chaves 1980). In humans, it causes intense localized pain, progressive hemorrhagic edema, and, in some cases, hemorrhagic blisters or hives, ecchymoses, and necrosis (Lomonte et al. 2008; Pla et al. 2017). Prezotto-Neto et al. (2016) studied the composition of the venom of specimens of *B. nigroadspersus* from Costa Rica and found that its properties differ drastically from specimens of *Bothriechis torvus* from Vegachi, Antioquia. When compared to the latter, the venom of *B. nigroadspersus* was found to be more edematous, hemorrhagic, and lethal. LD50 estimated as 1.7–5.6 mg/kg in *B. nigroadspersus* vs 9.24 mg/kg in *B. torvus* (Bolaños 1972; Gutiérrez and Chaves 1980; Lomonte et al. 2008, 2012; Prezotto-Neto et al. 2016).

Distribution. *Bothriechis nigroadspersus* is known from at least 335 localities (listed in Suppl. material 3) throughout much of the Mesoamerican biome, from the isthmus of Tehuantepec in Mexico to extreme

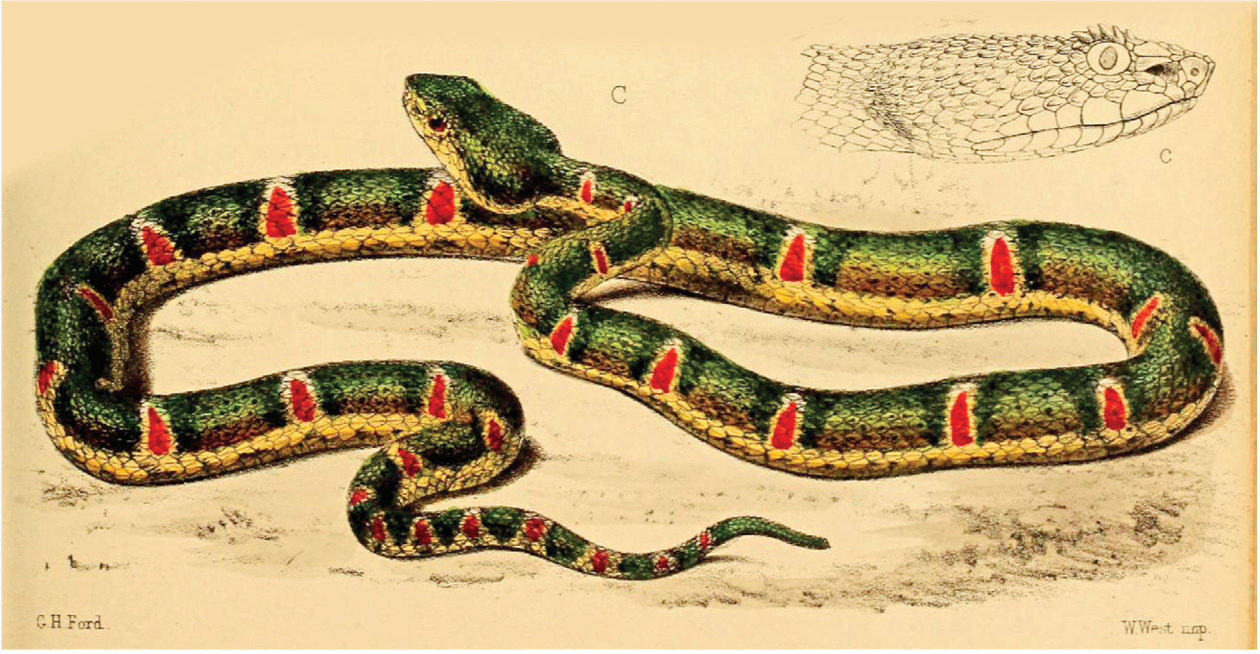

Figure 7. Illustration of the holotype of *Bothriechis nitidus* by George Henry Ford taken from the original description (Günther 1859) at the Biodiversity Heritage Library.

northwestern Colombia (Cerro Tacarcuna) along the Panamanian border. The species occurs over an estimated 352,139 km^2 area and has been recorded at elevations 0–1,434 m above sea level (Fig. 3). Approximately 1.0% of the predicted area of distribution of *B. nigroadspersus* overlaps with that of *B. supraciliaris*. Although sympatry has not been reported, we bring attention to a photographic record of an individual of *B. nigroadspersus* from the vicinity of San Pedro, Puntarenas province, just 4 km away from a record of *B. supraciliaris* (Photo by Ethyn Maki on iNaturalist; Suppl. material 3). An estimated 1.0% of the predicted area of distribution of *B. nigroadspersus* overlaps with that of *B. torvus*, and we found evidence of sympatry between the two species in Cordillera de Pirré, Darién province, Panama. MHCH 1664 is a *B. nigroadspersus* and MVUP 1384 is a *B. torvus*; both from the same mountain range.

Etymology. The specific epithet *nigroadspersus* comes from the Latin words *nigrum* (meaning "black") and *adspersus* (meaning "sprinkled"). It refers to the minute black specks scattered throughout the dorsum of the holotype (Fig. 4).

Conservation status. We consider *Bothriechis nigroadspersus* to be included in the Least Concern category following IUCN Red List criteria (IUCN 2012) because the species is widely distributed (but see Discussion), present in dozens of protected areas, tolerates moderate habitat degradation, and is presumably not declining fast enough to qualify in a threatened category. In a rainforest locality in Panama, the occurrence rates of *B. nigroadspersus* have increased by a factor of ten in the period from 2006 to 2012 (Zipkin et al. 2020). In another locality in Panama, the species was found to be extremely common in forest islands within a matrix of pastures (Sorrell 2007). However, it is unsure whether such "forest islands" will sustain the species without the presence of a dense population nearby that may act as a source of individuals that can immigrate to the fragmented habitat (Sorrell 2007).

Bothriechis supraciliaris (Taylor, 1954)

Figs 9–11

Bothrops schlegelii supraciliaris Taylor, 1954: 791. Holotype KU 31997 (Fig. 9), an adult female from mountains near San Isidro del General, San José province, Costa Rica.

Bothriechis supraciliaris Solórzano et al., 1998: 453–462.

Referred specimens. all labeled *Bothriechis supraciliaris* in Suppl. material 1.

Standard English name. Blotched Eyelash-Pitviper.

Standard Spanish name. Bocaracá manchada.

Diagnosis. *Bothriechis supraciliaris* is diagnosed based on the following combination of characters: (1) two or three raised and spinelike supraciliary scales; (2) anterior dorsal head scales smooth or barely keeled; (3) gular scales much smaller than chinshields; (4) 7–24 interoculolabials; (5) 4–5 canthals, most of which have raised triangular projections; (6) loreal in contact with preocular; (7) *oropel* morph extremely rare and with noticeably dark dorsal blotches on a yellowish background, rather than uniform golden-yellow; (8) reddish-brown irregular oval blotches or bands on dorsum; (9) opposing kidney shaped dorsal marks absent; (10) black speckles on dorsal scales absent; (11) black speckling on ventral surfaces usually absent or faint; (12) ventral surfaces not entirely white but yellowish anteriorly and light bluish-green posteriorly;

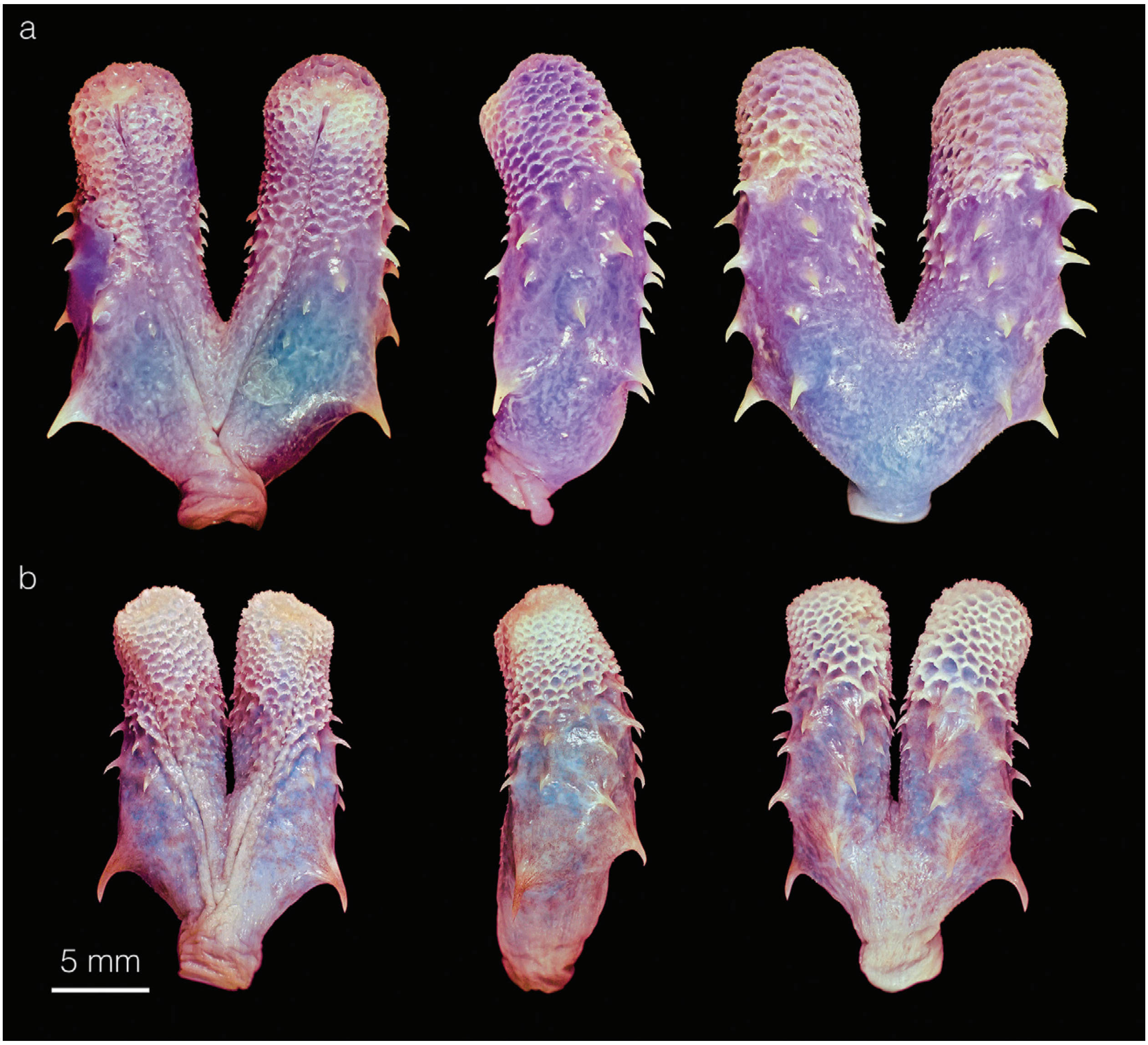

Figure 8. Hemipenial architecture of *Bothriechis nigroadspersus* in sulcate, lateral, and asulcate views. **a.** UTA R-32143 from Limón province, Costa Rica; **b.** UTA R-12957 from Aldea Vista Hermosa, Izabal department, Guatemala. Photos by Eric N. Smith.

(13) iris straw yellow with fine black speckles; (14) 21–23 dorsal scale rows at mid-body; (15) 144–150 ventrals in males, 139–148 in females; (16) maximum total length in males 382 mm, in females 583 mm.

Comparisons. *Bothriechis supraciliaris* occurs near and is most similar to *B. nigroadspersus*. It can be identified from this other much more variable and widely distributed viper species primarily by having fewer ventral scales (Table 2), loreal in contact with preocular, anterior head scales smooth or barely keeled (vs keeled in *B. nigroadspersus*), and by having reddish dorsal blotches or bands (Fig. 10). Lotzkat (2014) accurately pointed out that the two species differ in the dorsal pattern of the snout in contrastingly marked specimens. In *B. supraciliaris* there is a single anteriormost dorsal blotch centrally on the snout (Figs 9a, 10b–e); in *B. nigroadspersus*, there are two paramedian blotches that may touch each other or be fused to form a short transverse band, but nevertheless remain discernible. Lotzkat (2014) also brings attention to the fact that specimens of *B. supraciliaris* have very little or no dark pigmentation on the ventral surfaces at least anteriorly in adult specimens (vs. extensive dark pigmentation throughout the venter which are not of the *oropel* morph in *B. nigroadspersus*).

Hemipenial morphology. (n = 2, Fig. 11) Everted and inflated, the organ is deeply bilobed, calyculate and semicapitate; hemipenial lobes cylindrical and with minimal separation between them; in sulcate and asulcate views, lobe crotch ornamented with densely packed spinules; sulcus spermaticus centrolineal, bifurcate and with walls weakly defined, bifurcation occurs below bilobation point and proximal to the base of the hemipenial body; sulcus spermaticus branch runs to lobe tips; distal one third of each hemipenal lobe densely ornamented by calyces with strongly defined spinulate edges. In sulcate view, hemipenial body surface nude medially, but with enlarged and strongly calcified lateral spines followed by 1–2 rows of smaller obliquely-arranged mesial spines

Figure 9. Adult female holotype of *Bothriechis supraciliaris* KU 31997 in **a.** Dorsal and **b.** Ventral view. Photos by Hannah Som.

that become gradually smaller towards each lobe's capitulum; each hemipenial lobe ornamented with 1–2 mesial spines and 3–4 lateral spines, all about half the size of the larger lateral spines at the base of the hemipenial body; the spines in each lobe are replaced distally by calyces with strongly spinulate edges. In lateral view, hemipenial body and lobes with rows of spines, but otherwise smooth; distal 1/4 strongly calyculate. In asulcate view, the center of hemipenial body is nude except for a pair of large lateral spines; hemipenial lobes ornamented with 3–4 rows of smaller spines that decrease in size towards the lobe crotch.

Natural history. *Bothriechis supraciliaris* is an arboreal snake that inhabits evergreen lower-montane forests, cloud forests, clearings with coffee and banana plantations, edges of farm fields, and rural gardens (Campbell and Lamar 2004; Lotzkat 2014; Leenders 2019; Solórzano et al. 2021). Solórzano et al. (1998) and

Figure 10. Photographs of some specimens of *Bothriechis supraciliaris* in life. **a, d, e.** From Reptilandia, Costa Rica; and **b, c.** From a private collection in Costa Rica. Photos by Jose Vieira.

Figure 11. Hemipenial architecture of *Bothriechis supraciliaris* in sulcate, lateral, and asulcate views. **a.** UTA R-35246 from Jardin Botánico Las Cruces, Puntarenas province, Costa Rica; **b.** UTA R-35192 from Jardin Botánico Las Cruces, Puntarenas province, Costa Rica. Photos by Eric N. Smith.

Solórzano (2004) report that individuals of *B. supraciliaris* are crepuscular, nocturnal, and tend to spend more time on the ground than other *Bothriechis*: they found 22 out of 25 individuals on the forest floor. However, all individuals seen by CLBA (n=10) were on bushes from 30 cm to 2 m above the ground. Another naturalist, Norberto Solano, with more than 80 sighting of the species, only found one on the ground (Norberto Solano, pers. comm. to CLBA). During the day, individuals have been found resting on the ground or at the base of trees and shrubs (Solórzano 2004). Lotzkat (2014) and Batista et al. (2020) found individuals on low understory vegetation 0.5–2 m above the ground, either in ambush posture, crawling, or hanging from the tail. These authors also report that SMF 89764 was in the process of swallowing a comparatively large *Craugastor fitzingeri*. Solórzano et al. (1998) found mostly small forest-floor rodents in the stomach contents of four dissected specimens. They also report that specimens found in resting positions were curled in a loose circle and mostly on horizontal, wide surfaces.

Venom. Lomonte et al. (2012) studied the venom of this species in Costa Rica and found that it has a potent hemorrhagic action, moderate myotoxicity, and very weak procoagulant activity. These authors estimated its lethal activity at LD50 6.04 mg/kg and found that the equine polyvalent (Viperidae) antivenom from Instituto Clodomiro Picado was able to neutralize the lethal effect of *B. supraciliaris* venom. The antivenom tested was produced from the plasma of equines immunized with a mixture of the venoms of *Bothrops asper*, *Crotalus simus*, and *Lachesis stenophrys* (Lomonte et al. 2012).

Distribution. *Bothriechis supraciliaris* is known from at least 24 localities (listed in Suppl. material 3) along the Pacific slopes of the Cordillera de Talamanca in southwestern Costa Rica and western Panama. The species occurs over an area of approximately 3,568 km^2 and has been recorded at elevations 734–1,506 m above sea level (Fig. 3). We found that 100% of the predicted area of distribution of *B. supraciliaris* is included in the predicted area of distribution of *B. nigroadspersus*.

Etymology. The specific epithet *supraciliaris*, which comes from the Latin words *supra* (meaning "above") and *cilium* (meaning "eyelash"), refers to the prominent spinelike scales above the eye.

Conservation status. We consider *Bothriechis supraciliaris* to be included in the Endangered category (instead of Least Concern; see Solórzano et al. 2021) following the IUCN criteria B1a, b (i, iii, iv) (IUCN 2012), because the species' extent of occurrence is estimated to be less than 5,000 km^2 (Fig. 3) and its habitat is severely fragmented and declining in extent and quality due to deforestation. Although *B. supraciliaris* occurs in three protected areas (Estación Biológica Las Cruces, Las Nubes Biological Reserve, and Parque Binacional La Amistad), the remaining localities where the species has been recorded (Suppl. material 3) are in forest patches surrounded by a matrix of pastures and small settlements. Based on the species distribution model presented in Fig. 3 in combination with maps of vegetation cover of Central America (CATHALAC 2011), we estimate that nearly 37% of the forest cover throughout the species' potential distribution area has been destroyed, mostly due to the expansion of the agricultural frontier.

Bothriechis schlegelii (Berthold, 1846)

Figs 6, 12–14

Trigonocephalus schlegelii Berthold, 1846: 13. Holotype ZFMK 32554 (Fig. 6), a juvenile from Popayán, Colombia.

Thanatophis colgadora García, 1896: 27. Syntypes: lost, from "Cordillera Occidental de los Andes, en Calima, en Dapa, en San Antonio y en otros sitios de las montañas del Cauca".

Referred specimens. All labeled *Bothriechis schlegelii* in Suppl. material 1.

Proposed standard English name. Highland Eyelash-Pitviper, Schlegel's Eyelash-Pitviper.

Spanish names. Víbora de tierra fría, víbora de pestañas de Schlegel, guaruma.

Diagnosis. *Bothriechis schlegelii* is diagnosed based on the following combination of characters: (1) two or three triangular and moderately raised supraciliary scales; (2) anterior dorsal head scales smooth; (3) gular scales much smaller than or similar in size to chinshields; (4) 3–11 interoculolabials; (5) 1–3 canthals which may be flat or slightly raised forming a ridge along the canthus; (6) loreal in contact with preocular in ~1/3 of specimens; (7) yellow morph absent; (8) dorsal bands black or dark brown; (9) no opposing kidney shaped dorsal marks; (10) black speckles on dorsal scales usually absent; (11) black speckling on ventral surfaces usually absent; (12) ventral surfaces never entirely white; (13) iris pale orange, light green, or golden yellow usually without speckles; (14) 21–25 dorsal scale rows at mid-body; (15) 139–156 ventrals in males, 135–153 in females; (16) maximum total length in males 834 mm, in females 969 mm.

Comparisons. *Bothriechis schlegelii sensu stricto* is compared to other species of the genus previously subsumed under *B. schlegelii sensu lato* (differences summarized in Table 2). It differs from the other species except *B. klebbai* sp. nov. and *B. khwargi* sp. nov. in having two triangular and moderately raised supraciliary scales (supraciliaries either low and granular or enlarged and spinelike in the other species). *Bothriechis schlegelii* occurs near the distribution of *B. torvus* (Fig. 3), from which it differs by having anterior dorsal head scales smooth, 1–3 canthals (rather than 3–8), bands black or dark brown (rather than pink or red), ventral surfaces never entirely white, a lower number of ventrals in both males and females, and larger basal hooks on the hemipenial body (Fig. 14). *Bothriechis schlegelii* is most similar to *B. klebbai* sp. nov. and *B. rasikusumorum* sp. nov., but these other species occur parapatrically and are characterized by having small supraciliary scales, dorsal and ventral surfaces heavily speckled with black, gular scales similar in size or larger than chinshields, and no enlarged basal hooks on the hemipenial body. *Bothriechis schlegelii* differs from *B. khwargi* sp. nov. by having smooth anterior dorsal head scales (instead of keeled), a lower number of canthals, no reddish bands, and an entirely pale white belly.

Hemipenial morphology. (n = 7; three depicted in Fig. 14) Everted and inflated, the organ is deeply bilobed, calyculate and noncapitate; hemipenial lobes uniformly cylindrical or tapering towards the tip; in sulcate and asulcate views, lobe crotch ornamented with densely packed spines that become larger distally; sulcus spermaticus centrolineal, bifurcate and with walls strongly defined (weakly in IAVH-R 5465 and ICN PR 13225), bifurcation occurs below bilobation point and proximal to the base of the hemipenial body; sulcus spermaticus branch runs to lobe tips; distal one third to one half of each hemipenal lobe densely ornamented with small calyces with strongly defined spinulate edges. In sulcate view, hemipenial body surface with small spinules medially, but with enlarged and strongly calcified lateral spines (basal hooks) followed by 2–4 rows of much smaller obliquely-arranged mesial spines that become gradually smaller towards each lobe's capitulum; each hemipenial lobe ornamented with mesial and lateral spines about 1/3–1/10 the size of the large basal hooks; the spines in each lobe are replaced distally by calyces with strongly spinulate edges. In lateral view, hemipenial body and lobes with rows of spines, but otherwise non-spiculate; distal 1/3–1/2 of lobes strongly calyculate. In asulcate view, the center of hemipenial body is nude to barely spiculate; hemipenial lobes ornamented with 3 rows of smaller spines that decrease in size towards the lobe crotch.

Figure 12. Photographs of some specimens of *Bothriechis schlegelii* from Antioquia department, Colombia, in life. **a, c, f.** From the live collection at INSV-SR, Serpentario Nacional de Colombia; **b, d, e, g.** from the live collection at SUA, Serpentario de la Universidad de Antioquia. Photos by Jose Vieira.

Figure 13. Photographs of some specimens of *Bothriechis schlegelii* in life. **a.** Adult from Planes de San Rafael, Risaralda department, Colombia; **b–f.** Adults from Popayán, Cauca department, Colombia, photographed at the Serpentario de la Universidad del Cauca. Photos by Jose Vieira and Amanda Quezada.

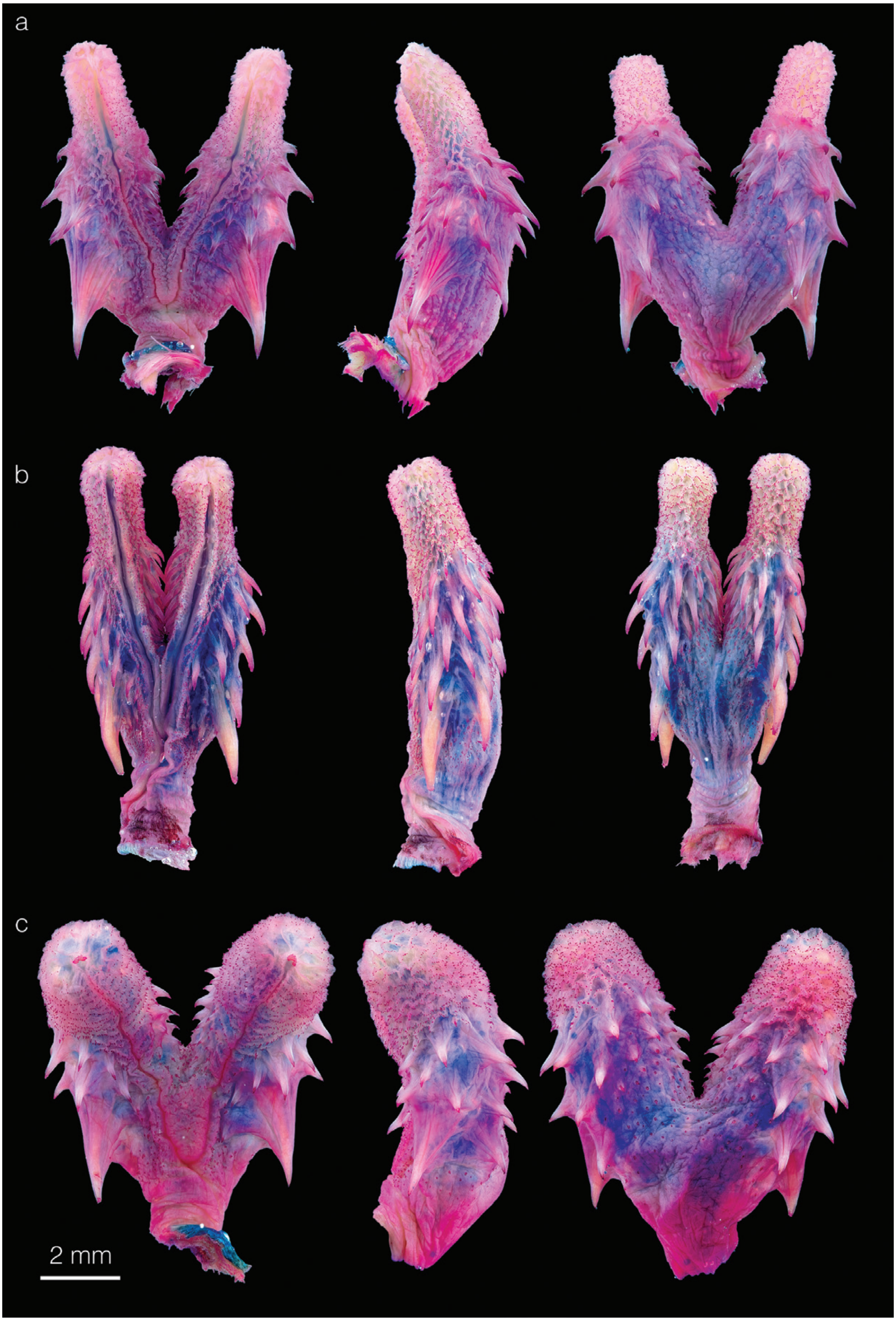

Figure 14. Hemipenial architecture of *Bothriechis schlegelii* in sulcate, lateral, and asulcate views. **a.** IAVH-R 6055 from Reserva Tesorito, Valle del Cauca department, Colombia; **b.** IAVH-R 6598 from Popayán, Cauca department, Colombia; **c.** IAVH-R 5465 from Fresno, Tolima department, Colombia. Photos by Duván Zambrano.

Description of holotype. A juvenile, SVL 183 mm, tail length 35.3 mm (19.3% SVL; tail-tip missing); head length 12.8 mm (7.0% SVL) from tip of snout to angle of jaw; head width 10.3 mm (80.5% head length) taken at broadest point; rostral broader than high (2.6 × 1.9 mm); nasal divided and not fused with first supralabial; loreal about 1/3 size of pit, in contact with preocular, postnasal, second canthal, 1 suprafoveal, 1 prefoveal, prelacunal, and supralacunal; prefoveals 3/4; subfoveals 2/2; postfoveals 0; prelacunal fused with second supralabial; sublacunals 2/2; supralacunal L-shaped and in contact with orbit; preoculars 1/1 (2/2 if supralacunal is considered a preocular); suboculars 1/1; postoculars 2/2; loreal pit large, directed anteriorly, located slightly below line drawn from center of eye to naris; supralabials 8/8 (including lacunolabial); infralabials 10/10, first pair meet posteriorly; mental broader than long (2.7 × 1.5 mm); 1 pair of chinshields; 3 pairs of gulars between chinshields and preventrals; preventrals 3; anterior internasals 3; canthals 2/2; 3/3 moderately triangular but low supraciliary scales; supraoculars oblong, 2.5× longer than wide; intersupraoculars 5; anterior dorsal head scales smooth; posterior head scales keeled; interrictals 25; dorsal scale rows 21/21/17; ventrals 150; cloacal plate entire; 50 undivided subcaudals; tail prehensile.

Natural history. *Bothriechis schlegelii* is an arboreal snake that inhabits evergreen montane forests, planted forests, coffee plantations, and urban/rural gardens. Based on our own field notes, iNaturalist records, and the literature (Rojas-Morales 2012; Galofre-Ruiz 2016), vipers of this species are found at night perched on low (less than 3 m above the ground) shrubby vegetation or at ground level during the day, either in ambush posture or moving. Most observations are of snakes active on rocks, leaf-litter, grass, and even crossing roads during daylight hours. Santiago Ayerbe (pers. comm. to AA) reports that in Cauca, individuals of this species prey upon lizards (anolines and *Pholidobolus*), rodents, and hummingbirds. Rojas-Morales (2012) reports that "though generally docile, individuals of *B. schlegelii* defend themselves with frontal displays and striking."

Venom. Galofre-Ruiz (2016) reports two cases of human envenomation by this snake species in departments Caldas and Tolima. The victims developed pain at the site of the wound, paresthesia, and edema, but recovered with the use of polyvalent antivenom. Sevilla-Sánchez et al. (2021) report that most accidents caused by this snake involve coffee plantation workers bitten on the hands or face during the harvesting work. In a series of 1,653 cases of envenomation in Cauca province between 2009 and 2018, 8.4% were caused by *B. schlegelii* (Sevilla-Sánchez et al. 2021), whereas 5.5% of 218 cases in departments Antioquia and Chocó between 1989 and 1990 were caused by a combination of *B. schlegelii* and *B. torvus* (Otero et al. 1992a). Of these, one envenomation was moderate, 11 were mild, and none involved systemic complications. The venom in this Antioquian-Chocoan mixed *Bothriechis* sample was found to be the least edematous, hemorrhagic, and lethal (LD50 estimated as 10.3 mg/kg) among a sample of eight Colombian venomous snakes (Otero et al. 1992b). Ayerbe et al. (1979) provided a report of a coffee plantation worker that died after being bitten on the tongue.

Distribution. *Bothriechis schlegelii* is endemic to Colombia. It is known from at least 161 localities (listed in Suppl. material 3) on Colombia's Cordillera Central and Occidental, from Cauca department in the south to Antioquia department in the north. The species occurs over an area of approximately 40,432 km^2 and has been recorded at elevations 1,034–2,597 m above sea level (Fig. 3). Approximately 8.8% of the predicted area of distribution of *B. schlegelii* overlaps with that of *B. torvus*, but we did not find evidence of sympatry. In Valle del Cauca department, we found the two species separated by an airline distance of about 10 km.

Etymology. The specific epithet *schlegelii* honors Hermann Schlegel (1804–1884), a renowned German ornithologist and herpetologist.

Conservation status. We consider *Bothriechis schlegelii* to be included in the Near Threatened category following IUCN Red List criteria (IUCN 2012) primarily because the species' extent of occurrence is estimated to be much larger than the 20,000 km^2 needed to meet B1 criteria for the Vulnerable category. However, although the species occurs in numerous protected areas (no less than 13; see Suppl. material 2), the majority of the species' montane forest habitat has been destroyed. Based on the distribution model presented in Fig. 3 in combination with maps of vegetation cover of Colombia (IDEAM 2014), we estimate that only ~23% of the species' forest habitat is still standing.

Bothriechis torvus (Posada Arango, 1889a)

Figs 15, 16

Thanatophis torvus Posada Arango, 1889a: 48. Type(s): not designated. Type locality: "lugares cálidos de Antioquia."

Thanatophis torvus Posada Arango, 1889b: 345. Type(s): not designated. Type locality: "regions chaudes d'Antioquia."

Teleuraspis birri Posada Arango, 1909: 231.

Referred specimens. All labeled *Bothriechis torvus* in Suppl. material 1.

Proposed standard English name. Birri Eyelash-Pitviper.

Spanish names. Víbora birrí, serpiente guinda, pestañona.

Diagnosis. *Bothriechis torvus* is diagnosed based on the following combination of characters: (1) 3 raised supraciliary scales, triangular, but rounded and not spine-like; (2) anterior dorsal head scales keeled; (3) gular scales much smaller than chinshields; (4) 6–18 interoculolabials; (5) 3–8 canthals, most of them with raised triangular projections; (6) loreal in contact with preocular in some populations; (7) yellow morph absent; (8) dorsal bands pink or red; (9) no opposing kidney shaped dorsal marks; (10) black speckles on dorsal scales absent; (11) black

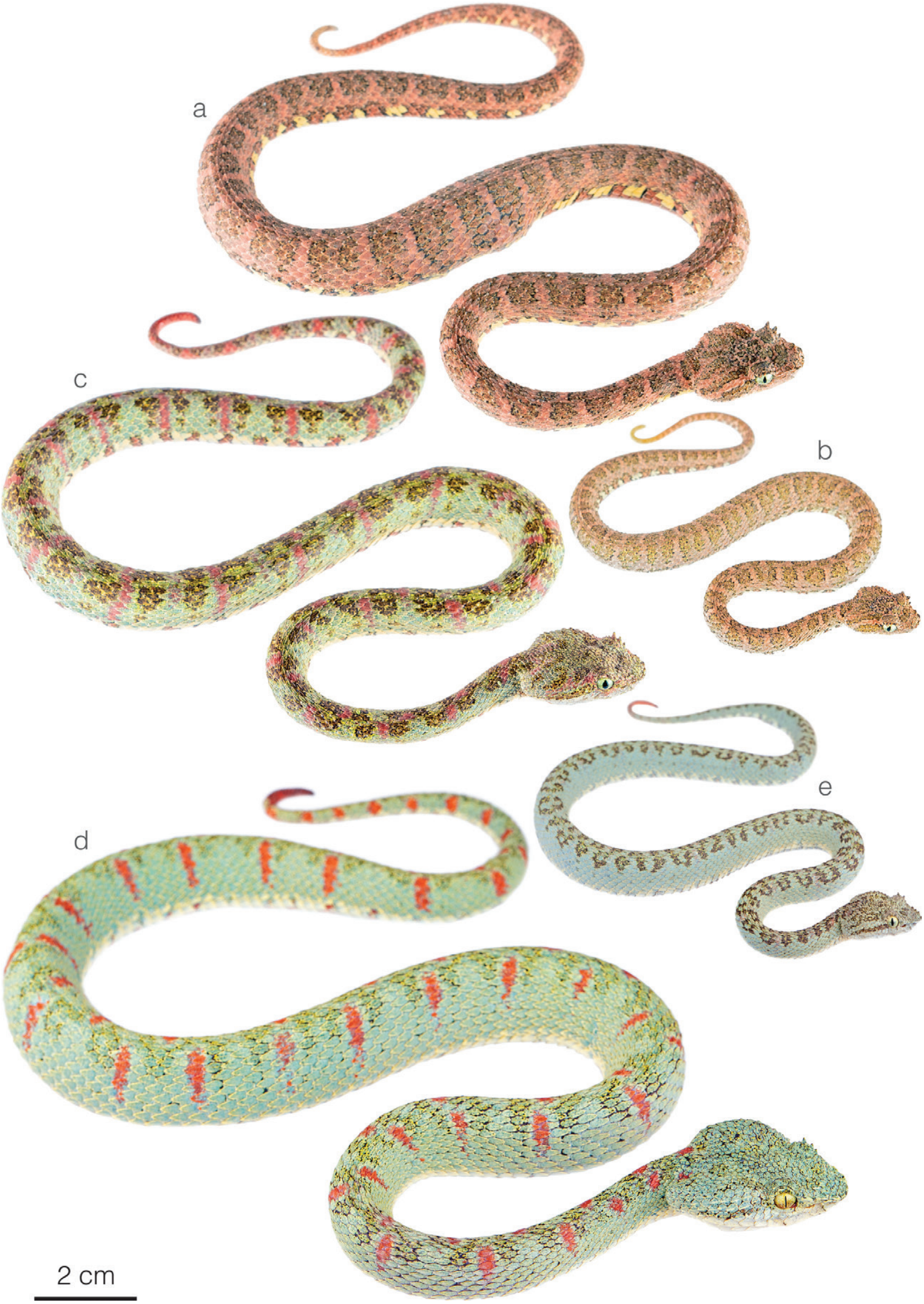

Figure 15. Photographs of some specimens of *Bothriechis torvus* in life. **a.** Adult and **b.** Juvenile (same individual) from Morromico Reserve, Chocó department, Colombia; **c.** TH 145 adult female from Morromico Reserve, Chocó department, Colombia; **d.** Adult and **e.** Juvenile from Urabá, Antioquia department, Colombia. Photos by Jose Vieira.

speckling on ventral surfaces absent or faint; (12) ventral surfaces entirely white in some individuals; (13) iris pale bluish-green or straw yellow with fine black speckling; (14) 21–23 dorsal scale rows at mid-body; (15) 148–159 ventrals in males, 141–153 in females; (16) maximum total length in males 378 mm, in females 657 mm.

Comparisons. *Bothriechis torvus* is compared to other species of the genus previously subsumed under *B. schlegelii sensu lato* (differences summarized in Table 2). It differs from all of them by having three raised and triangular supraciliary scales, anterior dorsal head scales keeled, a high number of canthals (3–8) and interoculolabials (6–18), thin pink dorsal bands, and entirely pale white belly. *Bothriechis torvus* differs from *B. schlegelii sensu stricto* by having anterior dorsal head scales keeled, 3–8 canthals (rather than 1–3), bands pink or red (rather than black or dark brown; Fig. 15), ventral surfaces entirely white, a higher number of ventrals in both males and females, smaller body size, and smaller basal hooks on the hemipenial body (Fig. 16). *Bothriechis torvus* is sympatric with *B. nigroadspersus* along the Serranía de Pirre, Darién province, Panama (lime green shade in Fig. 3). The two species differ in coloration (Figs 5, 15). *Bothriechis torvus* has dorsal bands (vs opposing kidney shaped marks), three broad and triangular enlarged supraciliary scales (vs two thin and spinelike), a lower number of ventral scales, and lateral surface of the hemipenial body and lobes more heavily packed with larger spines (Fig. 16). *Bothriechis torvus* resembles *B. rahimi* sp. nov., but this other species occurs south of the known distribution of *B. torvus* and is characterized by having a lower number of ventrals, enlarged spinelike supraciliary scales, faint dorsal banding, and presence of the yellow morph. *Bothriechis torvus* differs from *B. khwargi* sp. nov. by having pink bands (bands absent or dark vertebral blotches in *B. khwargi* sp. nov.).

Hemipenial morphology. (n = 1; Fig. 16) Everted and inflated, the organ is deeply bilobed, calyculate and non-capitate; hemipenial lobes cylindrical, but tapering towards the capitulum; in sulcate and asulcate views, lobe crotch ornamented with densely packed spines that become larger distally; sulcus spermaticus centrolineal, bifurcate and with walls strongly defined, bifurcation occurs below bilobation point and proximal to the base of the hemipenial body; sulcus spermaticus branch runs to lobe tips; distal half of each hemipenal lobe densely ornamented with small calyces with defined spinulate edges. In sulcate view, hemipenial body surface spinulate medially with enlarged and strongly calcified lateral basal hooks followed by rows of smaller obliquely arranged mesial spines that become gradually smaller towards each lobe's capitulum; each hemipenial lobe ornamented with mesial and lateral spines about 1/5 the size of the large basal hooks; the spines in each lobe are replaced distally by calyces with strongly spinulate edges. In lateral view, hemipenial body and lobes non-spiculate but with rows of spines; distal half of lobes strongly calyculate. In asulcate view, the center of hemipenial body is nude to barely spiculate; hemipenial lobes ornamented with 2 rows of smaller spines (about 1/3 the size of the lateral basal hooks) that decrease in size towards the lobe crotch.

Natural history. *Bothriechis torvus* is an arboreal snake that inhabits evergreen lowland/foothill forests. We have found vipers of this species active at night crawling on ferns, tree branches, and trunks from 1.8 to 5 m above the ground or during the day perched on arboreal vegetation up to 3.5 m above the ground. Specimens from Cerro Sapo and Pirre Mountain ranges, eastern Panama, were found in a cloud forest consisting predominantly of trees

Figure 16. Hemipenial architecture of *Bothriechis torvus* in sulcate, lateral, and asulcate views. ICN 0421 from Valle del Cauca department, Colombia. Photos by Duván Zambrano.

covered with moss and a large variety of understory and midstory bromeliads. In captivity, TH 145 (Fig. 15c) and another specimen from Morromico, Chocó department, Colombia, consumed lizards (*Anolis* and *Lepidodactylus*) and frogs (*Craugastor* and *Pristimantis*).

Venom. Prezotto-Neto et al. (2016) studied the composition of the venom of specimens of *Bothriechis torvus* from Vegachi, Antioquia, and found that its properties differ drastically from specimens of *B. nigroadspersus* from Costa Rica. When compared to the latter, the venom of *B. torvus* was found to be less edematous, hemorrhagic, and lethal (LD50 estimated as 9.24 mg/kg vs 5.60 mg/kg).

Distribution. *Bothriechis torvus* is known from at least 37 localities (listed in Suppl. material 3) along the Chocó and Río Magdalena valley regions of northern Colombia and extreme southeastern Panama. The species occurs over an area of approximately 61,466 km^2 and has been recorded at elevations 61–1,413 m above sea level (Fig. 3). The ENM suggests the distribution is not continuous across the valley of the Río Atrato (see Discussion).

Etymology. The specific epithet *torvus* is a Latin word meaning “fierce.” It refers to the protruding supraciliary scales, which according to Andrés Posada Arango, give this species an “*air féroce*.”

Conservation status. We consider *Bothriechis torvus* to be included in the Least Concern category following IUCN Red List criteria (IUCN 2012) because the species is distributed over a region that holds large areas of continuous unspoiled forest. Based on the species distribution model presented in Fig. 3 in combination with maps of vegetation cover of Colombia (IDEAM 2014) and Panama (CATHALAC 2011), we estimate that more than half (~59%) of the species’ forest habitat is still standing. Unfortunately, vast areas of the Chocó rainforest in northern Colombia and towards central Panama have already been converted to pastures (Myers et al. 2000). However, *B. torvus* occurs over an area greater than 50,000 km^2 and is presumably not declining fast enough to qualify for a threatened category.

Bothriechis khwargi sp. nov.

https://zoobank.org/8BFF5643-306F-435A-B074-668B41C43291

Figs 17, 18

Type material. ***Holotype*:** UIS-R 4294 (Figs 17, 18b), adult female collected by Elson Meneses-Pelayo on November 15, 2017 at Finca la Esperanza, vereda Sogamoso, municipio de Betulia, Santander department, Colombia (7.00600, -73.421608; 1195 m).

***Paratypes*:** All labeled *Bothriechis khwargi* sp. nov. in Suppl. material 1.

Proposed standard English name. Khwarg’s Eyelash-Pitviper.

Proposed standard Spanish name. Víbora de pestañas de Khwarg.

Diagnosis. *Bothriechis khwargi* sp. nov. is diagnosed based on the following combination of characters: (1) two triangular and moderately raised supraciliary scales; (2) anterior dorsal head scales keeled; (3) gular scales much smaller than chinshields; (4) 7–14 interoculolabials; (5) 3–4 canthals, some of which with raised triangular projections; (6) loreal in contact with preocular in ~1/3 of specimens; (7) yellow morph absent; (8) dorsal bands absent, faint, or restricted to top of dorsum in

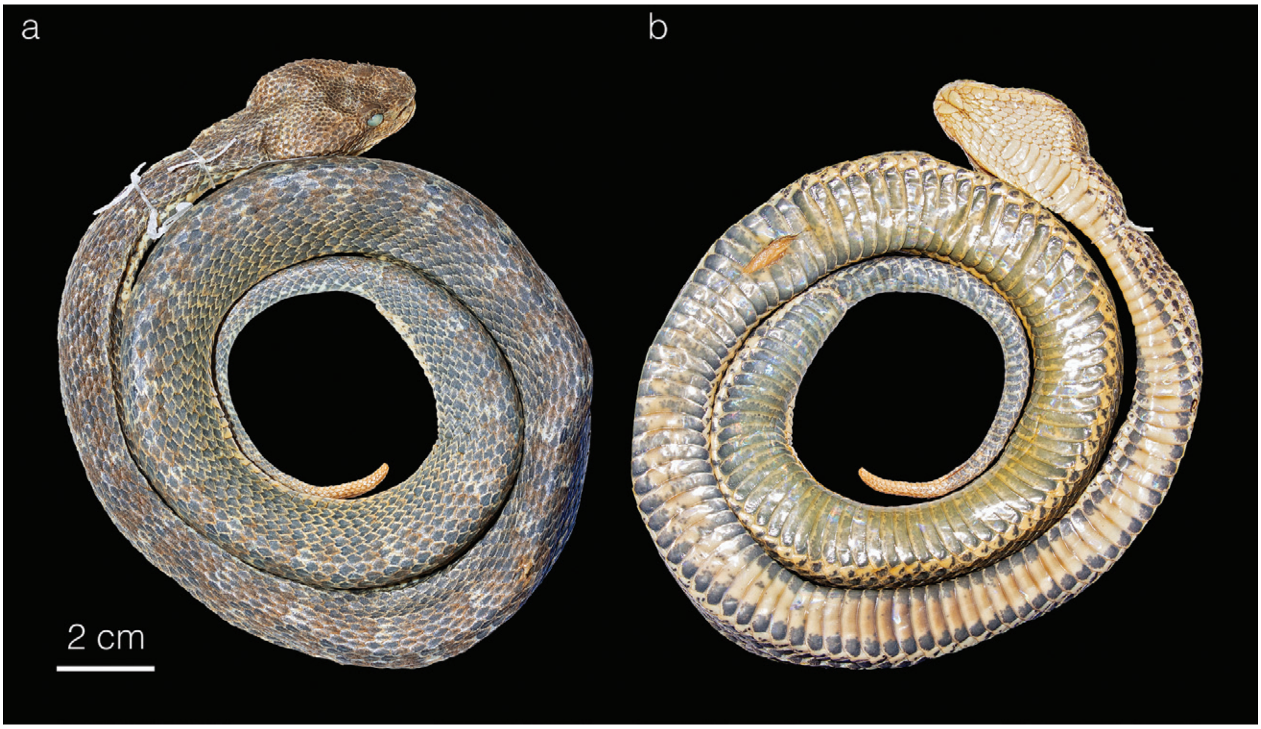

Figure 17. Female holotype of *Bothriechis khwargi* sp. nov. UIS-R 4294 in **a.** Dorsal and **b.** Ventral view. Photos by Johan Cordón-Rangel.

Figure 18. Photographs of some specimens of *Bothriechis khwargi* sp. nov. in life. **a.** UIS-R-4921 female from Quebrada La Boquerona, vereda La Putana, municipio de Betulia, Santander department **b.** UIS-R 4294 female holotype from Finca La Esperanza, vereda Sogamoso, municipio de Betulia, Santander department, Colombia. Photos by Jose Vieira and Elson Meneses Pelayo.

juveniles; (9) opposing kidney shaped dorsal marks absent; (10) black speckles on dorsal scales absent; (11) black speckling on ventral surfaces absent; (12) ventral surfaces entirely white in most individuals; (13) iris pale green or straw yellow; (14) 21–23 dorsal scale rows at mid-body; (15) 144–153 ventrals in males, 145–154 in females; (16) maximum total length in males 219 mm, in females 610 mm.

Comparisons. *Bothriechis khwargi* sp. nov. is compared to other species of the genus previously subsumed under *B. schlegelii sensu lato* (differences summarized in Table 2). It differs from all of them by having two triangular and moderately raised supraciliary scales, anterior head scales keeled, gular scales much smaller than chinshields, entirely pale white belly in most individuals, and dorsal bands faint or absent. *Bothriechis khwargi* sp. nov. resembles *B. torvus*, but this other species does not occur along the slopes of the Cordillera Oriental and is characterized by having thin pink bands on the dorsum (Fig. 15). *Bothriechis khwargi* sp. nov. occurs in sympatry with *B. klebbai* sp. nov. along the upper slopes of the Serranía De Los Yariguíes, but it differs from this other species by being smaller in body size, having an entirely white belly, pale greenish iris, keeled anterior dorsal head scales, gulars much smaller than chinshields, and by lacking black speckling on dorsal and ventral surfaces (Fig. 18).

Description of holotype. An adult female, SVL 511 mm, tail length 99 mm (19.4% SVL); head length 31.7 mm (6.2% SVL) from tip of snout to angle of jaw; head width 21.8 mm (68.8% head length) taken at broadest point; rostral broader than high (3.0 × 2.6 mm); nasal divided and not fused with first supralabial; loreal about same size of pit, contacting postnasal, 1 canthal, 1 prefoveal, supralacunal, lacunolabial, and preocular; prefoveals 4/4; subfoveals 3/3; postfoveals 0; prelacunal fused with second supralabial; sublacunals 1/1; supralacunal elongated and barely reaches orbit; preoculars 1/1 (2/2 if supralacunal is considered a preocular); suboculars 1/1; postoculars 1/1; loreal pit large, directed anteriorly, located slightly below line drawn from center of eye to naris; supralabials 9/8 (including lacunolabial); infralabials 11/12, first pair meet posteriorly; mental broader than long (4.0 × 1.4 mm); 1 pair of chinshields; 5 pairs of gulars between chinshields and preventrals; preventrals 2; anterior internasals 2; canthals 3/3; supraciliary scales low and granular with the exception of a pair of low, but triangular and pointed scales; supraoculars bean-shaped, 2.3× longer than wide; intersupraoculars 8; anterior dorsal head scales lightly keeled; posterior head scales strongly keeled; interrictals 25; dorsal scale rows 23/23/19; ventrals 154; cloacal plate entire; 54 undivided subcaudals; tail prehensile.

Natural history. *Bothriechis khwargi* sp. nov. is an arboreal snake that inhabits evergreen foothill forests. Vipers of this species have been seen at night or during the day, either at ground level or on low understory vegetation. EMP and CLBA found one individual at 1 m above the ground in a fern foraging at night.

Venom. We know of no snakebites caused by this species in Colombia.

Distribution. *Bothriechis khwargi* sp. nov. is known from at least 19 localities (listed in Suppl. material 3) along the western foothills of Colombia's Cordillera Oriental. The species occurs over an area of approximately 14,697 km^2 and has been recorded at elevations 167–1,800 m above sea level (Fig. 3). Approximately 29% of the predicted area of distribution of *B. khwargi* sp. nov. overlaps with that of *B. klebbai* sp. nov., and we found evidence of sympatry between the two species at elevations above 1,700 m on the western slopes of the Serranía de los Yariguíes. UIS-R 4447 is a *B. klebbai* sp. nov. and was found just 3.7 km airline distance from a photographic record of an *B. khwargi* sp. nov.

Etymology. This species is named in honor of Dr. Juewon Khwarg, in recognition of his support of the discovery and protection of new species of vipers in the Andes of Colombia. Khwarg is a conservationist with a special love for the lesser-known reptiles of the unexplored regions of the tropics.

Conservation status. We consider *Bothriechis khwargi* sp. nov. to be included in the Vulnerable category following the IUCN criteria B1a, b (i, iii, iv) (IUCN 2012), because the species' extent of occurrence is estimated to be less than 20,000 km^2 (Fig. 3) and its habitat is severely fragmented and declining in extent and quality due to deforestation. Although *B. khwargi* sp. nov. occurs in one national park (Serranía de los Yariguíes) and a privately protected area (Reserva Natural Reinita Cielo Azul), most localities where the species has been recorded (Suppl. material 3) are in heavily human-modified areas. Based on the species distribution model presented in Fig. 3 in combination with maps of vegetation cover of Colombia (IDEAM 2014), we estimate that nearly 79% of the forest cover throughout the species' potential distribution area has been destroyed, mostly due to the expansion of the agricultural frontier.

Bothriechis klebbai sp. nov.

https://zoobank.org/20308073-EA2E-474F-951C-C2ABBFB7562F

Figs 19–21

Type material. ***Holotype*:** ICN 2786 (Fig. 19), adult male collected by Pedro M. Ruiz-Carranza on September 21, 1979 at Vereda Virolín, municipio de Charalá, Santander department, Colombia (6.10720, -73.19781; 1819 m).

***Paratypes*:** All labeled *Bothriechis klebbai* sp. nov. in Suppl. material 1.

Proposed standard English name. Klebba's Eyelash-Pitviper.

Proposed standard Spanish name. Víbora de pestañas de Klebba.

Diagnosis. *Bothriechis klebbai* sp. nov. is diagnosed based on the following combination of characters: (1) supraciliary scales low and granular or two raised, but not sharp; (2) anterior dorsal head scales smooth; (3) gular scales similar in size to chinshields; (4) 7–11 interoculolabials; (5) 2–4 canthals which may be flat or slightly raised forming a ridge along the canthus; (6) loreal in contact with preocular in ~1/3 of specimens; (7) yellow morph absent; (8) dorsal bands black; (9) opposing kidney shaped dorsal marks absent; (10) black speckles on dorsal scales present; (11) black speckling on ventral surfaces present, prominent; (12) ventral surfaces never entirely white; (13) iris rich dark reddish brown;

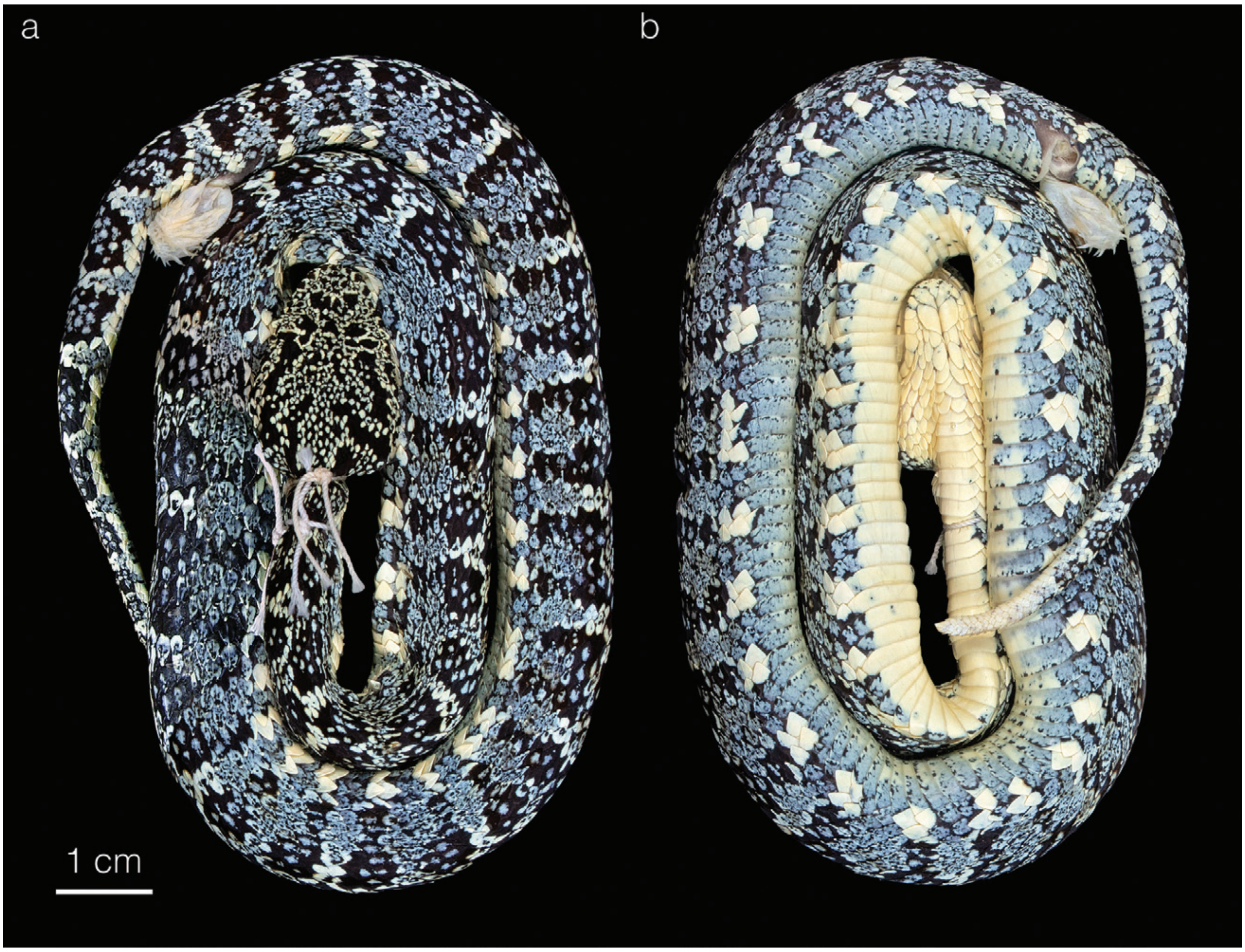

Figure 19. Adult male holotype of *Bothriechis klebbai* sp. nov. ICN 2786 in **a.** Dorsal and **b.** Ventral view. Photos by Duván Zambrano.

(14) 21–23 dorsal scale rows at mid-body; (15) 144–151 ventrals in males, 141–151 in females; (16) maximum total length in males 671 mm, in females 874 mm.

Comparisons. *Bothriechis klebbai* sp. nov. is compared to other species of the genus previously subsumed under *B. schlegelii sensu lato* (differences summarized in Table 2). It differs from most of them by having supraciliaries low and granular or two raised but not sharp, a low number of canthals (2–4), gular scales similar in size to chinshields, and dorsal and ventral surfaces heavily speckled with black pigment. *Bothriechis klebbai* sp. nov. resembles *B. rasikusumorum* sp. nov., but this other species occurs on the southern portion of Colombia's Cordillera Oriental and is characterized by having more than 151 ventrals in females, loreal not in contact with preocular, and by lacking paired oblong blotches on each side of the dorsum (a characteristic variably present in *B. klebbai* sp. nov.). Furthermore, *Bothriechis klebbai* sp. nov. lacks enlarged basal hooks on the hemipenial body (present in *B. rasikusumorum* sp. nov.). *Bothriechis klebbai* sp. nov. also resembles *B. schlegelii*, but this other species does not occur on the Cordillera Oriental and is characterized by having two triangular and moderately raised supraciliary scales, a different iris color (pale orange, light green, or golden yellow usually without speckles vs rich dark reddish brown), and by lacking black speckles on the dorsal and ventral scales in most populations (Figs 19, 20). *Bothriechis klebbai* sp. nov. occurs in sympatry with *B. khwargi* sp. nov. along the upper slopes of the Serranía De Los Yariguíes, but it differs from this other species by being larger in body size, having a dark green belly, smooth anterior dorsal head scales, gulars similar in size to or large than chinshields, and by having prominent black speckling on dorsal and ventral surfaces.

Hemipenial morphology. (n = 3; Fig. 21) Everted and inflated, the organ is deeply bilobed, calyculate and noncapitate; hemipenial lobes cylindrical or spindle shaped; in sulcate and asulcate views, lobe crotch ornamented with densely packed small spines that become larger distally; sulcus spermaticus centrolineal, bifurcate and with walls weakly defined (strongly in ICN 2786), bifurcation occurs below bilobation point and proximal to the base of the hemipenial body; sulcus spermaticus branch runs to lobe tips; distal half of each hemipenal lobe densely ornamented with small calyces with strongly defined spinulate edges. In sulcate view, hemipenial body surface spinulate medially; calcified spines at the base of the hemipenial body only slightly larger than adjacent rows of smaller obliquely arranged mesial spines that become gradually smaller towards each lobe's capitulum

Figure 20. Photographs of some specimens of *Bothriechis klebbai* sp. nov. in life from Finca la Arrinconada, vereda Esparta, municipio de Santa Bárbara, Santander department, Colombia. **a.** UIS-R 4139 adult male; **b.** INSV-SR-00098 adult of undetermined sex; and **c.** UIS-R-4296 juvenile male. Photos by Elson Meneses Pelayo and Jose Vieira.

Figure 21. Hemipenial architecture of *Bothriechis klebbai* sp. nov. in sulcate, lateral, and asulcate views. **a.** ICN 2786 and **b.** ICN 2855 from Virolín, municipio de Charalá, Santander department, Colombia; **c.** UIS-R 4803 from vereda Naranjal, municipio de Coromoro, Santander department, Colombia. Photos by Duván Zambrano and Elson Meneses Pelayo.

(=no obvious basal hooks); each hemipenial lobe ornamented with mesial and lateral spines homogenous in size; the spines in each lobe are replaced distally by calyces with strongly spinulate edges. In lateral view, hemipenial body and lobes with rows of spines, but otherwise non-spiculate; distal half strongly calyculate. In asulcate view, the center of hemipenial body is nude to barely spiculate; hemipenial lobes ornamented with 4 rows of spines that decrease in size towards the lobe crotch.

Description of holotype. An adult male, SVL 349 mm, tail length 75 mm (21.5% SVL); head length 27.8 mm (8.0% SVL) from tip of snout to angle of jaw; head width 20.1 mm (72% head length) taken at broadest point; rostral broader than high (2.7 × 1.8 mm); nasal divided and not fused with first supralabial; loreal about 1/2 size of pit, contacting postnasal, 2 canthals, lacunolabial, supralacunal, and preocular; prefoveals 4/4; subfoveals 3/4; postfoveals 0; prelacunal fused with second supralabial; sublacunals 2/2 (one of which is small and granular); supralacunal elongated and in contact with orbit; preoculars 1/1 (2/2 if supralacunal is considered a preocular); suboculars 1/1; postoculars 1/1; loreal pit large, directed anteriorly, located slightly below line drawn from center of eye to naris; supralabials 8/8 (including lacunolabial); infralabials 11/11, first meet posteriorly; mental broader than long (3.3 × 3.0 mm); 1 pair of chinshields; 5 pairs of gulars between chinshields and preventrals; preventral 1; anterior internasals 2; canthals 3/3; supraciliary scales low and granular with the exception of one raised and triangular scale; supraoculars oblong, 1.8× longer than wide; intersupraoculars 6; anterior dorsal head scales smooth; posterior head scales barely keeled; interrictals 27; dorsal scale rows 21/21/18; ventrals 148; cloacal plate entire; 56 undivided subcaudals; tail prehensile.

Natural history. *Bothriechis klebbai* sp. nov. is an arboreal snake that inhabits montane cloud forests. Vipers of this species have been seen at night or during the day, either at ground level or on low understory vegetation. In captivity, this species is known to hybridize with *B. khwargi* sp. nov. INSV-SR-1015 is believed to be a hybrid between the two species. We (JV and EMP) have seen this species preying upon *Anolis heterodermus*.

Venom. We know of no snakebites caused by this species in Colombia nor any studies on the venom that could be assigned to this species.

Distribution. *Bothriechis klebbai* sp. nov. is known from at least 23 localities (listed in Suppl. material 3) along the western slopes of Colombia's Cordillera Oriental. The species occurs over an estimated 20,061 km^2 and has been recorded at elevations 1,418–2,380 m above sea level (Fig. 3). Approximately 21% of the predicted area of distribution of *B. klebbai* sp. nov. overlaps with that of *B. khwargi* sp. nov.

Etymology. Named after Casey Klebba, whose dedication to the preservation of tropical biodiversity is exemplary. As a co-founder of Minifund.org, alongside Carly Jones, he has been one of the most invaluable supporters of AA's field expeditions to remote areas of Ecuador and Colombia, resulting in the discovery of many new species to science.

Conservation status. We consider *Bothriechis klebbai* sp. nov. to be included in the Near Threatened category following IUCN Red List criteria (IUCN 2012) primarily because the species' extent of occurrence is estimated to be greater than the 20,000 km^2 needed to meet B1 criteria for the Vulnerable category. However, although the species occurs in at least two national parks (Serranía de los Yariguíes and Guanentá-alto Río Fonce), the majority of the species' montane forest habitat has been destroyed. Based on the distribution model presented in Fig. 3 in combination with maps of vegetation cover of Colombia (IDEAM 2014), we estimate that only ~14% of the species' forest habitat is still standing.

Bothriechis rasikusumorum sp. nov.

https://zoobank.org/AF46F519-7700-46C7-9E97-B2E30B56D77D

Figs 22–24

Type material. ***Holotype*:** CZUT-R 349 (Fig. 22), adult male collected by Hugo Hernández at Vereda El Regenero, Huila department, Colombia (1.82023, -75.99867; 1616 m).

***Paratypes*:** All labeled *Bothriechis rasikusumorum* sp. nov. in Suppl. material 1.

Proposed standard English name. Shah's Eyelash-Pitviper.

Proposed standard Spanish name. Víbora de pestañas de los Shah.

Local Spanish name. Culebra de tiro.

Diagnosis. *Bothriechis rasikusumorum* sp. nov. is diagnosed based on the following combination of characters: (1) supraciliary scales low and granular, or two pointy but not raised; (2) anterior dorsal head scales smooth; (3) gular scales similar in size to chinshields; (4) 5–10 interoculolabials; (5) 2–3 canthals which may be flat or slightly raised forming a ridge along the canthus; (6) loreal not in contact with preocular; (7) yellow morph absent; (8) dorsal bands dark brown or black; (9) opposing kidney shaped dorsal marks absent; (10) black speckles on dorsal scales present; (11) black speckling on ventral surfaces prominent posteriorly; (12) ventral surfaces never entirely white; (13) iris light green, yellow, or reddish brown with black reticulations; (14) 21–23 dorsal scale rows at mid-body; (15) 144–152 ventrals in males, 152–153 in females; (16) maximum total length in males 650 mm, in females 799 mm.

Comparisons. *Bothriechis rasikusumorum* sp. nov. is compared to other species of the genus previously subsumed under *B. schlegelii sensu lato* (differences summarized in Table 2). It differs from most of them by having supraciliaries not raised, a low number of canthals (2–3), a low number of ventral scales, and dorsal and ventral surfaces heavily speckled with black pigment (Figs 22, 23a). *Bothriechis rasikusumorum* sp. nov. resembles *B. klebbai* sp. nov., but this other species occurs on the

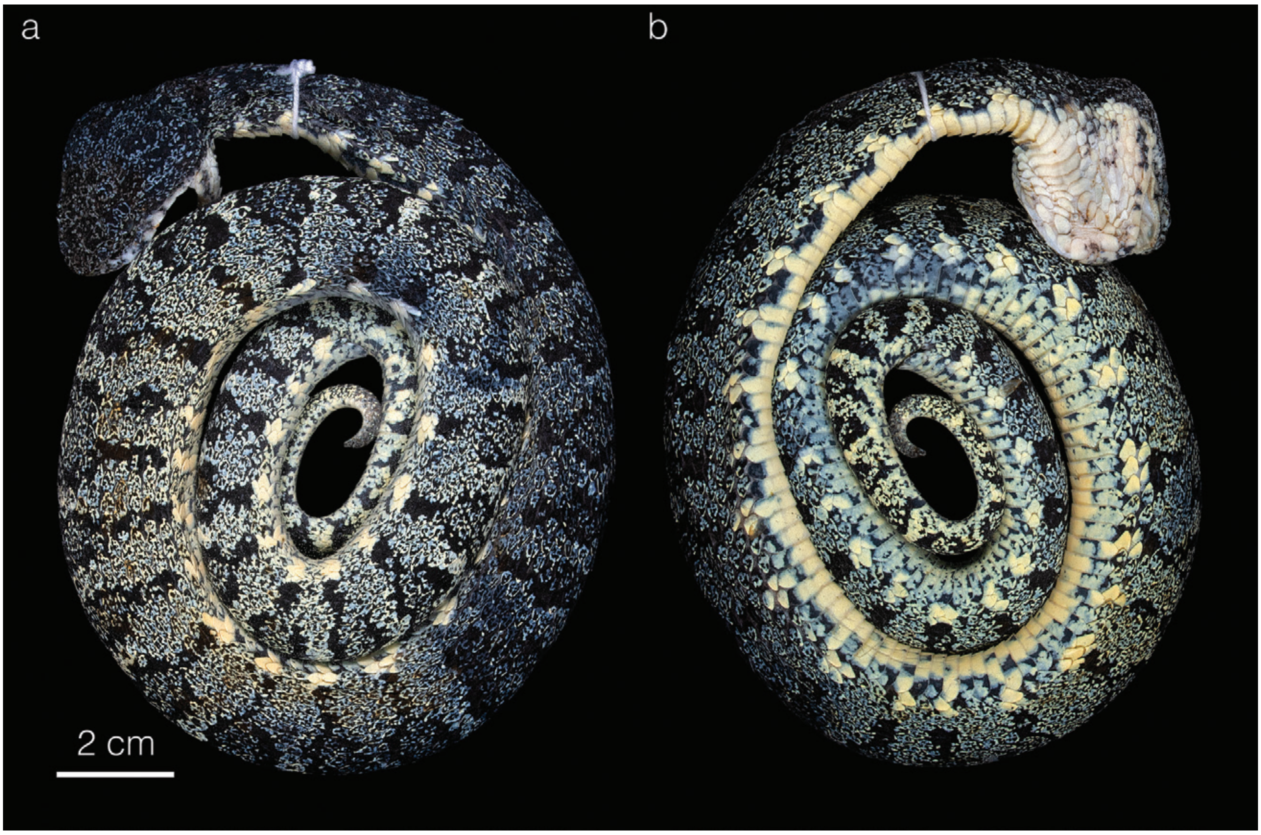

Figure 22. Adult male holotype of *Bothriechis rasikusumorum* sp. nov. CZUT-R 349 in **a.** Dorsal and **b.** Ventral view. Photos by Duván Zambrano.

northern portion of Colombia's Cordillera Oriental and is characterized by having less than 152 ventrals in females, a rich reddish-brown iris, a series of paired oblong blotches on each side of the dorsum, and no basal hooks on the hemipenial body (Fig. 21). *Bothriechis rasikusumorum* sp. nov. is also similar to *B. schlegelii*, but this other species occurs northwest of the known distribution of *B. rasikusumorum* sp. nov. and is characterized by lacking black speckles on the dorsal and ventral scales in most populations (Figs 12, 13), having two triangular and moderately raised supraciliary scales, and basal hooks that are 3–10X the size of adjacent spines at the base of the hemipenial body (Fig. 14).

Hemipenial morphology. (n = 1; Fig. 24) Everted and inflated, the organ is deeply bilobed, calyculate and non-capitate; hemipenial lobes cylindrical, narrower towards the capitulum; in sulcate and asulcate views, lobe crotch ornamented with densely packed small spines that become larger distally; sulcus spermaticus centrolineal, bifurcate and with walls strongly defined, bifurcation occurs below bilobation point and proximal to the base of the hemipenial body; sulcus spermaticus branch runs to lobe tips; distal half of each hemipenal lobe densely ornamented with small calyces with strongly defined spinulate edges. In sulcate view, hemipenial body surface with small spinules medially, but with a pair of enlarged and strongly calcified lateral spines (basal hooks) followed by 4–5 rows of smaller obliquely-arranged mesial spines that become gradually smaller towards each lobe's capitulum; each hemipenial lobe ornamented with mesial and lateral spines about half the size of the basal hook; the spines in each lobe are replaced distally by calyces with strongly spinulate edges. In lateral view, hemipenial body and lobes with rows of spines, but otherwise non-spiculate; distal half of lobes strongly calyculate. In asulcate view, the center of hemipenial body covered with scattered spinules; hemipenial lobes ornamented with 3 rows of spines.

Description of holotype. An adult male, SVL 536 mm, tail length 114 mm (21.3% SVL); head length 30.2 mm (5.6% SVL) from tip of snout to angle of jaw; head width 24.2 mm (80.1% head length) taken at broadest point; rostral broader than high (4.2 × 2.3 mm); nasal not entirely divided; incomplete suture between nasal and first supralabial; loreal about 1/3 size of pit, contacting postnasal, 1 canthal, 1 suprafoveal, 2 prefoveals, lacunolabial, and preocular; prefoveals 5/4; subfoveals 2/2; postfoveals 0; prelacunal fused with second supralabial on the right side; sublacunals 2/2 (one of which is small and granular); supralacunal elongated and in contact with orbit; preoculars 1/1 (2/2 if supralacunal is considered a preocular); suboculars 1/1; postoculars 1/1; loreal pit large, directed anteriorly, located slightly below line drawn from center of eye to naris; supralabials 8/8 (including lacunolabial); infralabials 11/11, first pair barely fail to meet posteriorly due to stretched skin; mental broader than long (4.2 × 2.3 mm); 1 pair of chinshields; 5 pairs of gulars between chinshields and preventrals; preventrals 1; anterior internasals 2; canthals 3/2; supraciliary scales

Figure 23. Photographs of living specimens of *Bothriechis rasikusumorum* sp. nov. from Huila department, Colombia. **a.** La Umbría; **b.** SUA 4659 Finca Miraflores. Photos by Jose Vieira and Duván Zambrano.

low and granular with the exception of a pair of raised and triangular scales; supraoculars B-shaped, 2.5× longer than wide; intersupraoculars 5; anterior dorsal head scales smooth; posterior head scales barely keeled; interrictals 27; dorsal scale rows 23/23/19; ventrals 147; cloacal plate entire; 56 undivided subcaudals; tail prehensile.

Natural history. *Bothriechis rasikusumorum* sp. nov. is an arboreal snake that inhabits montane cloud forests and coffee plantations (Erik Gaitan, pers. comm.). Vipers of this species have been seen at night or during the day, either on leaf-litter or on low understory vegetation less than 2 m above the ground.

Venom. We know of no snakebites caused by this species in Colombia nor any studies on the venom that could be assigned to this species.

Distribution. *Bothriechis rasikusumorum* sp. nov. is known from at least 26 localities (listed in Suppl. material 3) along the upper watershed of the Río Magdalena in Huila department, Colombia. The species occurs over an area of approximately 6,578 km^2 along the eastern slope of Cordillera Central and on both slopes of Cordillera Oriental at elevations 1,298–2,180 m above sea level (Fig. 3).

Etymology. The specific epithet rasikusumorum is a patronym honoring Rasik Shah (1939–2022), Kusum Shah (1942–present), and their grandson Oscar Shah. The Shah family helped fund the expedition that led to the discovery of this new species.

Conservation status. We consider *Bothriechis rasikusumorum* sp. nov. to be included in the Vulnerable category following the IUCN criteria B1a, b (i, iii, iv) (IUCN 2012), because the species' extent of occurrence is estimated to be much less than 20,000 km^2 (Fig. 3) and its habitat is severely fragmented and declining in extent and quality due to deforestation. Although *B. rasikusumorum* sp. nov. occurs in three protected areas (Parque Nacional Cueva de los Guácharos, Bosque Protector Pompeya, and Reverva Chiyurco), most localities where the species has been recorded (Suppl. material 1) are in forest patches surrounded by a

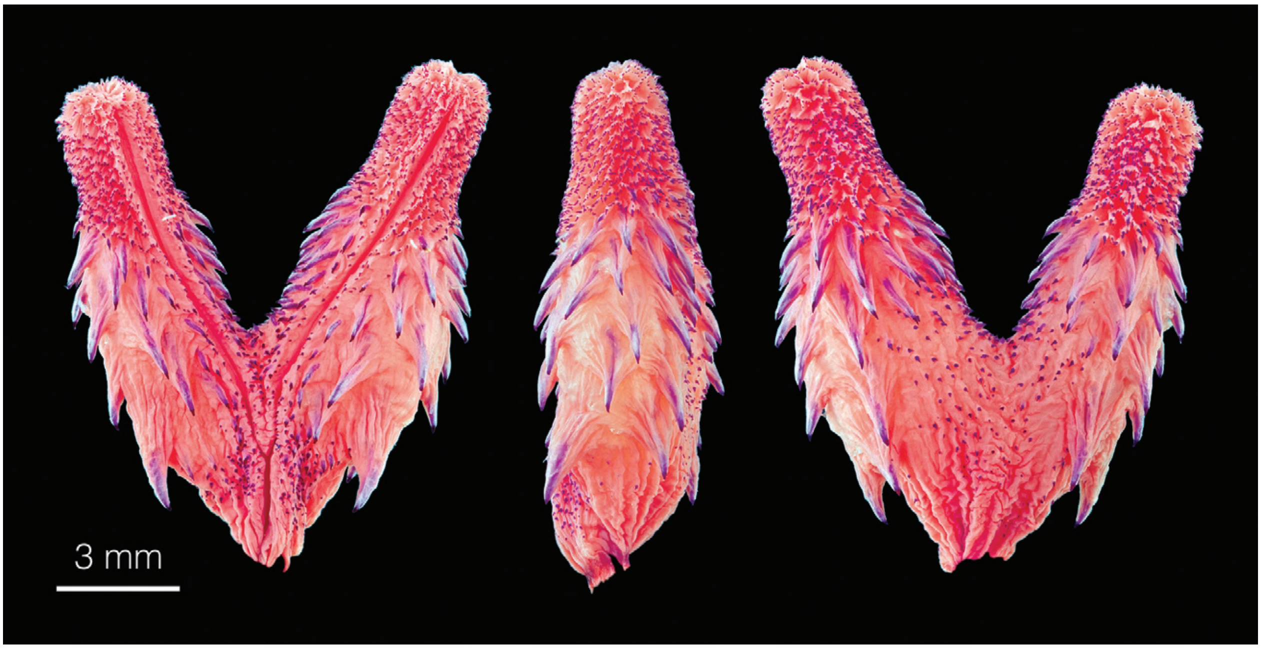

Figure 24. Hemipenial architecture of *Bothriechis rasikusumorum* sp. nov. in sulcate, lateral, and asulcate views. ICN 11164 from Parque Nacional Cueva de Los Guácharos, Huila department, Colombia. Photos by Duván Zambrano.

matrix of pastures and plantations. Based on the species distribution model presented in Fig. 3 in combination with maps of vegetation cover of Colombia (IDEAM 2014), we estimate that nearly 71% of the forest cover throughout the species' potential distribution area has been destroyed, mostly due to the expansion of the agricultural frontier. One of us (JV) interviewed coffee plantation workers in Huila, who report that they routinely kill these vipers when found. During a 10-night expedition to Huila department, a group of three field biologists led by JV failed to find a single specimen in four of the localities where the species has been reported nearby the city Pitalito.

Bothriechis rahimi sp. nov.

https://zoobank.org/0178468C-8CE0-433A-8DEB-356E26FFA3BD

Figs 25, 26

Type material. ***Holotype***: ZSFQ 5055 (18), adult female collected on January 1, 2017 at Tundaloma Lodge, Esmeraldas province, Ecuador (1.18236, -78.7525; 74 m).

Paratypes: All labeled *Bothriechis rahimi* sp. nov. in Suppl. material 1.

Proposed standard English name. Rahim's Eyelash-Pitviper.

Proposed standard Spanish name. Víbora de pestañas de Rahim.

Diagnosis. *Bothriechis rahimi* sp. nov. is diagnosed based on the following combination of characters: (1) two or three raised triangular or spinelike supraciliary scales; (2) anterior dorsal head scales keeled; (3) gular scales much smaller than chinshields; (4) 8–13 interoculolabials; (5) 4–5 canthals, some raised slightly forming a ridge along the canthus; (6) loreal not in contact with preocular; (7) yellow morph present; (8) dorsal bands pink and faint; (9) opposing kidney shaped dorsal marks absent; (10) black speckles on dorsal scales absent; (11) black speckling on ventral surfaces absent; (12) ventral surfaces entirely white in some individuals; (13) iris pale straw yellow with fine black speckles; (14) 21–23 dorsal scale rows at mid-body; (15) 137–145 ventrals in males, 146–151 in females; (16) maximum total length in males 336 mm, in females 494 mm.

Comparisons. *Bothriechis rahimi* sp. nov. is compared to other species of the genus previously subsumed under *B. schlegelii sensu lato* (differences summarized in Table 2). It differs from all of them by having supraciliaries raised, anterior dorsal head scales keeled, 8–13 interoculolabials, faint pink dorsal bands, and low number of ventrals in both males and females. It is one of the two members of the SA *Bothriechis* group where the golden morph has been recorded (Fig. 26c), albeit it is not identical to the *B. nigroadspersus* golden morph (Fig. 5a–c), as it has faint pink bands. *Bothriechis rahimi* sp. nov. resembles *B. torvus* and *B. nitidus*. From the former, the new species differs by being smaller in body size, having less than 148 ventrals in males, supraciliaries spinelike (vs broad and triangular), and presence of yellow morph and red morph. From *B. nitidus*, the new species differs by being smaller in body size, having raised supraciliaries (vs low), a higher number of canthals (4–5 vs 2–3) and interoculolabials (8–13 vs 3–8), scales on the anterior dorsal surface of the head keeled (vs smooth), presence of a yellow morph, and lack of a green morph (all *B. nitidus* examined are green; Suppl. material 1).

Description of holotype. An adult female, SVL 374 mm, tail length 71 mm (18.9% SVL); head length 26.8 mm (7.2% SVL) from tip of snout to angle of jaw; head width 20.5 mm (76.5% head length) taken at broadest point; rostral broader than high (3.2 × 2.5 mm); nasal

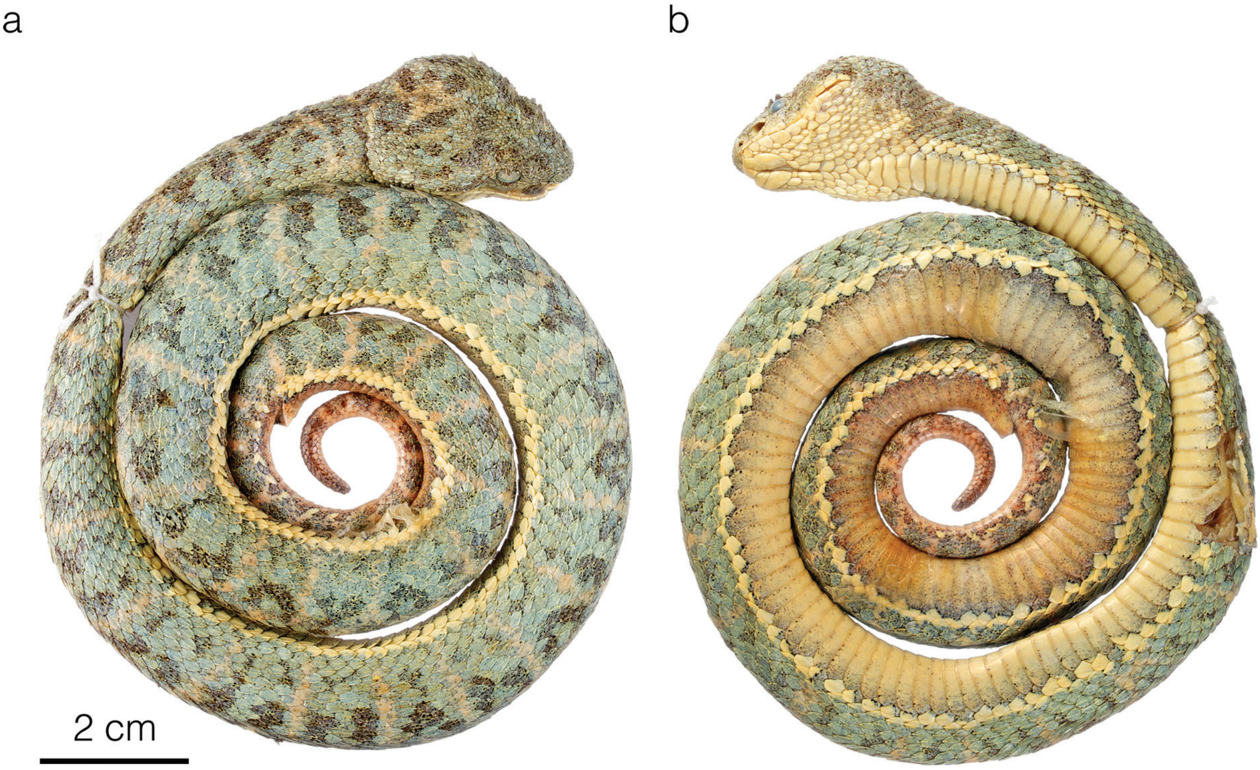

Figure 25. Adult female holotype of *Bothriechis rahimi* sp. nov. ZSFQ 5055 in **a.** Dorsal and **b.** Ventral view. Photos by Alejandro Arteaga.

divided and not fused with first supralabial; loreal about 1/5 size of pit, contacting postnasal, canthals, prelacunal, and supralacunal; prefoveals 4/2; subfoveals 1/0; postfoveals 0; prelacunal fused with second supralabial; sublacunals 2/2; supralacunal elongated and in contact with orbit; preoculars 1/1 (2/2 if supralacunal is considered a preocular); suboculars 1/1; postoculars 2/3; loreal pit large, directed anteriorly, located slightly below line drawn from center of eye to naris; supralabials 9/8 (including lacunolabial); infralabials 12/12, first pair meet posteriorly; mental broader than long (3.2 × 2.8 mm); 1 pair of chinshields; 6 pairs of gulars between chinshields and preventrals; preventrals 1; anterior internasals 3; canthals 4/5; 2/2 triangular and raised supraciliary scales; supraoculars oblong with irregular borders, 1.8× longer than wide; intersupraoculars 6; anterior dorsal head scales keeled; posterior head scales keeled; interrictals 30; dorsal scale rows 27/23/19; ventrals 151; cloacal plate entire; 51 undivided subcaudals; tail prehensile.

Natural history. *Bothriechis rahimi* sp. nov. is an arboreal snake that inhabits evergreen lowland forests usually within 25 km from the coastline. We have found vipers of this species active at night perched on stems, branches, and tangled vegetation 0.4–8 m above the ground. One individual was perched in hunting posture on a heliconia stem facing the flowers of the plant, presumably on the wait for hummingbirds. One individual was found feeding on a treefrog (*Smilisca phaeota*) and another one regurgitated an unidentified *Pristimantis* species, probably *P. esmeraldas* (field observations by Javier Aznar and JHV).

Venom. We know of only one snakebite cause by this species in Ecuador. A photographer was bitten on the right index finger by MZUTI 3332. The victim experienced intense local pain and swelling but recovered shortly after receiving three doses of polyvalent antivenom (elaborated by Instituto Clodomiro Picado).

Distribution. *Bothriechis rahimi* sp. nov. is known from at least 12 localities (listed in Suppl. material 3) along the mouths of the rivers Esmeraldas, Santiago, Cayapas, and Mira in extreme northwestern Ecuador and southwestern Colombia. The species occurs over an area of approximately 6,003 km^2 of the Chocó biome and has been recorded at elevations of 11–200 m above sea level (Fig. 3). Approximately 7.6% of the predicted area of distribution of *B. rahimi* sp. nov. overlaps with that of *B. nitidus*, but we did not find evidence of sympatry between the two species.

Etymology. The specific epithet *rahimi* is a patronym honoring Prince Rahim Aga Khan, a firm environmentalist who has inspired many with his work focused on tackling climate change, primarily in countries where the Aga Khan Development Network is active, alongside his brother Prince Hussain Aga Khan.

Conservation status. We consider *Bothriechis rahimi* sp. nov. to be included in the Vulnerable category following the IUCN criteria B1a, b (i, iii, iv) (IUCN 2012), because the species' extent of occurrence is estimated to be much less than 20,000 km^2 (Fig. 3) and its habitat is severely fragmented and declining in extent and quality due to deforestation. Although *B. rahimi* sp. nov. occurs in two protected areas (Reserva Awá and

Figure 26. Photographs of living specimens of *Bothriechis rahimi* sp. nov. from Tundaloma Lodge, Esmeraldas province, Ecuador. **a.** Adult female; **b.** MZUTI 3325 adult male; and **c.** ZSFQ 5053 adult female. Photos by Lucas Bustamante, Alejandro Arteaga, and Frank Pichardo.

Refugio de Vida Silvestre La Chiquita), the remaining ten localities where the species has been recorded (Suppl. material 3) are in heavily human-modified areas. Based on the species distribution model presented in Fig. 3 in combination with maps of vegetation cover of Colombia (IDEAM 2014) and Ecuador (MAE 2012), we estimate that nearly 49% of the forest cover throughout the species' potential distribution area has been destroyed, mostly due to the expansion of the agricultural frontier.

Bothriechis nitidus (Günther, 1859)

Figs 7, 27, 28

Lachesis nitidus Günther, 1859: 414. Holotype BMNH 1946.1.17.73 (Fig. 7), an adult of undetermined sex from "Western Andes of Ecuador".

Bothrops boussingaultii Jan, 1863: 127. Holotype MNHN 0.227, an adult (male?) from the trail between Latacunga and Guayaquil, Cotopaxi province, Ecuador.

Teleuraspis nitida Cope, 1871: 206.

Referred specimens. All labeled *Bothriechis nitidus* in Suppl. material 1.

Proposed standard English name. Ecuadorian Eyelash-Pitviper.

Spanish names. Víbora de pestañas ecuatoriana, cabeza de candado, equis voladora (Manabí), papagayo (Santo Domingo de los Tsáchilas), zampiña (cloud forest populations).

Diagnosis. *Bothriechis nitidus* is diagnosed based on the following combination of characters: (1) supraciliary scales low and granular or two pointed but not raised; (2) anterior dorsal head scales smooth or barely keeled; (3) gular scales usually much smaller than chinshields or similar in size to chienshields in some cloud forest populations; (4) 3–8 interoculolabials; (5) 2–3 canthals, raised slightly and forming a ridge along the canthus; (6) loreal not in contact with preocular; (7) yellow morph absent; (8) dorsal bands dark reddish brown, red, or pink; (9) no opposing kidney shaped dorsal marks; (10) black speckles on dorsal scales usually absent, but present in some cloud forest populations; (11) black speckling on ventral surfaces usually absent, but present in some cloud forest populations; (12) ventral surfaces entirely white in some individuals; (13) iris pale golden yellow to light green with or without black speckles or spots; (14) 19–23 dorsal scale rows at mid-body; (15) 129–156 ventrals in males, 132–158 in females; (16) maximum total length in males 594 mm, in females 857 mm.

Comparisons. *Bothriechis nitidus* is compared to other species of the genus previously subsumed under *B. schlegelii sensu lato* (differences summarized in Table 2). It differs from all of them by having supraciliaries not raised, a low number of canthals (2–3) and interoculolabials (3–8), a low number of ventral scales, and a green dorsum with dark reddish brown, red, or pink bands (Fig. 27). *Bothriechis nitidus* resembles *B. hussaini* sp. nov., but this other species occurs south of the known distribution of *B. nitidus* and is characterized by having the loreal scale in contact with the preocular (not in contact in *B. nitidus*), supralabials spotted, dorsal scales densely stippled with black pigment, black-spotted ventral surfaces, and basal hooks of hemipenial body 4X (instead of 2X) the size of adjacent spines. Also, unlike *B. nitidus*, the golden morph is present in *B. hussaini* sp. nov. *Bothriechis nitidus* also resembles *B. rahimi* sp. nov., but this other species occurs north of the known distribution of *B. nitidus* and is characterized by having two or three raised and spinelike supraciliary scales, keeled anterior dorsal head scales, a higher number of interoculolabials (8–13 vs 3–8), canthals (4–5 vs 2–3), and a completely different array of color morphs, including red and pink.

Hemipenial morphology. (n = 3; Fig. 28) Everted and inflated, the organ is deeply bilobed, calyculate and noncapitate; hemipenial lobes cylindrical, but tapering towards the capitulum; in sulcate and asulcate views, lobe crotch ornamented with densely packed spinules and spines that become larger distally; sulcus spermaticus centrolineal, bifurcate and with walls strongly defined, bifurcation occurs below bilobation point and proximal to the base of the hemipenial body; sulcus spermaticus branch runs to lobe tips; distal one third to one half of each hemipenal lobe densely ornamented with small calyces with strongly defined spinulate edges. In sulcate view, hemipenial body surface with small spinules medially, basal hooks present, and 2–3 rows of smaller obliquely-arranged mesial spines that become gradually smaller towards each lobe's capitulum; each hemipenial lobe ornamented with mesial and lateral spines about half the size of the basal hooks; the spines in each lobe are replaced distally by calyces with strongly spinulate edges. In lateral view, hemipenial body and lobes with rows of spines, but otherwise non-spiculate; distal half strongly calyculate. In asulcate view, the center of hemipenial body is nude to sparsely spiculate; hemipenial lobes ornamented with 5 rows of smaller spines (about 1/3 the size of the lateral basal hooks) that decrease in size towards the lobe crotch.

Natural history. *Bothriechis nitidus* is an arboreal snake that inhabits evergreen lowland and montane forests, cloud forests, planted forests, plantations (cacao, coffee, and banana), and rural gardens. We have found vipers of this species at night perched on arboreal vegetation up to 10 m above the ground or, rarely, moving at ground level during the daytime. Individuals of *B. nitidus* are known to prey on frogs (*Craugastor longirostris*, *Pristimantis achatinus*, *P. walkeri*, and *Trachycephalus jordani*; Valencia et al. 2016; Meza-Ramos et al. 2019), anoles (personal observation by JV), hummingbirds, and mice (Meza-Ramos et al. 2019). Valencia et al. (2016) reports that females of this species give birth to litters of 5–9 young. One specimen (FHGO 7795) lived for 12 years in captivity.

Venom. In coastal Ecuador, 0.2–10.3% of snakebites are attributed to this species (Betancourt 2012; Valencia et al. 2016). Kuch et al. (1996) found the venom of this species to be not hemorrhagic, weakly coagulant, and only moderately lethal (LD50 estimated as 6.5 mg/kg).

Distribution. *Bothriechis nitidus* is endemic to Ecuador. It is known from at least 121 localities (listed in Suppl. material 3) along the Chocoan lowlands and adjacent foothills of the Andes in Ecuador. The species occurs over an area of approximately 37,400 km^2 and has been recorded at elevations of 0–2,230 m above sea level (Fig. 3). An estimated 4.2% of the predicted area of distribution of *B. nitidus* overlaps with that of *B. hussaini* sp. nov. and we found evidence (MZUA.Re.288 and JM 75; Fig. 3) of sympatry between the two species where the model predicted it. Both species co-occur along the foothills of the Andes in the sector between Naranjal, Guayas province, and Cumandá, Chimborazo province. An estimated 3.3% of the predicted area of distribution of *B. nitidus* overlaps with that of *B. rahimi* sp. nov., but we did not find evidence of sympatry between the two species.

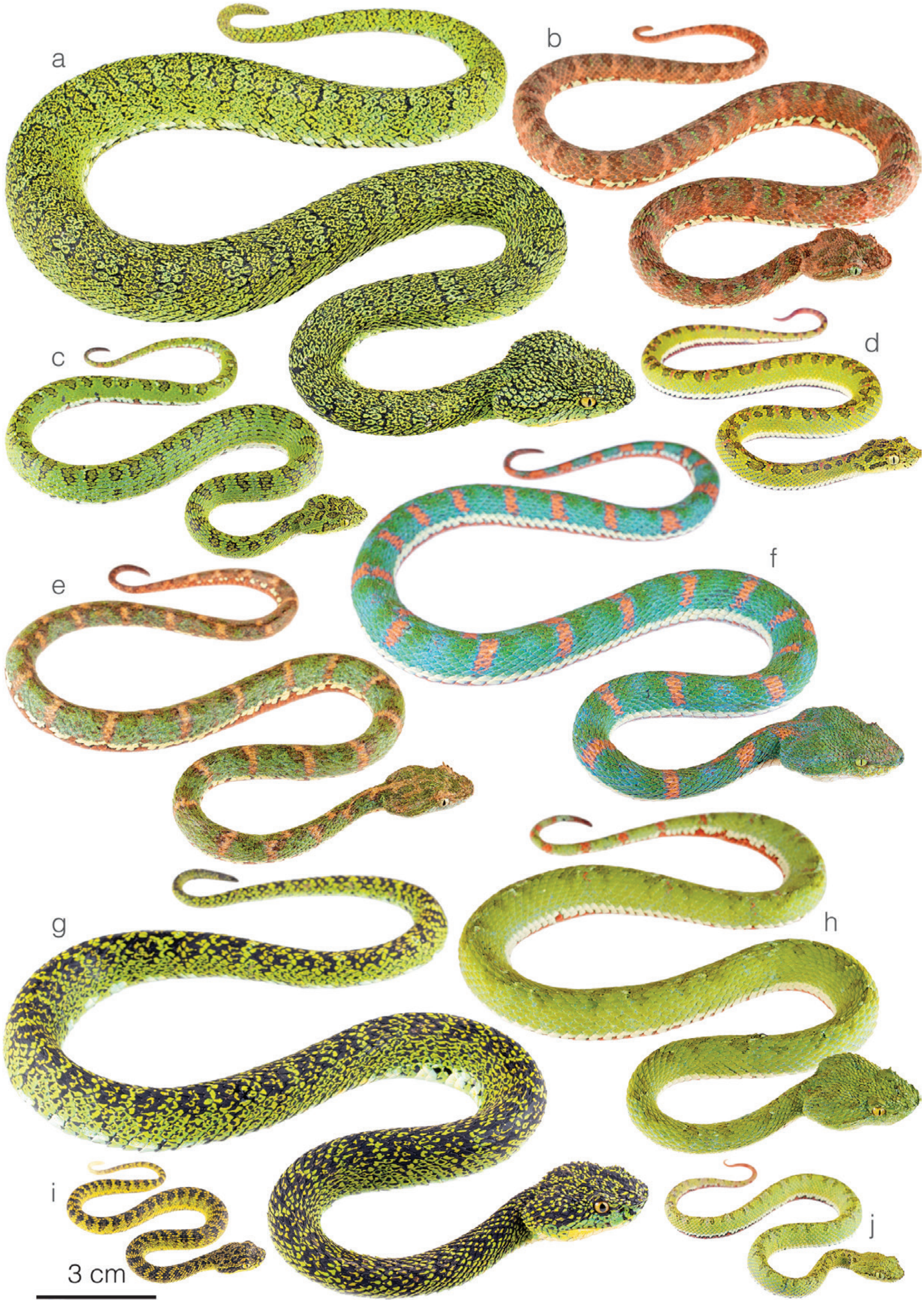

Figure 27. Photographs of living specimens of *Bothriechis nitidus* from Ecuador. **a.** AMARU SN adult female from Santa Rosa, Pichincha province; **b.** Adult from Reserva FCAT, Esmeraldas province; **c.** Juvenile from Verde Bambú, Pichincha province; **d.** Juvenile from Canandé Biological Reserve, Esmeraldas province; **e.** Adult from Gualpi, Esmeraldas province; **f.** Adult from Bosque Protector La Perla, Esmeraldas province; **g.** Adult from Santa Lucía Reserve, Pichincha province; **h.** Adult from Reserva FCAT, Esmeraldas province; **i.** Juvenile from Santa Lucía Reserve, Pichincha province; **j.** Juvenile from Canandé Biological Reserve, Esmeraldas province. Photos by Jose Vieira, Alejandro Arteaga, and Sebastián Di Doménico.

Figure 28. Hemipenial architecture of *Bothriechis nitidus* in sulcate, lateral, and asulcate views. **a.** MZUTI 3753 from Sachatamia Lodge, Pichincha province, Ecuador; **b.** MZUTI 3754 from Santa Rosa de Intag, Imbabura province, Ecuador; **c.** ZSFQ 5054 from Canandé Biological Reserve, Esmeraldas province, Ecuador. Photos by Amanda Quezada.

Etymology. The specific epithet *nitidus* is a Latin word meaning "shining" or "elegant." It refers to the eye-catching dorsal pattern of the holotype, which is shared by most individuals of lowland populations of this species (Fig. 27).

Conservation status. We consider *Bothriechis nitidus* to be included in the Near Threatened category following IUCN Red List criteria (IUCN 2012) primarily because the species' extent of occurrence is estimated to be larger than the 20,000 km^2 needed to meet B1 criteria for the Vulnerable category. However, although the species occurs in numerous protected areas (no less than 30; see Suppl. material 3), the majority of the species' forest habitat has been destroyed. Based on the distribution model presented in Fig. 3 in combination with maps of vegetation cover of Ecuador (MAE 2012), we estimate that only ~32% of the species' forest habitat is still standing.

Bothriechis hussaini sp. nov.

https://zoobank.org/43A03715-A0CB-439B-ACAC-220569BB993A

Figs 29–31

Type material. ***Holotype***: ZSFQ 5056 (Figs 29, 30g), subadult male collected on June 11, 2019 at Buenaventura Biological Reserve, El Oro province, Ecuador (-3.65467, -79.76794; 524 m).

Paratypes: All labeled *Bothriechis hussaini* sp. nov. in Suppl. material 1 from museum collections AMNH, DHMECN, MZUA, and ZSFQ.

Proposed standard English name. Hussain's Eyelash-Pitviper.

Proposed standard Spanish name. Víbora de pestañas de Hussain.

Local Spanish names. Cabeza de candado, víbora sol (yellow morph).

Diagnosis. *Bothriechis hussaini sp. nov.* is diagnosed based on the following combination of characters: (1) supraciliary scales low and granular to triangular; (2) anterior dorsal head scales smooth or barely keeled; (3) gular scales 1/2 size of chinshields; (4) 5–8 interoculolabials; (5) 2–3 canthals, which may be flat or slightly raised forming a ridge along the canthus; (6) loreal in contact with preocular; (7) yellow morph present; (8) dorsal bands black or pink or a combination of both; (9) opposing kidney shaped dorsal marks absent; (10) black speckles on dorsal scales present; (11) black speckling on ventral surfaces prominent; (12) ventral surfaces never entirely white; (13) iris green to yellow with fine black speckles, spots, or reticulations; (14) 21–23 dorsal scale rows at mid-body; (15) 140–151 ventrals in males, 147–153 in females; (16) maximum total length in males 608 mm, in females 656 mm.

Comparisons. *Bothriechis hussaini* sp. nov. is compared to other species of the genus previously subsumed under *B. schlegelii sensu lato* (differences summarized in Table 2). It differs from all of them by having supraciliaries not raised, a low number of canthals (2–3) and interoculolabials (5–8), a low number of ventral scales, and a green dorsum heavily speckled with black pigment (Fig. 30). *Bothriechis hussaini* sp. nov. is similar to *B. nitidus*, but differs from this species by having the loreal scale in contact with the preocular (not in contact in *B. nitidus*), supralabials heavily marked by black speckles, dorsal and ventral scales densely stippled with black pigment, basal hooks of hemipenial body 4X (instead of 2X) the size of adjacent spines (Fig. 31), and by the presence of a yellow morph (=*víbora sol*; Fig. 30b). *Bothriechis nitidus* also resembles *B. rahimi* sp. nov., but this other species occurs north of the known distribution of *B. nitidus* and is characterized by having two or three raised and spinelike supraciliary scales, keeled anterior dorsal head scales, a higher number of interoculolabials (8–13 vs 3–8), canthals (4–5 vs 2–3), and a completely different array of color morphs, including pale gray and pink (Fig. 26).

Hemipenial morphology. (n=2; Fig. 31) Everted and inflated, the organ is deeply bilobed, calyculate and noncapitate; hemipenial lobes cylindrical to spindle-shaped; in sulcate and asulcate views, lobe crotch ornamented with scattered spinules proximally and larger spines distally; sulcus spermaticus centrolineal, bifurcate and with walls strongly defined, bifurcation occurs below bilobation point and proximal to the base of the hemipenial body; sulcus spermaticus branch runs to lobe tips; distal half of each hemipenal lobe densely ornamented with small calyces with strongly defined spinulate edges. In sulcate view, hemipenial body surface with small mesial spinules, a pair of enlarged calcified spines (basal hooks), and 3–4 rows of smaller obliquely-arranged mesial spines that become gradually smaller towards each lobe's capitulum; each hemipenial lobe ornamented with mesial and lateral spines about 1/4 the size of the basal hooks; the spines in each lobe are replaced distally by calyces with strongly spinulate edges. In lateral view, hemipenial body and lobes with rows of spines, but otherwise non-spiculate; distal half strongly calyculate. In asulcate view, the center of hemipenial body is nude to sparsely spiculate; hemipenial lobes ornamented with 4 rows of smaller spines (about 1/4 the size of the lateral basal hooks) that decrease in size towards the lobe crotch.

Description of holotype. An adult male, SVL 358 mm, tail length 51+ mm (14.3% SVL; tail-tip missing); head length 29.2 mm (8.2% SVL) from tip of snout to angle of jaw; head width 21.6 mm (73.9% head length) taken at broadest point; rostral broader than high (3.1 × 1.9 mm); nasal completely divided and not fused with first supralabial; loreal about 1/2 size of pit, contacting nasal, canthals, 2 prefoveals, prelacunal, supralacunal, and preocular; prefoveals 4/5; subfoveals 2/2; postfoveals 0; prelacunal not fused with second supralabial; sublacunals 2/2; supralacunal elongated and in contact with orbit; preoculars 1/1 (2/2 if supralacunal is considered a preocular); suboculars 1/1; postoculars 1/1; loreal pit large, directed anteriorly, located slightly below line drawn from center of eye to naris; supralabials 8/9 (including lacunolabial); infralabials 11/11, first pair meet posteriorly; mental broader than long (3.2 × 2.0 mm); 1 pair of chinshields; 5 pairs of gulars between chinshields

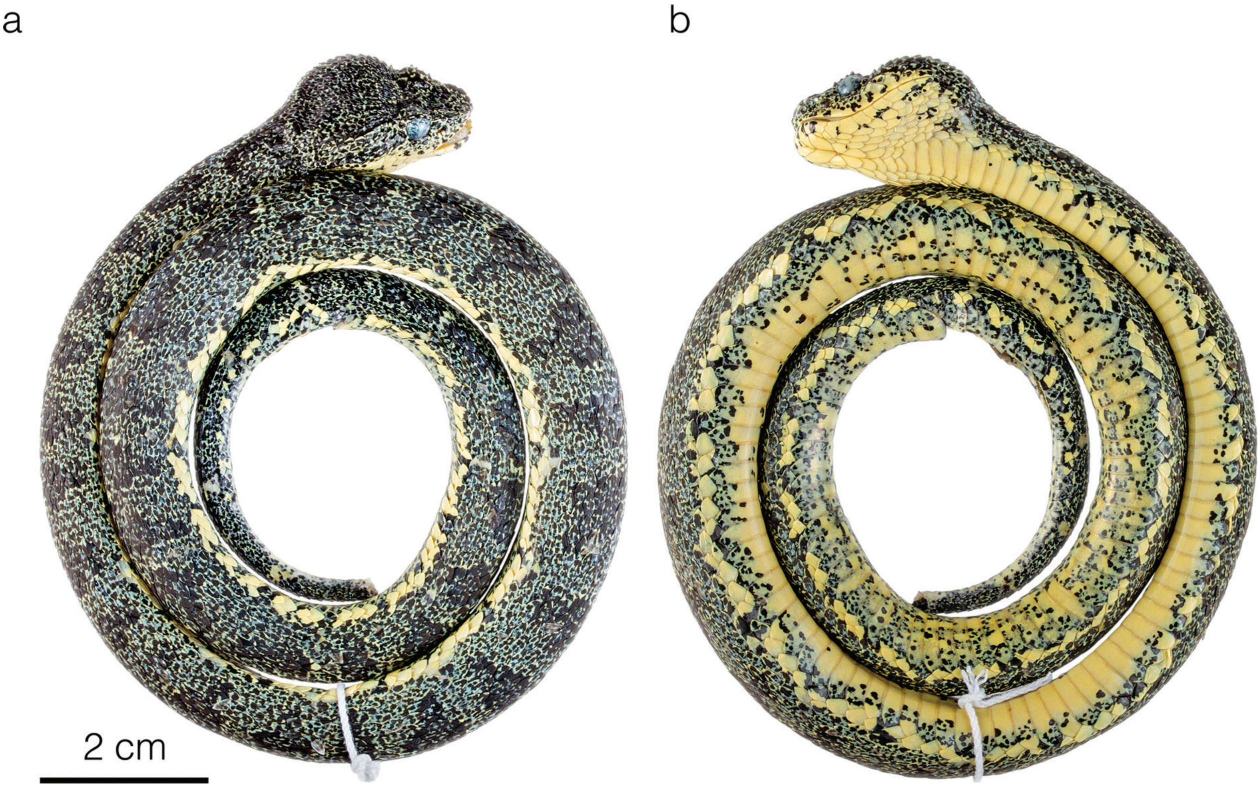

Figure 29. Adult male holotype of *Bothriechis hussaini* sp. nov. ZSFQ 5056 in **a.** Dorsal and **b.** Ventral view. Photos by Alejandro Arteaga.

and preventrals; preventrals 0; anterior internasals 3; canthals 3/3; supraciliary scales low and granular, with one on the left side triangular; supraoculars kidney-shaped, 2.2× longer than wide; intersupraoculars 7; anterior dorsal head scales slightly keeled; posterior head scales strongly keeled; interrictals 28; dorsal scale rows 25/23/17; ventrals 152; cloacal plate entire; 38+ undivided subcaudals (tail incomplete); tail prehensile.

Natural history. *Bothriechis hussaini* sp. nov. is an arboreal snake that inhabits evergreen lowland/foothill forests and plantations (coffee and banana). We have found vipers of this species at night or during the day perched on vegetation up to 1.2–3 m above the ground. Meza-Ramos et al. (2010) found a mouse of the genus *Oryzomys* in the stomach of DHMECN 2580 from El Oro province, Ecuador.

Venom. One biologist who was bitten in the shoulder by a *Bothriechis hussaini* sp. nov. from Azuay province reports intense localized pain, swelling, and tachycardia with two hours from the incident (personal comments to AA, 2022). The patient received antihistamines as treatment and recovered fully within 24 hours of the bite.

Distribution. *Bothriechis hussaini* sp. nov. is known from at least 55 localities (listed in Suppl. material 3) along the Pacific lowlands and adjacent Andean foothills in southwestern Ecuador and extreme northwestern Peru. The species occurs over an area of approximately 13,591 km^2 in the transition area between the humid Chocó rainforests and the Tumbesian dry forests and has been recorded at elevations 1–1,680 m above sea level (Fig. 3). Approximately 11.7% of the predicted area of distribution of *B. hussaini* sp. nov. overlaps with that of *B. nitidus* and we found evidence (MZUA.Re.288 and JM 75) of sympatry between the two species where the model predicted it. Both species co-occur along the foothills of the Andes in the sector between Naranjal, Guayas province, and Cumandá, Chimborazo province.

Etymology. The specific epithet *hussaini* is a patronym honoring Prince Hussain Aga Khan, who has devoted his life, influence, and wealth to environmental conservation since he was eleven years old. In 2014, he created a Swiss-based non-profit called Focused On Nature (FON), through which he protects endangered global biodiversity through local organizations worldwide.

Conservation status. We consider *Bothriechis hussaini* sp. nov. to be included in the Vulnerable category following the IUCN criteria B1a, b (i, iii, iv) (IUCN 2012), because the species' extent of occurrence is estimated to be much less than 20,000 km^2 (Fig. 3) and its habitat is severely fragmented and declining in extent and quality due to deforestation. Although *B. hussaini* sp. nov. occurs in four protected areas (Reserva Ecológica Manglares Churute, Reserva Biológica Buenaventura, Reserva Forestal Cerro Samama, and Reserva Nacional Tumbes), the remaining localities where the species has been recorded (Suppl. material 3) are historical and probably do not represent extant populations. Based on the species distribution model presented in Fig. 3 in combination with maps of Ecuador (MAE 2012), we estimate that approximately 78% of the forest cover throughout the species' potential distribution area has been destroyed, mostly due to the expansion of the agricultural frontier.

Figure 30. Photographs of living specimens of *Bothriechis hussaini* sp. nov. from Ecuador. **a.** CZ-003 adult female from El Colorado, El Oro province; **b.** CZ-001 adult male from El Colorado, El Oro province; **c.** CZ-004 adult male from San Carlos, Guayas province; **d.** ZSFQ 5058 juvenile female from Buenaventura Biological Reserve, El Oro province; **e.** AMARU SN adult female Buenaventura Biological Reserve, El Oro province; **f.** subadult of undetermined sex from Buenaventura Biological Reserve, El Oro province; **g.** ZSFQ 5056 adult male holotype from Buenaventura Biological Reserve, El Oro province. Photos by Alejandro Arteaga and Jose Vieira.

Discussion

This work marks the first comprehensive attempt elucidating the systematics of *Bothriechis schlegelii sensu lato* from an integrated taxonomic perspective. It solves the paraphyly of the species with respect to the previously recognized *B. supraciliaris*, finds support for the recognition of ten species within this complex, and provides a framework for future studies on this group. Hurtado-Gómez (2009) pointed out that the paraphyly of *B. schlegelii sensu lato* was probably the result of this nomen (or name) being a species complex. Our results confirm this view. We uncovered as much variation at the molecular level within *B. schlegelii sensu lato* as among

Figure 31. Hemipenial architecture of *Bothriechis hussaini* sp. nov. in sulcate, lateral, and asulcate views. **a.** MZUTI 5872 and **b.** ZSFQ 5056 from Reserva Biológica Buenaventura, El Oro province, Ecuador. Photos by Amanda Quezada.

all other currently recognized members of this genus (Fig. 2). We found that this genetic variation is geographically structured (Fig. 3) and corresponds to the existence of ten species that are easily diagnosed based on characters of coloration, lepidosis, hemipenial morphology, natural history, and venom properties (Table 2).

The name *Bothriechis schlegelii* (Berthold, 1846) is restricted to the species distributed on the central highlands of Colombia (magenta clade in Figs 2, 3) based on the inclusion of the holotype ZFMK 32554 (Fig. 6) in the phylogeny. The holotype was labeled as coming from Popayán. At the time of description, the city Popayán existed but the same name was applied to a colonial province that included nearly all of what is now western Colombia (Myers and Böhme 1996). Although broad, this region is inhabited by only two *Bothriechis* clades that correspond to species (magenta and turquoise clades in Figs 2, 3). The holotype (Fig. 6) belongs to the highland population and is genetically most similar to another sample from Colombia's Cordillera Occidental, rather than to samples from Cordillera Central. Although this is not enough to restrict the type locality to the city Popayán rather than the broader "Provinz Popoyán," it narrows down the provenance of the holotype to the highlands of the Cordillera Occidental of Colombia.

The name *Bothriechis torvus* (Posada Arango, 1889a) is restricted to the species distributed on the Chocó-Río Magdalena valley biome in Colombia (turquoise clade in Figs 2, 3) based on the description of the species as well as its type locality. In our phylogenetic analyses, we included samples of specimens assignable to this species from Darién province, Panama, and Chocó department, Colombia. However, all of these come from a western population, which according to our species distribution models, is separated from the one bordering the foothills of Colombia's Cordillera Occidental and Cordillera Central. In agreement with the model, we did not find records of *Bothriechis torvus* between the two populations, which now, appear to be isolated by the valley of the Río Atrato. We examined specimens from both populations and found minor differences among them, most notably in the condition of the loreal-preocular contact and in the color of the ventral surfaces. The inclusion of genetic samples from the eastern population will help shed light into the relationship between the two populations.

The name *Bothriechis nitidus* (Günther, 1859) is restricted to the species endemic to west-central Ecuador (purple clade in Figs 2, 3) based on the illustration and description of the holotype as well as on the travel logs of its collector. The illustration of the holotype (Fig. 7) resembles some individuals of *B. nitidus* in having immaculate supralabials and a bright green dorsum with red bands and minimal speckling on the dorsum (see Fig. 27f). The number of ventral scales in this specimen is 154, which is outside the range reported for *B. hussaini* sp. nov. but falls within the range reported for *B. nitidus* (Table 2). The holotype was collected by British zoologist and naturalist Louis Fraser in "western Ecuador" and almost surely in late 1858 or early 1859 based on the illustration of the holotype by George Henry Ford, which is based on a freshly preserved snake. This specimen was sent to the British Museum of Natural History in a shipment that contained hundreds of birds, fish, and reptiles, including the holotypes of *Anadia rhombifera*, *Anolis fraseri*, *Leptophis occidentalis*, *Stenocercus iridescens*, and *Gonatodes caudiscutatus*, all described by Günther in 1859. The birds were labeled as having been collected at Pallatanga, Chimborazo province, Ecuador where Mr. Fraser spent the months of August, September, and October 1858 (see Sclater 1859). Although during his trip to Ecuador, which lasted from 1857 to 1859, Mr. Fraser collected in other localities (see Gardner 1983 and references therein), none of these were in the range where *B. hussaini* sp. nov. is known to occur.

We bring attention to the comparatively low topological divergence between *Bothriechis nitidus* and *B. hussaini* sp. nov. in our phylogeny (Fig. 2). The relationship between these two species as reciprocally monophyletic is strongly supported but the genetic distances (1.5% in a 700 bp of the mitochondrial CYTB; Table 1) gene among them are smaller than intraspecific distances in another species of the group: *B. nigroadspersus*. We do not think this is an artifact of sampling because our molecular analysis includes samples from throughout each species' areas of distribution, including regions where the two species co-occur (Fig. 3). The fact that we found evidence of sympatry between the sister species where the model predicted it suggests a scenario of secondary contact after isolation. However, a comprehensive study of the genetics of this population is needed to evaluate whether introgressive hybridization is taking place in this contact zone. Although there is overlap in scale counts between *B. nitidus* and *B. hussaini* sp. nov. (Table 2), the two species can be diagnosed based on hemipenial morphology (Figs 28, 31) and coloration (Figs 27, 30) and the overlap between their predicted areas of distribution is small (4.2–11.7%; Fig. 3). The existence of *B. hussaini* sp. nov. mirrors the distribution of other recently described squamates that are restricted to the transition area between the humid Chocó rainforests and the Tumbesian dry forests in southwestern Ecuador and extreme northwestern Peru, including *Enyalioides touzeti* Torres-Carvajal et al. 2008, *Anadia buenaventura* Betancourt et al., 2018, *Dipsas bobridgelyi* Arteaga et al., 2018, *Sibon bevridgelyi* Arteaga et al., 2018, and *Anolis nemonteae* Ayala-Varela et al., 2021. All these reptiles are like *B. hussaini* sp. nov. not only in their distribution but in that their respective sister species are endemic to west-central Ecuador much like *B. nitidus*. We believe this shared pattern suggests the existence of a unique biogeographical province between the humid Chocó rainforests and the Tumbesian dry forests.

One of the new species described here, *Bothriechis rahimi* sp. nov., occurs along the mouths of the rivers Esmeraldas, Santiago, Cayapas, and Mira in extreme northwestern Ecuador and southwestern Colombia (orange clade in Fig. 2, 3). We find the existence and distribution of this species puzzling given that we know of no other snakes having a similar range and we found overlap between the predicted areas of distribution of these two species but no evidence of sympatry. Along the northern coast of Esmeraldas province, we found *B. nitidus* only west of the Río Esmeraldas and *B. rahimi* sp. nov. only east of the river, a pattern that could point to the role of this river as a potential biogeographical barrier between the two species. However, *B. nitidus* does occur east of the river towards the Andean foothills in Ecuador. The Río Esmeraldas has already been identified as a potential biogeographical barrier (Arteaga et al. 2016; Guayasamin et al. 2022) for other taxa and could likely help explain the distribution of *B. rahimi* sp. nov.

The name *Bothriechis nigroadspersus* (Steindachner, 1870) is restricted to the widespread Central American (red clade in Figs 2, 3) species based on the inclusion of the holotype NMW 18811 (Fig. 4) in the phylogeny. We found two major clades within this species: one distributed throughout Panama and another distributed from Mexico to extreme western Panama. Although we did not identify major biogeographical barriers separating the two populations, we did find a gap in Comarca Ngäbe Buglé, western Panama, where the species has not been reported. The *oropel* (=golden morph; Fig. 5a–c) has so far only been recorded in the western population, mainly from central and eastern Panama northwards to Honduras. Despite the genetic distances and differential occurrence of the *oropel* morph, we did not find additional morphological differences that could allow the separation of the two clades into distinct species. Additionally, we only examined the hemipenial architecture of specimens of the western population (Fig. 8). Finally, when locality records of this Panamanian clade were excluded from the species distribution model of *B. nigroadspersus*, the resulting model still predicted the species occurrence throughout Panama, including the gap in Comarca Ngäbe Buglé (Fig. 3). A more comprehensive sampling of this region in Panama is needed to ascertain whether the model is correct and *B. nigroadspersus* has a continuous distribution throughout Mesoamerica. We found that an estimated 1.0% of the predicted area of distribution of *B. nigroadspersus* overlaps with that of *B. torvus* and *B. supraciliaris*, and we found records of *B. nigroadspersus* 3.9–4.4 km airline distance away from those of the other two species, respectively. It remains to be seen whether *B. nigroadspersus* occurs syntopically with one of the other two species and if they occupy different microhabitats when this is the case.

The status of *Bothriechis supraciliaris* as a valid species is maintained. We found that this species can easily be diagnosed from *B. nigroadspersus* based on characters of dorsal coloration (Figs 5, 9) and number of ventrals (Solórzano et al. 1998), pattern on dorsal aspect of the head (Lotzkat 2014), natural history, venom properties (Lomonte et al. 2012), and hemipenial morphology (all summarized under Table 2). Lomonte et al. (2012) estimated the lethal activity of their sampled *B. supraciliaris* venom at LD50 6.04 mg/kg, less potent than what has been recorded for *B. nigroadspersus*, which has LD50 values in the range of 1.7–5.6 mg/kg (Bolaños 1972; Gutiérrez and Chaves 1980; Lomonte et al. 2008, 2012; Prezotto-Neto et al. 2016). The fact that we did not find evidence of syntopy between *B. nigroadspersus* and *B. supraciliaris* is intriguing given that the distribution area of the latter is the only region where the presence of *B. nigroadspersus* is predicted but not confirmed (Fig. 3). The absence of *B. nigroadspersus* probably points out to a scenario of ecological exclusion. Solórzano et al. (1998) and Solórzano (2004) pointed out that the two species have different habits: primarily arboreal in *B. nigroadspersus* and mainly terrestrial in *B. supraciliaris*. However, all *B. supraciliaris* found by us were on bushes (see account for the species above).

Natera-Mumaw et al. (2015) discussed the presence of *Bothriechis* in Venezuela and suggested that this species be removed from the herpetofauna of this country given the lack of confirmed museum vouchers. Our results agree with this view. We did not find any museum voucher or photographic assignable to this genus in Venezuela, and neither the distribution models of *B. klebbai* sp. nov. or *B. khwargi* sp. nov. (the easternmost species in the group) predict their presence in Venezuela (Fig. 3).

Although this work marks the first comprehensive attempt at elucidating the systematics of *Bothriechis schlegelii*, it is still far from complete. First, the relationships between the Colombian members of the genus are not strongly supported in our phylogeny and they will likely benefit from an improved sampling that includes more nuclear loci. Adult males of *B. khwargi* sp. nov. and their corresponding hemipenial morphology remain unknown. Likewise, we were unable to produce information on the hemipenial architecture of *B. rahimi* sp. nov. The low genetic differentiation but notable morphological difference between cloud forest and lowland population of *B. nitidus* is intriguing. Conversely, the lack of major morphological differences but comparatively high genetic variation between Panamanian *B. nigroadspersus* and the population distributed elsewhere throughout Central America deserves further study. Finally, it remains to be seen whether populations of *B. torvus* from Darién and northwestern Colombia are conspecific with the Central Colombian population. We suggest that any future work focused on the systematics of the *B. schlegelii* species group include a more comprehensive sampling of molecular characters. Such work would gain much clarity by sampling species of *Bothriechis* occurring on Colombia's Cordillera Oriental. Until then, we hope that our work helps guide future studies into the biogeography and venom composition in this charismatic group of vipers.

Author contributions

Conceived and designed the work: AA. Performed the analyses: AA. Gathered morphological data: AA RAP AB JV EMP ENS CLBA CK SA JHV. Analyzed the data: AA. Contributed reagents/materials/analysis tools: ENS EMP. Wrote the paper: AA RAP AB JV EMP ENS CLBA CK SA JHV LB KJH.

Acknowledgments

This article was greatly improved by comments of Javier Sunyer. We are indebted to Juan Pablo Hurtado for providing morphological data and scale counts of *Bothriechis* of Colombia and Panama as well as for his suggestions to improve earlier versions of this manuscript. For granting access to the protected forests under their care, we are grateful to Daniel Arias and Raúl Arias of the Canopy Family lodges, to Martin Schaefer and David Agro of Fundación Jocotoco, to Guido Berguido of Fundación Adopta Bosque, and to Jason Crespo Whitney and Veronica Buenaño Bonilla (Bosque Protector la Perla). For providing DNA sequence data of *Bothriechis schlegelii*, we are grateful to Juan Manuel Daza (MHUA), Christopher Parkinson (Clemson University), and the whole team at Parkinson Lab. Thanks to Michael Preick and Axel Barlow for their assistance in generating a DNA library from the holotype ZMFK 32554. For granting access to museum specimens under their care, we are grateful to Martha Lucia Calderón Espinosa (ICN); Sandra Patricia Galeano Muñoz (IAvH); Kevin de Queiroz, Addison Wynn, Steve Gotte, Kenneth Tighe, and Esther Langan (USNM); Juan Manuel Daza (MHUA); Yelenny López Aguirre (ARUQ); Gerardo Chaves (UCR); Sebastián Padrón (MZUA); Mario Yánez Muñoz (DHMECN); Katty Garzón-Tello and María Elena Barragán (FHGO); David Salazar-Valenzuela (MZUTI); Gustavo Adolfo Londoño Guerrero (CZI); Jhon Tailor Rengifo-Mosquera (COLZOOCH); Wilmar Bolívar (CPZ-UV); Raúl Sedano (UV-C); Silke Schweiger (NMW); Omar Torres-Carvajal (QCAZ); and Martha Patricia Ramírez Pinilla (UIS-R). For granting access to live specimens under their care, we are grateful to Quetzal Dwyer (Reptilandia, Costa Rica); César Quiroga Giraldo and Francisco Ruiz (Serpentario Nacional, Colombia); Gordon Shumway (private collection); Ernesto Arbeláez (Bioparque AMARU); César Zambrano (Centro de Rescate de Vida Silvestre), José Beltrán and Jimmy Guerrero (Serpentario del MHNUC, Colombia); and Jeisson Gómez, Jorge Asprila, Santiago Tavares, Paola Rey Suárez (Serpentario Universidad de Antioquia). JV would like to thank Simón Ulloa Rengifo, Mary Bueno Ospina, Karen Angel Camilo for their help in creating white background images of *Bothriechis*. Angie Tovar-Ortiz, Sophia Hurtado, Duván Zambrano, Hannah Som, Xilena Rueda, Alejandra Salazar, Carlos Gómez, Johan Cordón-Rangel, Morris Flecks, Santiago Orozco, Alice Schumacher, and Ivan Ineich examined museum vouchers and created photographs of alcohol-preserved specimens. Duván Zambrano also prepared the hemipenes of Colombian and Ecuadorian *Bothriechis* and created images of *B. rasikusumorum* sp. nov. Special thanks to Frank Pichardo, Amanda Quezada, Eric Osterman, Diana Troya, Matteo Espinosa, Pablo Montoya, Jorge Peña, Erik Gaitán, Lior Berman, Fernando Castro, and Luisa Jaramillo for their assistance and companionship in the field. Valentina Nieto created the illustrations of the *Bothriechis* cephalic shield terminology. Duván Zambrano and Amanda Quezada prepared and photographed the hemipenes of six *Bothriechis*. Daniela Franco and Gabriela Gavilanes provided invaluable assistance in accessioning material at ZSFQ and generating DNA sequence data; Lorena Benitez created the topographical maps. Fieldwork was made possible with the support of Focused on Nature (FON), *The Explorers Club Discovery Expedition Grants*, Khamai Foundation, and Tropical Herping. The 2022 fieldwork in Colombia, Ecuador, and Panama was made possible thanks to the generous support of Casey Klebba, Tushar Shah, Walter Jennings, Joseph Beck, Tim Paine, Alain Kormann, Matthew Pérez, Martin Koeppert, Philippe Monthemont, Nicolas Devos, Brian Monk, Michael Lavery, Daniel Zamora, Suzana Lightman, Peter Joost, Robert Thomas, Stefano Medri, Ashlee Vaughn, Ewald Wassink, Rodolphe Jouxtel, Roy and Laurie Averill-Murray, Jules Wyman, Carter Joost, Anna Renz, Catherine Evans, Wilson Jen-Lee, Jamael Gray, Fran Baccouche, JD Paes, Mami Okura, Petr Bambousek, Kelly Geer, Paul Hoskisson, Joe Goldufsky, Jarrett Koenemund, Christian Cave, Julian Duval, and Guy Clairiot. Work by AA and KJH in Colombia during 2023 was possible thanks to the support of Liberty University. Sequencing was made possible with support of the Inédita Program from the Ecuadorian Science Agency SENESCYT (Respuestas a la Crisis de Biodiversidad: La Descripción de Especies como Herramienta de Conservación; INEDITA PIC-20-INE-USFQ-001). Work by AB was supported by Sistema Nacional de Investigación (SNI) of the Secretaría Nacional de Ciencia, Tecnología e Innovación (SENACYT, Panama). RAP was supported by startup funding from the George Washington University. Sequencing of the holotypes NMW 18811 and ZMFK 32554 was funded by the German Research Foundation (DFG; project number 351649567 within the DFG SPP 1991 "Taxon-Omics").

References

Arteaga A, Pyron RA, Peñafiel N, Romero-Barreto P, Culebras J, Bustamante L, Yánez-Muñoz MH, Guayasamin JM (2016) Comparative phylogeography reveals cryptic diversity and repeated patterns of cladogenesis for amphibians and reptiles in northwestern Ecuador. PLoS ONE 11: e0151746. https://doi.org/10.1371/journal.pone.0151746

Arteaga A, Salazar-Valenzuela D, Mebert K, Peñafiel N, Aguiar G, Sánchez-Nivicela JC, Pyron RA, Colston TJ, Cisneros-Heredia DF, Yánez-Muñoz MH, Venegas PJ, Guayasamin JM, Torres-Carvajal O (2018) Systematics of South American snail-eating snakes (Serpentes, Dipsadini), with the description of five new species from Ecuador and Peru. ZooKeys 766: 79–147. https://doi.org/10.3897/zookeys.766.24523

Agne S, Preick M, Straube N, Hofreiter M (2022) Simultaneous barcode sequencing of diverse museum collection specimens using a mixed RNA bait set. Frontiers in Ecology and Evolution 10: 909846. https://doi.org/10.3389/fevo.2022.909846

Antonio FB (1980) Mating behavior and reproduction of the eyelash viper (*Bothriechis schlegelii*) in captivity. Herpetologica 36: 231–233.

Ayala-Varela F, Valverde S, Poe S, Narváez AE, Yánez-Muñoz MH, Torres-Carvajal O (2021) A new giant anole (Squamata: Iguanidae: Dactyloinae) from southwestern Ecuador. Zootaxa 4991: 295–317. https://doi.org/10.11646/zootaxa.4991.2.4

Ayerbe S, Paredes A, Gávez DA (1979) Estudio retrospectivo sobre ofidiotoxicosis en el departamento del Cauca. 2da parte. Aspectos clínicos, epidemiológicos y complicaciones. Cuadernos de Medicina, Facultad de Medicina, Universidad del Cauca 4: 33–45.

Arévalo E, Davis SK, Sites 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

Barrio-Amorós C (2015) *Bothriechis schlegelii*. Predation and color pattern. Mesoamerican Herpetology 2: 117–119.

Batista A, Hertz A, Ponce M, Lotzkat S (2020) Notes on amphibians and reptiles from western Panama. Herpetology Notes 13: 219–229.

Berthold AA (1846) Über verschiedene neue oder seltene Reptilien aus Neu-Granada und Crustaceen aus China. Abhandlungen der Königlichen Gesellschaft der Wissenschaften zu Göttingen 3: 3–32. https://doi.org/10.5962/bhl.title.5485

Betancourt RM (2012) Incidencia, zonas de riesgo y prevención de accidentes ofídicos en áreas rurales de las provincias de Manabí y Los Ríos, Ecuador, años 2007 a 2009. BSc thesis, Universidad Central de Ecuador, 165 pp.

Betancourt R, Reyes-Puig C, Lobos SE, Yánez- Muñoz MH, Torres-Carvajal O (2018) Sistemática de los saurios *Anadia* Gray, 1845 (Squamata: Gymnophthalmidae) de Ecuador: límite de especies, distribución geográfica y descripción de una especie nueva. Neotropical Biodiversity 4: 82–101. https://doi.org/10.1080/23766808.2018.1487694

Blody DA (1983) Notes on the reproductive biology of the Eyelash Viper *Bothrops schlegelii* in captivity. Herpetological Review 14: 45–46.

Boulenger GA (1896) Catalogue of the snakes in the British Museum. Taylor & Francis, London, 727 pp.

Bolaños R (1972) Toxicity of Costa Rican snake venoms for the white mouse. American Journal of Tropical Medicine and Hygiene 21: 60–63. https://doi.org/10.4269/ajtmh.1972.21.360

Bolaños RA (1984) Serpientes, venenos, y ofidismo en Centroamérica. Editorial Universidad de Costa Rica, San José, 136 pp.

Böhme W (2010) A list of the herpetological type specimens in the Zoologisches Forschungsmuseum Alexander Koenig, Bonn. Bonn zoological Bulletin 59: 79–108.

Burbrink FT, Lawson R, Slowinski JB (2000) Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): A critique of the subspecies concept. Evolution 54(6): 2107–2188. https://doi.org/10.1111/j.0014-3820.2000.tb01253.x

Burbrink FT, Crother BI, Murra CM, Smith BT, Ruane S, Myers EA, Pyron RA (2022) Empirical and philosophical problems with the subspecies rank. Ecology and Evolution 12: e9069. https://doi.org/10.1002/ece3.9069

Campbell JA, Lamar WW (2004) The venomous reptiles of the western hemisphere. Cornell University Press, Ithaca, 774 pp.

Carstens BC, Pelletier TA, Reid NM, Satler JD (2013) How to fail at species delimitation. Molecular Ecology 22: 4369–4383. https://doi.org/10.1111/mec.12413

Castoe TA, Daza JM, Smith EN, Sasa MM, Kuch U, Campbell JA, Chippindale PT, Parkinson CL (2009) Comparative phylogeography of pitvipers suggests a consensus of ancient Middle American highland biogeography. Journal of Biogeography 36: 88–103. https://doi.org/10.1111/j.1365-2699.2008.01991.x

CATHALAC (2011) Mapa Centroamericano de cobertura y uso de la tierra, cambios de cobertura y uso de la tierra 1980-1990-2000-2010. Programa Regional para la Reducción de la Vulnerabilidad y Degradación Ambiental, Ciudad de Panamá, 166 pp.

Chavarría M, Barrio-Amorós C (2014) *Clelia clelia*. Predation. Mesoamerican Herpetology 1: 286.

Daniel H (1949) Las serpientes en Colombia. Revista Facultad Nacional de Agronomía 9: 301–333.

Daza JM, Castoe, TA, Parkinson CL (2010) Using regional comparative phylogeographic data from snake lineages to infer historical processes in Middle America. Ecography 33: 343–354. https://doi.org/10.1111/j.1600-0587.2010.06281.x

de Queiroz K (2007) Species concepts and species delimitation. Systematic Biology 56: 879–886. https://doi.org/10.1080/10635150701701083

Doan TM, Mason AJ, Castoe TA, Sasa M, Parkinson CL (2016) A cryptic palm-pitviper species (Squamata: Viperidae: Bothriechis) from the Costa Rican highlands, with notes on the variation within *B. nigroviridis*. Zootaxa 4138: 271–290. https://doi.org/10.11646/zootaxa.4138.2.3

Dowling HG (1951) A proposed standard system of counting ventrals in snakes. British Journal of Herpetology 1: 97–99.

Dowling HG, Savage JM (1960) A guide to the snake hemipenis: a survey of basic structure and systematic characteristics. Zoologica 45: 17–28. https://doi.org/10.5962/p.203350

Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Heled J, Kearse M, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2021) Geneious version 2021.1. https://www.geneious.com

Elith J, Leathwick JR (2009) Species distribution models: ecological explanation and prediction across space and time. Annual Review of Ecology, Evolution, and Systematics 40: 677–697. https://doi.org/10.1146/annurev.ecolsys.110308.120159.

Elith J, Phillips SJ, Hastie T, Dudík M, Chee YE, Yates CJ (2011) A statistical explanation of MaxEnt for ecologists. Diversity and Distributions 17: 43–57. https://doi.org/10.1111/j.1472-4642.2010.00725.x.

Entiauspe-Neto OM, Chavarría Chaves GG, Martins Silva RH (2021) *Bothriechis schlegelii* (Eyelash Viper): diet and mortality. Herpetological Review 52: 657.

Felsenstein J (2004) Inferring phylogenies. Sinauer Associates, Sunderland, 664 pp.

Galofre-Ruiz M (2016) Emponzoñamiento causado por mordedura de la serpiente *Bothriechis schlegelii*. Reporte de dos casos ocurridos en Colombia. Case Reports 3: 1–8. https://doi.org/10.15446/cr.v3n1.58625

García E (1896) Los ofidios venenosos del Cauca: métodos empíricos y racionales empleados contra los accidentes producidos por la mordedura de esos reptiles. Libreria é Imprenta de la V[da] de Ch. Bouret, Paris, 102 pp. https://doi.org/10.5962/bhl.title.12461

Gardner AL (1983) *Proechimys semispinosus* (Rodentia: Echimydae): distribution, type locality, and taxonomic history. Proceedings of the Biological Society of Washington 96: 134–144.

Garman S (1884) The reptiles and batrachians of North America. Memoirs of the Museum of Comparative Zoology at Harvard College 8: 1–185. https://doi.org/10.5962/bhl.title.10754

Gerhardt RP, Harris PM, Vásquez MA (1993) Food habits of nesting great black hawks in Tikal National Park, Guatemala. Biotropica 25: 349–352. https://doi.org/10.2307/2388794

Gómez A, Chacón D, Rodríguez S, Corrales G (2015) Reproduction and sperm storage in a captive female *Bothriechis schlegelii* (Serpentes: Viperidae) in Costa Rica. Mesoamerican Herpetology 2: 581–584.

Günther A (1858) Second list of cold-blooded vertebrata collected by Mr. Fraser in the Andes of western Ecuador. Proceedings of the Zoological Society of London 1859: 402–422.

Gutiérrez JM, Chaves F (1980) Efectos proteolítico, hemorrágico y mionecrótico de los venenos de serpientes costarricenses de los géneros *Bothrops*, *Crotalus* y *Lachesis*. Toxicon 18: 315–321. https://doi.org/10.1016/0041-0101(80)90011-2

Hahn C, Bachmann L, Chevreux B (2013) Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads–a baiting and iterative mapping approach. Nucleic Acids Research 41: e129-e129. https://doi.org/10.1093/nar/gkt371

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965–1978. https://doi.org/10.1002/joc.1276

Hurtado-Gómez JP (2009) Patrones de variación morfológica de *Bothriechis schlegelii* (Serpentes:Viperidae) en un gradiente altitudinal. BSc thesis, Universidad de Antioquia, 39 pp.

IDEAM (2014) Mapa de cobertura de la tierra adaptada para Colombia.

IUCN (2012) IUCN Red List categories and criteria: Version 3.1. Second edition. IUCN Species Survival Commission, Gland and Cambridge, 32 pp.

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

Kuch U, Freire A (1995) Bemerkungen zur geographischen Verbreitung und Variabilität von Schlegels Palmen-Lanzenotter, *Bothriechis schlegelii* (Berthold, 1846), in Ecuador. Herpetozoa 8: 49–58.

Kuch U, Mebs D, Gutiérrez JM, Freire A (1996) Biochemical and biological characterization of the Ecuadorian pitvipers (genera *Bothriechis*, *Bothriopsis*, *Bothrops*, and *Lachesis*). Toxicon 34: 714–717. https://doi.org/10.1016/0041-0101(96)00016-5

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2016) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34: 772–773. https://doi.org/10.1093/molbev/msw260

Laurencio D (2005) *Bothriechis schlegelii* (Eyelash Palm Pit-viper, Bocacá). Attempted predation. Herpetological Review 36: 188.

Leenders T (2019) Reptiles of Costa Rica: a field guide. Cornell University Press, Ithaca, 625 pp. https://doi.org/10.7591/9781501739545

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R (2009) The sequence alignment/map format and SAMtools. Bioinformatics 25: 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Lomonte B, Escolano J, Fernández J, Sanz L, Angulo Y, Gutiérrez JM, Calvete JJ (2008) Snake venomics and antivenomics of the arboreal Neotropical pitvipers *Bothriechis lateralis* and *Bothriechis schlegelii*. Journal of Proteome Research 7: 2445–2457. https://doi.org/10.1021/pr8000139

Lomonte B, Tsai WC, Bonilla F, Solórzano A, Solano G, Angulo Y, Gutiérrez JM, Calvete JJ (2012) Snake venomics and toxicological profiling of the arboreal pitviper *Bothriechis supraciliaris* from Costa Rica. Toxicon 59: 592–599. https://doi.org/10.1016/j.toxicon.2012.01.005

Lotzkat S (2014) Diversity, taxonomy, and biogeography of the reptiles inhabiting the highlands of the Cordillera Central (Serranía de Talamanca and Serranía de Tabasará) in western Panama. PhD thesis, Goethe-Universität in Frankfurt am Main, 931 pp.

MAE (2012) Línea base de deforestación del Ecuador continental. Ministerio del Ambiente del Ecuador, Quito, 30 pp.

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. Journal 17: 10–12. https://doi.org/10.14806/ej.17.1.200

Mason AJ, Grazziotin FG, Zaher H, Lemmon AR, Moriarty Lemmon E, Parkinson CL (2019) Reticulate evolution in nuclear Middle America causes discordance in the phylogeny of palm-pitvipers (Viperidae: *Bothriechis*). Journal of Biogeography 46: 833–844. https://doi.org/10.1111/jbi.13542

Mekbel ST, Céspedes R (1963) Las lesiones renales en el ofidismo. Acta Médica Costarricense 6: 111–118.

Meza-Ramos PM, Almendáriz AL, Yánez-Muñoz MH (2019) Datos sobre la dieta de *Bothriechis schlegelii* (Berthold, 1846) (Serpentes-Viperidae) en el occidente del Ecuador. Boletín Técnico 6: 15–18.

Morgan, R, Barrio-Amorós CL (2015) *Bothriechis schlegelii*: diet. Mesoamerican Herpetology 2: 345–347.

Murphy JB, Mitchell LA (1984) Miscellaneous notes on the reproductive biology of reptiles. Thirteen varieties of the genus *Bothrops* (Serpentes, Crotalidae). Acta Zoologica et Pathologica Antverpiensia 78: 199–214.

Murphy RW, Crawford AJ, Bauer AM, Che J, Donnellan SC, Fritz U, Haddad CFB, Nagy ZT, Poyarkov NA, Vences M, Wang W, Zhang Y (2013) Cold Code: The global initiative to DNA barcode amphibians and non-avian reptiles. Molecular Ecology Resources 13(2): 161–167. https://doi.org/10.1111/1755-0998.12050

Myers CW, Böhme W (1996) On the type specimens of two Colombian poison frogs described by A. A. Berthold (1845), and their bearing on the locality "Provinz Popayan". American Museum Novitates 3185: 1–20.

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

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 835–838. https://doi.org/10.1038/35002501

Natera-Mumaw M, Esqueda-González LF, Castelaín-Fernández M (2015) Atlas serpientes de Venezuela. Dimacofi Negocios Avanzados S.A., Santiago de Chile, 456 pp.

Noonan BP, Chippindale PT (2006) Dispersal and vicariance: the complex evolutionary history of boid snakes. Molecular Phylogenetics and Evolution 40: 347–358. https://doi.org/10.1016/j.ympev.2006.03.010

Otero R, Tobón GS, Gómez LF, Osorio R, Valderrama R, Hoyos D, Urreta JE, Molina S, Arboleda JJ (1992a) Accidente ofídico en Antioquia y Chocó. Acta Médica Colombiana 17: 229–249.

Otero R, Osorio RG, Valderrama R, Giraldo CA (1992b) Efectos farmacológicos y enzimáticos de los venenos de serpientes de Antioquia y Chocó (Colombia). Toxicon 30: 611–620. https://doi.org/10.1016/0041-0101(92)90855-Y

Padial JM, Miralles A, de la Riva I, Vences M (2010) The integrative future of taxonomy. Frontiers in Zoology 7: 16. https://doi.org/10.1186/1742-9994-7-16

Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991) The simple fool's guide to PCR, version 2.0. University of Hawaii, Honolulu, 94 pp.

Peñafiel N, Flores DM, Rivero De Aguilar J, Guayasamin JM, Bonaccorso E (2020) A cost-effective protocol for total DNA isolation from animal tissue. Neotropical Biodiversity 5: 69–74. https://doi.org/10.1080/23766808.2019.1706387

Pesantes OS (1994) A method for preparing hemipenis of preserved snakes. Journal of Herpetology 28: 93–95. https://doi.org/10.2307/1564686

Peters JA, Orejas-Miranda B (1970) Catalogue of Neotropical Squamata: part I, snakes. Bulletin of the United States National Museum, Washington, D.C., 347 pp. https://doi.org/10.5962/bhl.title.46653

Peters WCH (1859) Über die von Hrn. Dr. Hoffmann in Costa Rica gesammelten und an das Königl. Zoologische Museum gesandten Schlangen. Monatsberichte der Königlichen Preussische Akademie des Wissenschaften zu Berlin 1859: 275–278.

Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. Ecological Modelling 190: 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Pla D, Sanz L, Sasa M, Acevedo ME, Dwyer Q, Durban J, Pérez A, Rodriguez Y, Lomonte B, Calvete JJ (2017) Proteomic analysis of venom variability and ontogeny across the arboreal palm-pitvipers (genus *Bothriechis*). Journal of Proteomics 152: 1–12. https://doi.org/10.1016/j.jprot.2016.10.006

Peterson AT, Soberon JR., Pearson RG, Anderson RP, Martínez-Meyer E, Nakamura M, Bastos Araújo M (2011) Ecological niches and geographic distributions. Princeton University Press, Princeton, 328 pp. https://doi.org/10.23943/princeton/9780691136868.003.0003

Posada Arango A (1889a) Apuntamientos para la ofiología Colombiana. Anales de la Academia de Medicina de Medellín 2: 45–49.

Posada Arango A (1889b) Note sure quelques solénoglyphes de Colombie. Bulletin de la Société Zoologique de France 1889: 343–345.

Posada Arango A (1909) Las serpientes. In: Molina CA (Ed.) Estudios científicos del Doctor Andrés Posada con algunos otros escritos suyos sobre diversos temas y con ilustraciones y grabados. Imprenta Oficial, Medellín, 252–284.

Prezotto-Neto JP, Kimura LF, Alves AF, Gutiérrez JM, Otero R, Suárez AM, Santoro ML, Barbaro KC (2016) Biochemical and biological characterization of *Bothriechis schlegelii* snake venoms from Colombia and Costa Rica. Experimental Biology and Medicine 241: 2075–2085. https://doi.org/10.1177/1535370216660214

Rambaut A, Suchard M, Drummond AJ (2022) Tracer version 1.7.1 http://www.beast.community

Rand AS, Myers CW (1990) The herpetofauna of Barro Colorado Island, Panama: an ecological summary. In: Gentry AH (Ed.) Four Neotropical fainforests. Yale University Press, New Haven, 386–409.

Renner IW, Warton DI (2013) Equivalence of MAXENT and poisson point process models for species distribution modeling in ecology. Biometrics 69: 274–281. https://doi.org/10.1111/j.1541-0420.2012.01824.x

Rojas-Morales JA (2012) Snakes of an urban-rural landscape in the central Andes of Colombia: species composition, distribution, and natural history. Phyllomedusa 11: 135–154. https://doi.org/10.11606/issn.2316-9079.v11i2p135-154

Ronquist F, Huelsenbeck JP (2013) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Royle JA, Chandler RB, Yackulic C, Nichols JD (2012) Likelihood analysis of species occurrence probability from presence-only data for modelling species distributions. Methods in Ecology and Evolution 3: 545–554. https://doi.org/10.1111/j.2041-210X.2011.00182.x

Taylor EH (1954) Further studies on the serpents of Costa Rica. University of Kansas Science Bulletin 36: 673–800.

Townsend J, Medina-Flores M, Wilson LD, Jadin J, Austin J (2013) A relict lineage and new species of green palm-pitviper (Squamata, Viperidae, *Bothriechis*) from the Chortís Highlands of Mesoamerica. ZooKeys 298: 77–105. https://doi.org/10.3897/zookeys.298.4834

Savage JM (2002) The amphibians and reptiles of Costa Rica, a herpetofauna between two continents, between two seas. The University of Chicago Press, Chicago, 934 pp.

Sclater PL (1859) List of the first collection of birds made by Mr. Louis Fraser at Pallatanga, Ecuador, with notes and descriptions of new species. Proceedings of the Zoological Society of London 27: 135–147.

Seifert RP (1983) *Bothrops schlegelii* (oropel [gold morph], bocaracá, eyelash viper, palm viper). In: Janzen DH (Ed.) Costa Rican natural history. University of Chicago Press, Chicago, 384–385.

Sevilla-Sánchez MJ, Ayerbe-González S, Bolaños-Bolaños E (2021) Aspectos biomédicos y epidemiológicos del accidente ofídico en el departamento del Cauca, Colombia, 2009–2018. Biomédica 41: 314–337. https://doi.org/10.7705/biomedica.5853

Soberón J, Peterson TA (2005) Interpretation of models of fundamental ecological niches and species' distributional areas. Biodiversity Informatics 2: 1–10. https://doi.org/10.17161/bi.v2i0.4

Solórzano A (2004) Serpientes de Costa Rica. Distribución, taxonomía e historia natural. Universidad de Costa Rica, San José, 792 pp.

Solórzano A, Gómez LD, Monge-Nájera J, Crother BI (1998) Redescription and validation of *Bothriechis supraciliaris* (Serpentes: Viperidae). Revista de Biología Tropical 46: 453–462. https://doi.org/10.15517/rbt.v46i2.19701

Solórzano A, Porras LW, Chaves G, Acosta Chaves V (2021) *Bothriechis supraciliaris*. The IUCN red list of threatened species. https://doi.org/10.2305/IUCN.UK.2021-3.RLTS.T203661A15875283.en

Sorrell GG (2007) Natural history and conservation of the Eyelash Palm-Pitviper (*Bothriechis schlegelii*) in Western Panama. MSc thesis, Auburn University, 54 pp.

Sorrell GG (2009) Diel movement and predation activity patterns of the Eyelash Palm-Pitviper, *Bothriechis schlegelii*. Copeia 2009: 105–109. https://doi.org/10.1643/CE-06-284

Steindachner F (1870) Herpetologische Notizen (II). Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften 62: 326–350.

Valencia JH, Garzón-Tello K, Barragán-Paladines ME (2016) Serpientes venenosas del Ecuador: sistemática, taxonomía, historial natural, conservación, envenenamiento y aspectos antropológicos. Fundación Herpetológica Gustavo Orcés, Quito, 653 pp.

Wallach V, Williams KL, Boundy J (2014) Snakes of the World: a catalogue of living and extinct species. Taylor and Francis, CRC Press, Boca Raton, 1237 pp.

Wüster W, Salomao MG, Quijada-Mascareñas JA, Thorpe RS, BBBSP (2002) Origins and evolution of the South American pit viper fauna: evidence from mitochondrial DNA sequence analysis. In: Campbell JA, Brodie ED (Eds) Biology of the vipers. Eagle Mountain Publishing, Eagle Mountain, 112–128.

Zaher H (1999) Hemipenial morphology of the South American xenodontine snakes, with a proposal for a monophyletic Xenodontinae and a reappraisal of Colubroid hemipenis. Bulletin of the American Museum of Natural History 240: 1–168.

Zaher H, Grazziotin FG, Cadle JE, Murphy RW, Moura-Leite JC, Bonatto SL (2009) Molecular phylogeny of advanced snakes (Serpentes, Caenophidia) with an emphasis on South American xenodontines: a revised classification and descriptions of new taxa. Papéis Avulsos de Zoologia 49: 115–153. https://doi.org/10.1590/S0031-10492009001100001

Zipkin EF, DiRenzo GD, Ray JM, Rossman S, Lips KR (2020) Tropical snake diversity collapses after widespread amphibian loss. Science 367: 814–816. https://doi.org/10.1126/science.aay5733

Appendix 1

Table A1. List of PCR and sequencing primers and their respective PCR conditions (denaturation, annealing, extension and number of corresponding cycles) used in this study. All PCR protocols included an initial 1.5–3 min step at 94 °C and a final extension of 7–10 min at 72 °C.

Locus	Primer	Sequence (5'-3')	Reference	PCR profile:
12S	H1557mod	GTACRCTTACCWTGTTACGACTT	Zaher et al. (2009)	93 °C (1 min), 54 °C (1 min), 72 °C (2–5 min) [x25–40]
	L1091mod	CAAACTAGGATTAGATACCCTACTAT		
16S	16Sar-L	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)	94 °C (45 sec), 53 °C (45 sec), 72 °C (1 min) [x30]
	16Sbr-H-R	CCGGTCTGAACTCAGATCACGT		
COI	RepCOI-F	TNTTMTCAACNAACCACAAAGA	Murphy et al. (2013)	94 °C (3 min), 48.5 °C (30 sec), 72 °C (1 min) [x40]
	RepCOI-R	ACTTCTGGRTGKCCAAARAATCA		
Cytb	L14910	GACCTGTGATMTGAAAACCAYCGTTGT	Burbrink et al. (2000)	94 °C (1 min), 58 °C (1 min), 72 °C (2 min) [x30–36]
	H16064	CTTTGGTTTACAAGAACAATGCTTTA		
ND4	ND4	CACCTATGACTACCAAAAGCTCATGTAGAAGC	Arévalo et al. (1994)	94 °C (25 sec), 56 or 60 °C (1 min), 72 °C (2 min) [x25–30]
	Leu	CATTACTTTTACTTGGATTTGCACCA		
NT3	NT3-F3	ATATTTCTGGCTTTTCTCTGTGGC	Noonan and Chippindale (2006)	94 °C (30 sec), 51 °C (sec), 72 °C (90 sec) [x30]
	NT3-R4	GCGTTTCATAAAAATATTGTTTGACCGG		
RAG1	Snake_RAG1_F	AGCTGCAGYCARTAYCAYAARATGTA	This work	95 °C (20 sec), 50 °C (25 sec), 72 °C (2 min) [x40]
	Snake_RAG1_R	AACTCAGCTGCATTKCCAATRTCA		

Supplementary material 1

Morphological and locality data for specimens of *Bothriechis* examined either directly or indirectly through digital photographs

Authors: Alejandro Arteaga
Data type: xlsx
Explanation note: Terminology for cephalic shields is explained in Fig. 1. Codes: SVL = snout-vent length; M = Male, F = Female.

Link: https://doi.org/10.3897/evolsyst.8.114527.suppl1

Supplementary material 2

GenBank accession numbers

Authors: Alejandro Arteaga
Data type: xls
Explanation note: GenBank accession numbers for loci and terminals of taxa and outgroups sampled in this study. Novel sequence data produced in this study are marked with an asterisk (*).

Link: https://doi.org/10.3897/evolsyst.8.114527.suppl2

Supplementary material 3

Locality data used to create distribution maps

Authors: Alejandro Arteaga
Data type: xls

Link: https://doi.org/10.3897/evolsyst.8.114527.suppl3