



A new species of *Bothrops* (Serpentes: Viperidae: Crotalinae) from Pampas del Heath, southeastern Peru, with comments on the systematics of the *Bothrops newwiedi* species group

PAOLA A. CARRASCO^{1,2,10}, FELIPE G. GRAZZIOTIN³, ROY SANTA CRUZ FARFÁN⁴,
CLAUDIA KOCH⁵, JOSÉ ANTONIO OCHOA^{6,7,8}, GUSTAVO J. SCROCCHI⁹,
GERARDO C. LEYNAUD^{1,2} & JUAN C. CHAPARRO^{6,7}

¹Universidad Nacional de Córdoba, Facultad de Ciencias Exactas, Físicas y Naturales, Centro de Zoología Aplicada, Rondeau 798, Córdoba 5000, Argentina.

²Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Diversidad y Ecología Animal (IDEA), Rondeau 798, Córdoba 5000, Argentina.

³Laboratório de Coleções Zoológicas, Instituto Butantan, Avenida Vital Brasil, 1500, São Paulo, SP, Brazil.

⁴Área de Herpetología, Museo de Historia Natural de la Universidad Nacional de San Agustín, 051, Arequipa 04000, Perú.

⁵Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany.

⁶Museo de Biodiversidad del Perú, Urbanización Mariscal Gamarra A-61, Zona 2, Cusco, Perú.

⁷Museo de Historia Natural de la Universidad Nacional de San Antonio Abad del Cusco, Paraninfo Universitario (Plaza de Armas s/n), Cusco, Perú.

⁸Frankfurt Zoological Society-Perú, Residencial Huancaro, Los Cipreses H-21, Santiago, Cusco, Perú.

⁹UEL-CONICET and Fundación Miguel Lillo, Miguel Lillo 251, San Miguel de Tucumán, Tucumán, Argentina

¹⁰Corresponding author. E-mail: ammodytoides@gmail.com

Abstract

We describe a new species of pitviper of the genus *Bothrops* from the Peruvian Pampas del Heath, in the Bahuaja-Sonene National Park. Pampas del Heath is an area of seasonally flooded savannas and a northwestern extension of the Gran Chaco Boliviano-Paraguayo. The new species is easily distinguished from its congeners by the exclusive combination of dorsal color pattern of body consisting of small C-shaped blotches, postocular stripe originating posteriorly to the eye, covering posterior supralabials, dorsum of the head with paired markings arranged symmetrically, venter cream heavily speckled with brown, prelacunal scale discrete in contact with second supralabial, three to five prefoveals, subfoveal single usually present, postfoveals absent to two, canthals two, seven intersupraoculars, one or two suboculars, two or three postoculars, seven or eight supralabials, nine to eleven infralabials, 26–27 interrials, 23–25 middorsal scales, 172 ventrals in the female and 169–173 in males, 45 subcaudals in the female and 50 in males. We performed separate and combined phylogenetic analyses based on morphology and five mitochondrial genes and recovered the new species as a member of the *Bothrops newwiedi* species group. All lineages of this clade inhabit the South American dry diagonal. This novel species of pitviper increases the known diversity of the genus *Bothrops* and adds to the number of described taxa from the unique and scarcely known ecosystem of Pampas del Heath.

Key words: *Bothrops mattogrossensis*, species delimitation, molecular phylogeny, morphological characters, total evidence

Introduction

The pitvipers of the genus *Bothrops* Wagler 1824, which includes 45 species (Uetz & Hošek 2018), inhabit most of the ecoregions along South America. Some of these ecoregions are included in the area known as the South American dry diagonal, which extends through the phytogeographical domains of the Caatinga, Cerrado and Chaco, from northeastern Brazil to the center of Argentina. The diagonal is a belt of seasonally dry forests, including other types of vegetation such as wet forests and savannas, located between the Amazon and Atlantic rain

forests (Vanzolini 1963; Prado & Gibbs 1993; Oliveira-Filho & Ratter 2002; Pennington *et al.* 2006; Werneck 2011). Most species of *Bothrops* inhabiting the dry diagonal belong to the *B. alternatus* Duméril, Bibron & Duméril 1854 and *B. neuwiedi* Wagler 1824 species groups (Campbell & Lamar 2004; Silva 2004; Silva & Rodrigues 2008; Nogueira *et al.* 2010; Fenker *et al.* 2014; Machado *et al.* 2014; Araújo 2015). Herein, we describe a new species of the *Bothrops neuwiedi* group from the Pampas del Heath in the Bahuaja-Sonene National Park, a natural protected area in southeastern Peru, near the Bolivian border.

The *Bothrops neuwiedi* group of species is distributed from northeastern Brazil to southern Argentina, through Bolivia, Peru, Paraguay and Uruguay, and includes: the former species, which inhabits the mountains in the southeast Brazilian coast; *B. diporus* Cope 1862 and *B. mattogrossensis* Amaral 1925, which occur mainly in the Chaco and Pantanal; *B. erythromelas* Amaral 1923, restricted to the Caatinga; *B. lutzi* Miranda-Ribeiro 1915, *B. marmoratus* Silva & Rodrigues 2008 and *B. pauloensis* Amaral 1925, which occur mainly in the Cerrado; and *B. pubescens* Cope 1870, which inhabit the Uruguayan savannas of southern Brazil and Uruguay (Silva 2004; Silva & Rodrigues 2008; Machado *et al.* 2014). The Peruvian part of Pampas del Heath, where the new species was found, is an area of 6,549 ha of humid tropical savanna or seasonally flooded grassland, interspersed with small woodlots and areas of palm swamp (Williams *et al.* 2012); it is a northwestern extension of the Gran Chaco of Bolivia and Paraguay and a natural limit with the surrounding Amazonia. The area is a National Sanctuary protected since 1983, and currently is part of the Bahuaja-Sonene National Park, created in 1996. The ecosystem of these “pampas” of Peru remains largely unknown, although a few biological surveys have demonstrated the singularity of the area and the presence of many potential new species of amphibians and reptiles (Roberto Gutiérrez pers. comm.).

Shortly before the foundation of the sanctuary, Hoffmann *et al.* (1976) reported the presence of two mammal species, the marsh deer (*Blastoceros dichotomus* Illiger 1815) and the maned wolf (*Chrysocyon brachyurus* Illiger 1815), in Pampas del Heath, which was the first evidence of these species in Peruvian territory. In addition, Graham *et al.* (1980) reported the first records for Peru of 17 species of birds from the same region. A multidisciplinary survey, performed in Pampas del Heath in 1996 as a Rapid Assessment Program (RAS), reported new records for Peru of birds, mammals, amphibians and reptiles (Stotz *et al.* 2002; Cadle *et al.* 2002; Luna *et al.* 2002). In this study, we describe a newly discovered and distinct population of pitvipers of the genus *Bothrops* from Pampas del Heath, and use phylogenetic analyses of morphological and molecular characters to assess its systematic position as well as its taxonomic status. We recovered the new species as a member of the *Bothrops neuwiedi* group on the basis of both morphological and molecular evidence.

Material and methods

Material and collection procedures. We conducted field-work as a part of the biological monitoring program of the Bahuaja-Sonene National Park, which has been carried out every year since 2013. We euthanized the specimens with Halatal, we fixed them in formalin solution, and preserved them in ethanol 70%. We took tissues samples of muscle and preserved them in ethanol 96% for subsequent molecular studies.

Specimens and morphological study. We collected three specimens during 2013 and 2015, which were preserved in the MUBI and MUSA. We examined a fourth specimen housed in the MUSM. Sex was determined by examination of the presence/absence of hemipenes through a ventral incision at the base of the tail. We considered adult specimens those with snout-vent lengths ≥ 500 mm (Hartmann *et al.* 2004; Barros *et al.* 2014). We took measurements for morphometric characters using a digital caliper to the nearest 0.01 mm and later all measurements were rounded to only one decimal. We used a stereoscope and digital images for morphological examinations. We recorded coloration through observation of live specimens, photographs, and examination of alcohol-preserved specimens. We examined the hemipenial morphology of two males (MUBI 14679, MUSA 4350). Hemipenes were everted and prepared for examination *ex situ*, following standard procedures (Pesantes 1994; Zaher 1999). In the description, a slash (/) is used for counts from left/right sides of the body. For comparative purpose, we examined a total of 534 specimens of *Bothrops* and related crotaline genera (*Bothrocophias* Gutberlet & Campbell 2001, *Porthidium* Cope 1871, *Cerrophidion* Campbell & Lamar 1992, *Atropoides* Werman 1992, *Bothriechis* Peters 1859, *Lachesis* Daudin 1803 and *Crotalus* Linnaeus 1758) (Appendix I). The comparative material is housed in scientific collections whose institutional abbreviations are as listed in

Sabaj (2016), except for MUBI (Museo de Biodiversidad del Perú, Cusco, Perú) and CZA (Centro de Zoología Aplicada, Córdoba, Argentina). We followed the taxonomy of Carrasco *et al.* (2012) and Silva & Rodrigues (2008) for alpha and beta taxonomy adopted for the genus *Bothrops* and the *B. neuwiedi* group, respectively.

We recorded continuous and discrete characters from external morphology (most from scalation and coloration) and hemipenial morphology. Our definition of characters of external morphology followed or were adapted [*] from Carrasco *et al.* (2012), while others were modified from Silva & Rodrigues (2008) [**] or defined in the present study [***]. Definition of characters of the hemipenis followed Pesantes (1989). We recorded the following morphological characters: scales between internasals***; loreals***; ventrals; subcaudals; intersupraoculars; scales contacting supraoculars; supralabials; infralabials; canthals; postcanthals; anterior intercanthals***; medial intercanthals; posterior intercanthals***; prefoveals; subfoveals; postfoveals; suboculars; postoculars; interoculabials between suboculars and 3rd–4th supralabials***; interoculabials between suboculars and 4th supralabial; interoculabials between suboculars and 4th–5th supralabials***; sublacunals***; dorsocephalic scales from the snout to the neck, longitudinally***; interrials; gulars; middorsal scales rows; distance between the eye and the anterior end of the head/length of the head; interorbital distance/maximum width of the head; internostril distance/width of the head; length of caudal spine/caudal length; postorbital stripe height (scored as number of scales at the level of 5th–6th supralabials); postorbital stripe length (scored as number of scales posteriorly to the rictus)***; supralabials encroached on by postorbital stripe; infralabials encroached on by postorbital stripe***; inferior/superior margins of mid-dorsolateral blotch**; height of mid-dorsolateral blotch**; minimum space between mid-dorsolateral blotches**; dorsolateral blotches***; length of hemipenial body/total hemipenial length; length of capitulum/length of hemipenial lobe; shape of internasals (rounded, oval)***; ventral markings (immaculate, heavily speckled on all the ventral region, heavily speckled on the lateroventral region, slightly speckled on the lateroventral region, very slightly speckled on the lateroventral region to almost immaculate)***; gular coloration (strongly pigmented, slightly pigmented, immaculate)***; ventral margin of postorbital stripe starts posterior, below, or anterior to the orbit; pair of parietal blotches on the head (absent, rounded, elongated posteriorly)***; pair of occipital blotches (elongated posteriorly beyond the neck, elongated posteriorly to the neck)***; superior portion of dorsolateral blotches (rectangular, subtriangular, rounded)***; markings on gulars (absent, diffused markings, conspicuous stripes); distinct white spots on posterior infralabials and gulars (absent, present); dorsal color pattern (trapezoidal “*Bothrocophias microphthalmus*-type” markings, rectangular “*Bothrops ammodytoides*-type” markings, C-shaped “*B. alternatus*-type” markings, trapezoidal “*B. neuwiedi*-type” markings, trapezoidal “*B. jararaca*-type” markings, trapezoidal “*B. jararacussu*-type” markings, banded “*B. taeniatus*-type” markings, trapezoidal “*B. atrox*-type” markings)*; green body coloration (absent, present); supraoculars (oval, rounded, elongated)*; middle preocular (not in contact with the orbit, contacting orbit); upper preocular (does not contribute, contributes to the canthus rostralis); lower preocular (not in contact, in contact with the orbit)***; prelacunal (discrete, fused with second supralabial)*; prelacunal discrete (contacting third supralabial, contacting second supralabial, separated from second supralabial)*; subfoveals (incomplete row, complete row)*; loreal (not elongated, elongated); internasals (not elongated, elongated); rostral (triangular or subtriangular, quadrangular, trapezoidal); rostral (not elevated, elevated); canthus rostralis (not elevated, elevated forming a distinct ridge); intersupraoculars (smooth or slightly keeled, keeled); canthorostrals (absent, present); canthals (same size or slightly smaller than internasals, bigger than internasals and rounded, bigger than internasals and elongated)*; intercanthals (smooth or slightly keeled, keeled)***; upper preocular (entire, divided); tuberculate keels on mid-posterior dorsal scales (absent, present); middle preocular and supralacunal (fused, not fused); lowest preocular and sublacunal (not fused, partially or totally fused); intersupraocular fusions in plate-like scales (absent, usually present); subcaudals (entire, entire and divided, divided)*; body compressed laterally (absent, present)***; rattle (present, absent); hemipenis (U-shaped and lobes well separated, not U-shaped and lobes close to each other); lobes of the hemipenis (subcylindrical, cylindrical, fusiform)*; orientation of hemipenial lobes (parallel, divergent); distribution of hemipenial spines (heterogeneous, homogeneous)*; big sized spines in the hemipenis (absent, positioned distally, positioned proximally); hemipenial spines (straight or curved, hooked); intralobular spines (absent, distally in the intralobular area, along the intralobular area)*; microornamentation in the capitulum (in all calyces, in half or more of the calyces, in the basal calyces, absent); microornamentation in the ventral surface of the hemipenial body (in all the surface, in part of the surface); microornamentation in the dorsal surface of the hemipenial body (in part of the surface, in all the surface, absent)*; microornamentation in the intrasulcar area (absent, present); swollen dorsal area in hemipenial body (absent, present); swollen dorsal area in

hemipenial body (well developed, poorly developed); lateral depression in hemipenial body (absent, present); lateral depression in hemipenial body (developed in both sides of the body, developed in one side of the body, poorly developed in one side of the body); swollen dorsal area in the intralobular area (absent, present)***.

Molecular sampling and phylogenetic approaches. To evaluate the systematic position of the new species within *Bothrops* we performed two different phylogenetic analyses, each with a different dataset. First, we analyzed a molecular dataset that included sequences of the new species, 43 species of *Bothrops* and nine outgroup taxa, based on three mitochondrial protein-coding genes (*cytb*: cytochrome b, *nd4*: NADH subunit 4, *cox1*: cytochrome c oxidase subunit I), two non-coding ribosomal genes (12S rRNA, 16S rRNA) and three tRNAs (histidine, serine, leucine) that flank the *nd4*. Given that preliminary morphological comparisons indicated that the new species shares synapomorphic characters with the *Bothrops neuwiedi* species group (see taxonomic account below), we hypothesized that the new species was a member of this clade, and to test such hypothesis we included all mitochondrial sequences available in GenBank for this group of species. Additionally, we generated 35 DNA sequences for 13 species of *Bothrops* to prepare our molecular matrix together with 441 DNA sequences retrieved from GenBank (Appendix 2). In this way, the analysis was performed using one terminal for each of 34 species of *Bothrops* and multiple terminals for each of the eight species of the *B. neuwiedi* group.

Using multiple terminals for the species of the *Bothrops neuwiedi* group allowed us, not only to evaluate the position of the new species, but also to identify geographic and morphologically cohesive lineages, which might have implications in the taxonomy of the group as currently known (*sensu* Silva & Rodrigues 2008). When possible, we revised the identification of the individuals used in molecular studies through the morphological evaluation of the voucher specimens (Appendix 2). Those molecular lineages that we found geographic and morphologically distinguishable were used as terminals in a subsequent phylogenetic analysis combining molecular and morphological data. The combined dataset included a morphological matrix conformed of continuous and discrete characters from the external morphology, hemipenial morphology and osteology. It was composed by the matrix used by Carrasco *et al.* (2012) with the addition of new data for some species and new characters (Appendix 3). Osteological characters were coded as missing data for the new species. The final combined dataset included 118 morphological characters (morphological matrix in Appendix 4) and the same 3143 aligned sites from the molecular matrix for 50 terminal taxa (which included the new species and all lineages for the *B. neuwiedi* group mentioned above) with a total of 923 parsimony-informative characters.

DNA sequencing and alignment. We extracted DNA from muscle following standard protocols (Hillis *et al.* 1996). Sequences were amplified via polymerase chain reaction (PCR) using the primers and protocols as described by Graziotin *et al.* (2012) and Machado *et al.* (2014). PCRs for coding genes were performed with addition of BSA (bovine serum albumin) to increase the efficiency of amplification. The annealing temperature for *cytb*, *nd4* and *cox1* ranged from 60°C to 58°C and 54°C for the rRNAs. The PCRs were purified with shrimp alkaline phosphatase and exonuclease I (GE Healthcare, Piscataway, NJ) and the sequences were processed using BigDye Terminator cycle sequencing kit in an ABI 373A sequencer (Applied Biosystems, Foster City, CA) at Laboratório Especial de Toxinologia Aplicada (LETA), Instituto Butantan, São Paulo, Brazil. Both strands were checked and contigs were assembled using Geneious 11.1.5 (Kearse *et al.* 2012). Sequences were aligned using MAFFT 1.3.6 (Katho *et al.* 2005) as implemented in Geneious. We used default parameters for gap opening and extension and the alignments for protein-coding genes were visually checked to verify the correct reading frame.

Phylogenetic methods. The analysis of our molecular dataset was performed using the maximum likelihood (ML) approach. We used PartitionFinder 2 (Lanfear *et al.* 2016) to choose the combined sets of partitioning schemes and models of molecular evolution based on the Akaike Information Criterion with correction (AICc). We treated the two rRNA genes and the tRNAs as separate partitions and partitioned protein coding genes by codon positions. We used the greedy search option in PartitionFinder, allowing only the selection GTR models as implemented in RAxML 8.2.3 (Stamatakis 2014) without any correction for proportion of invariant sites, as recommended in the RAxML's manual. We used RAxML 8.2.3 to perform the ML analyses conducting a rapid bootstrap analysis (1000 replicates) and search for the best scoring ML tree in the same run (option -f a).

We performed a combined molecular and morphological analysis under maximum parsimony (MP) using TNT 1.5 (Goloboff & Catalano 2016). Continuous morphological characters were analyzed without discretization, represented as ranges of two standard deviations around the mean (Goloboff *et al.* 2006) and standardized to the same range (0–2) to avoid scaling problems. Additionally, we performed separate analysis of the molecular and morphological partitions. The total evidence analysis was performed under the New Technology search option with

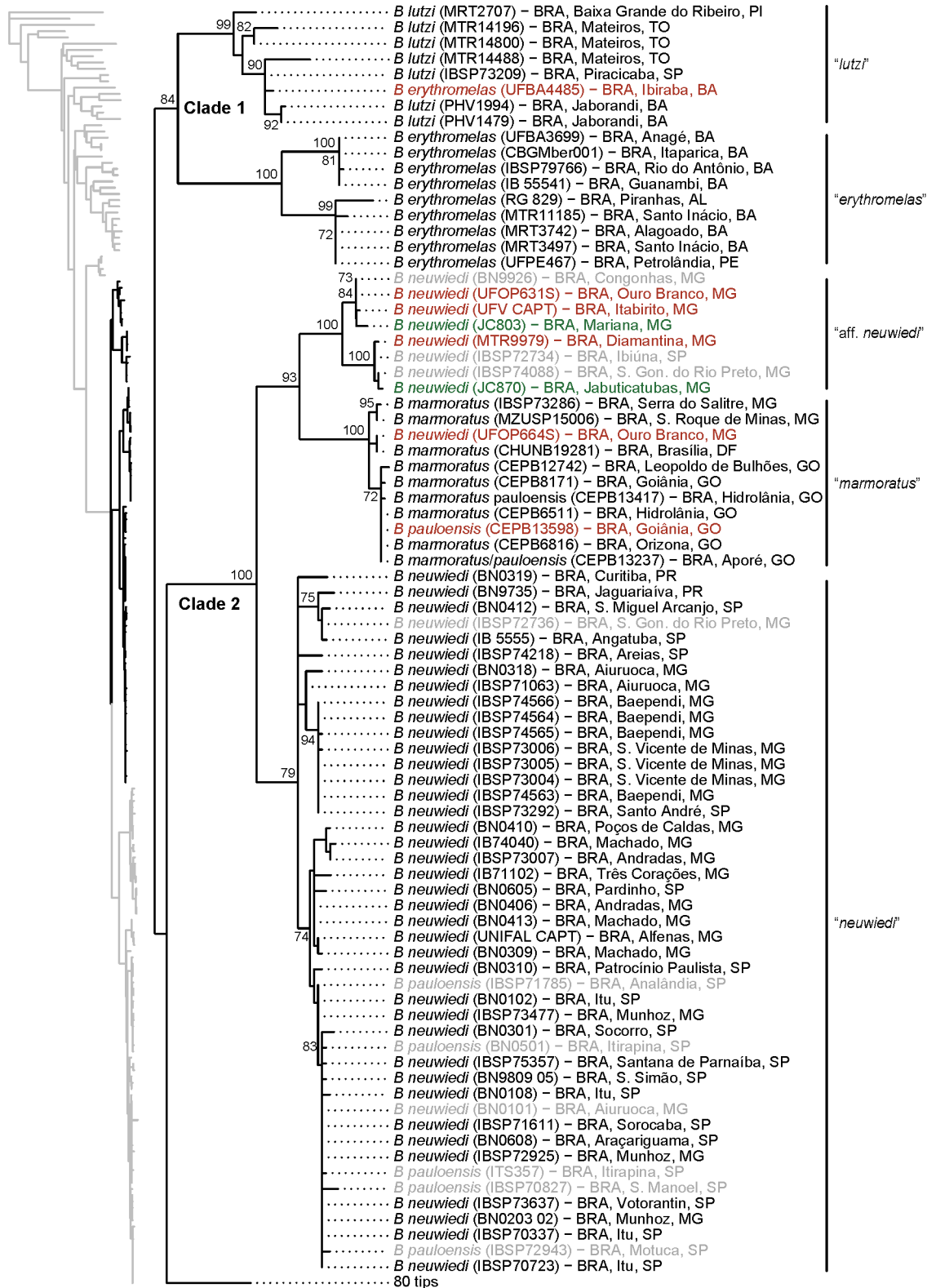
default parameters. Partitioned analyses were performed under the Traditional search option, using random addition sequences of Wagner trees, followed by the TBR algorithm, making 500 replications and saving up to 10 trees per replicate. In both total evidence and partitioned analyses, all characters were analyzed under equal weights and implied weights (Goloboff 1993; Goloboff *et al.* 2008). For implied weighting we used values for the concavity constant (k) between 8–15 in total evidence and molecular-only analysis and 3–10 in the morphology-only analysis. To evaluate branch support we calculated group frequencies using jackknife and bootstrap methods, performing 500 pseudoreplicates (10 random addition sequences each) followed by TBR swapping, saving up to 10 trees, and using a probability of elimination of P=0.36 for jackknife values.

Results

Analysis based on molecular evidence. Our final aligned dataset included 3143 base pairs (415 bp from 12S; 504 bp from 16S; 643 bp from *cox1*; 783 bp from *cytb*; 694 bp from *nd4*; and 104 bp from the tRNAs adjacent to *nd4*) and 204 terminal taxa. The ML analysis of mtDNA haplotypes (Fig. 1) recovered the new species within *Bothrops* and nested within the monophyletic *B. neuwiedi* group. *Bothrops neuwiedi* was recovered as paraphyletic, while *B. mattogrossensis*, *B. pauloensis*, *B. marmoratus*, and *B. diporus* were recovered as polyphyletic; the only species recovered as monophyletic was *B. pubescens*. Most, if not all, the paraphyly and polyphyly of species may be attributed to misidentification of samples (terminals in green and red in Fig. 1). The new species was recovered within a well-supported clade composed of individuals identified in GenBank as *Bothrops pauloensis* from northwestern Bolivia (Ñuflo de Chávez, department of Santa Cruz) and as *B. mattogrossensis* from Serra da Borda, western Brazil (Vila Bela da Santíssima Trindade, state of Mato Grosso) (Figs. 1, 2). The evaluation of the genetic distance between the new species and the close related individuals was difficult due to the lack of overlap among gene fragments sampled in our dataset. Between the specimen from Serra da Borda and the new species only a small fragment (270 bp) of the *cytb* is comparable, and we did not find any difference between these two sequences. On the other hand, when evaluating the *nd4* alignment the new species presents a p-distance of 0.0046 (three differences in 657 bp) from the individuals from Ñuflo de Chávez. Unfortunately, there is no sequence overlap for the individual from Serra da Borda and the individuals from Ñuflo de Chávez. Voucher specimens of the haplotypes from Ñuflo de Chávez and Serra da Borda were not available for morphological revision in this study. Then, we provisionally assigned these DNA sequences to a terminal named as *Bothrops* cf. **sp. nov.** and for the MP analysis we used the sequence of one of the specimens from Ñuflo de Chávez, and morphological characters from geographically close specimens (Beni Department, in northern Bolivia) that lacked DNA sequences.

From the tree topology obtained in the ML analysis, we were able to identify 15 geographically and morphologically distinguishable lineages within the *Bothrops neuwiedi* group (Fig. 1; see an estimated distribution in Fig. 2 and morphological characterization in Table 2). All lineages are well supported by bootstrap values. We assigned to *Bothrops diporus*, *B. erythromelas*, *B. lutzi*, *B. mattogrossensis*, *B. marmoratus*, *B. neuwiedi*, *B. pauloensis* and *B. pubescens* those lineages that included identified specimens from and/or near to the type locality of each species respectively. We named the rest of the lineages as follows: *Bothrops* aff. *diporus*, *B. aff. mattogrossensis* 1, *B. aff. mattogrossensis* 2, *B. aff. mattogrossensis* 3, *B. aff. neuwiedi*, *B. aff. pubescens*, and *B. cf. sp. nov.* We also reassigned some terminals to their specific lineages based on the re-identification of the voucher specimens (terminals in green in Fig. 1) or based on the phylogenetic position and the morphological characters of geographically similar individuals (terminals in red in Figure 1). It was not possible to assess the identification of some terminals in the molecular matrix, since they probably had their voucher specimens destroyed by the fire at Butantan Institute in 2010 (terminals in gray in Fig. 1).

The general topology of the ML tree (Fig. 1) indicates four main highly supported clades, as follows: clade 1 (84% of bootstrap value), composed by *B. lutzi* and *B. erythromelas*; clade 2 (100%), formed by *B. neuwiedi*, *B. aff. neuwiedi* and *B. marmoratus*; clade 3 (100%), including individuals of *B. mattogrossensis*, *B. aff. mattogrossensis* 1, *B. aff. mattogrossensis* 2 and *B. aff. mattogrossensis* 3; clade 4 (100%), comprised by *B. diporus*, *B. aff. diporus*, *B. pauloensis*, *B. pubescens*, *B. aff. pubescens*, *B. sp. nov.* and the individuals assigned to *B. cf. sp. nov.* Clade 1 is retrieved as the sister group of all the other clades, but with low bootstrap values (<70%). Clade 2 is the sister group of a clade composed by Clade 3 and Clade 4, both relationships were supported by low values of bootstrap.



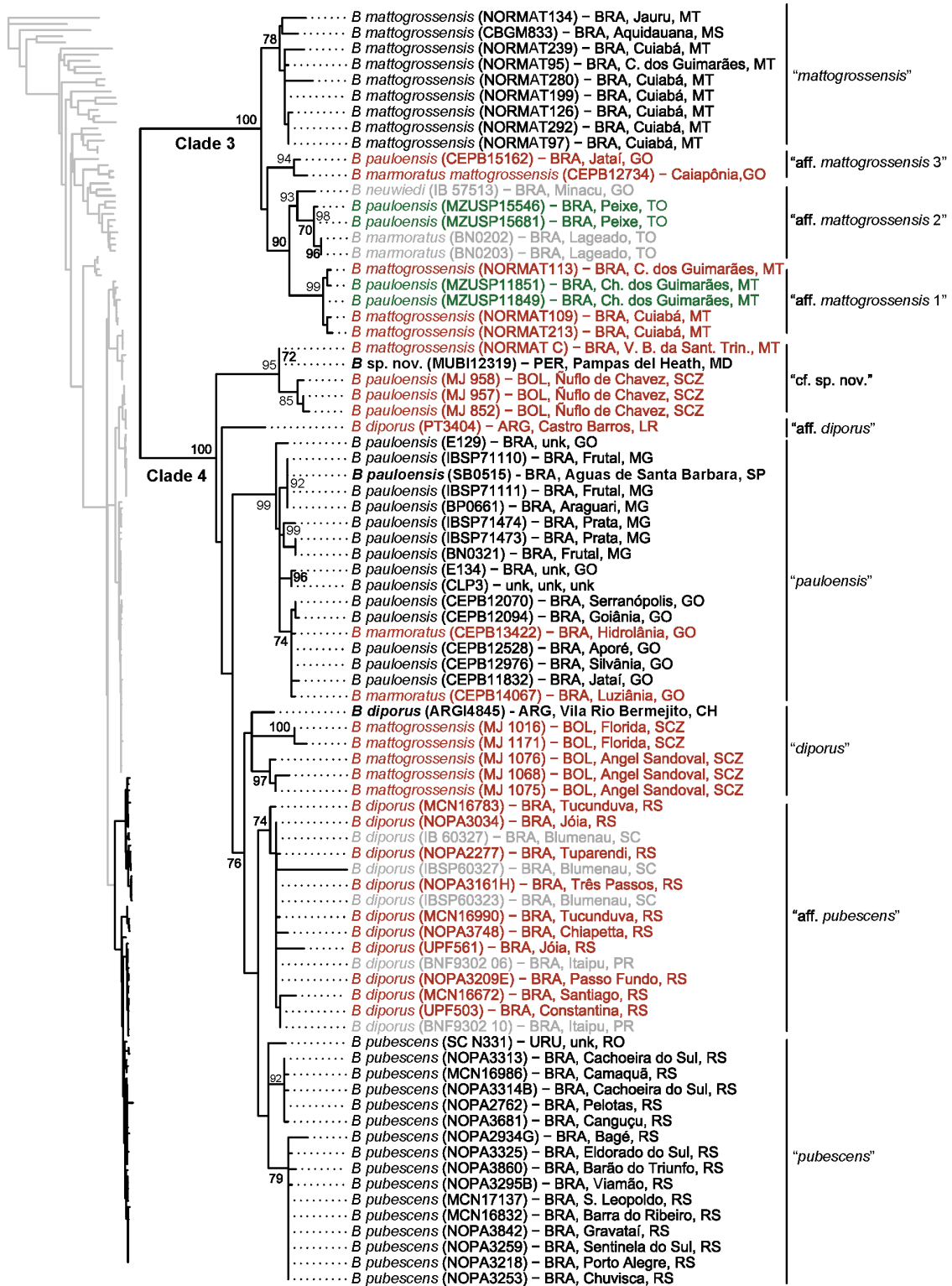


FIGURE 1. Maximum likelihood tree obtained in the analysis of mtDNA haplotypes, showing the relationships within the *Bothrops neuwiedi* species group. The topology of the complete ML tree is provided on the left. Numbers are bootstrap proportions. Color of terminal taxa: black=identification matches the assigned lineage, green= identification does not match the assigned lineage and voucher specimen was re-identified as belonging to the lineage after morphological examination, red= identification does not match the assigned lineage and voucher specimen could not be examined in this study, grey= identification does not match the assigned lineage and voucher specimens were lost during the fire at the Butantan collection in 2010.

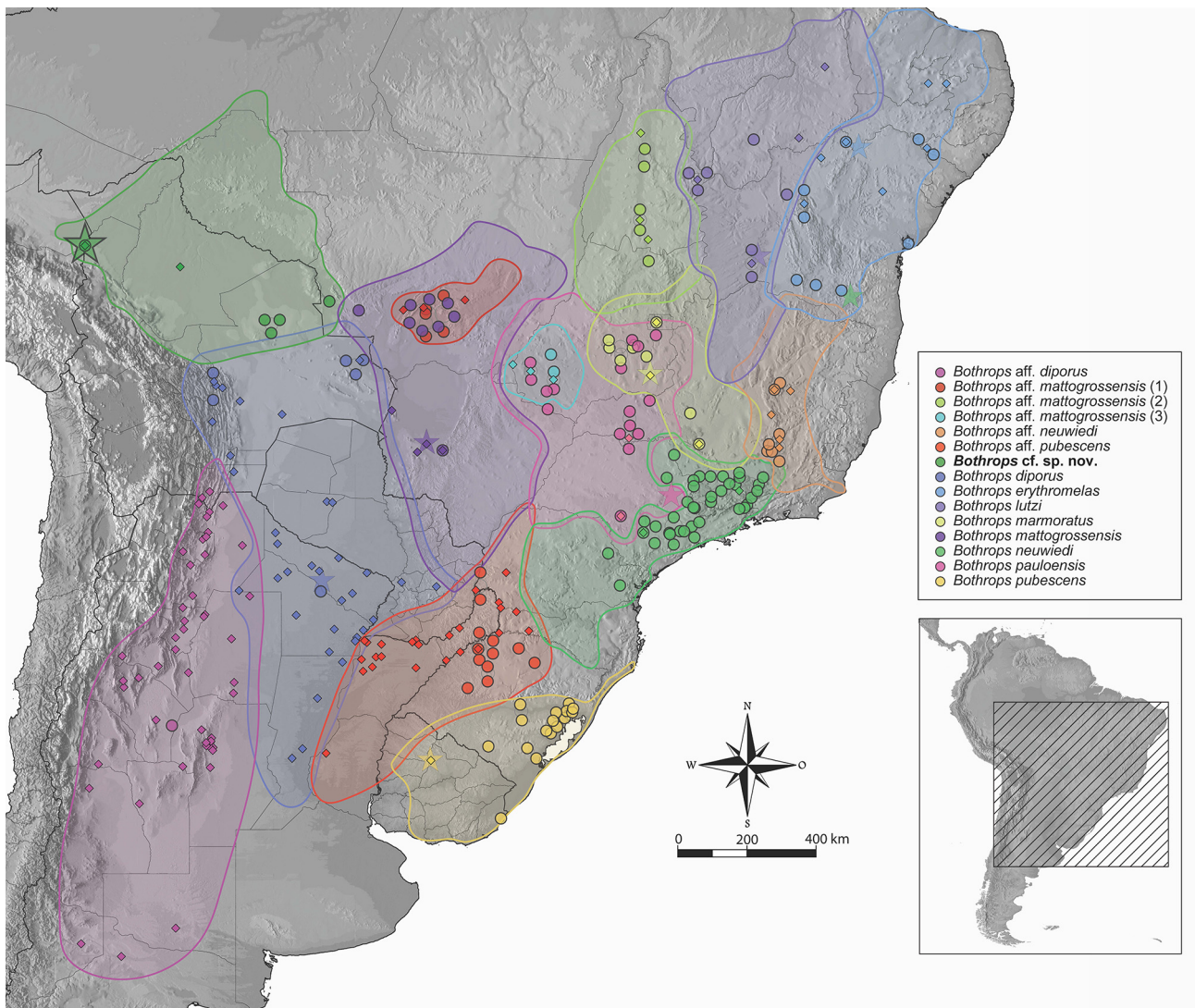


FIGURE 2. Distribution of the lineages of the *Bothrops neuwiedi* species group. Symbols are as follow: stars = type localities, circles = records of specimens with DNA samples, squares = records of specimens that were only examined morphologically.

Since the overlap among gene fragments is not complete in our dataset, we took the branch length/patristic distance (absolute time x mutation rate) among lineages as a proxy for the mitochondrial genetic distance. We estimated the mean branch length among the MRCAs (Most Recent Common Ancestor) for lineages within each main clade as follows: 0.0415 (4.1%) for clade 1, 0.0341 (3.4%) for clade 2, 0.0165 (1.7%) for clade 3 and 0.0221 (2.2%) for clade 4. Taking into account the average branch length of each terminal, the mean distance among lineages based on our ML tree are the following: 0.0473 (4.7%) for clade 1, 0.0360 (3.6%) for clade 2, 0.0199 (2.0%) for clade 3 and 0.0246 (2.5%) for clade 4. The complete table showing the distances among lineages can be seen in Appendix 5. The MRCA of the lineage that includes the new species presents a minimum distance of 0.0199 (2%) in relation to the MRCA of *B. aff. diporus* and a maximum distance of 0.1119 (11%) in relation to *B. marmoratus*. Therefore, the patristic distance estimated among the new species and the other lineages falls into the average distance range among species/lineages of the *B. neuwiedi* group (Appendix 5).

MP total evidence analyses. The MP total evidence analysis recovered the same single tree under both EW and IW (Fig. 3). Similarly to the results of molecular ML analyses, the topology recovered *Bothrops* *sp. nov.* within the *B. neuwiedi* group and, together with *B. cf. sp. nov.*, in a basal position to a clade conformed by *B. diporus*, *B. pauloensis*, *B. pubescens* and the related *B. aff. diporus* and *B. aff. pubescens*. The sister relationship between *Bothrops* *sp. nov.* and *B. cf. sp. nov.* was supported by two morphological synapomorphies (number of dorsolateral blotches and rounded or C-shaped dorsolateral blotches) and high jackknife and bootstrap values. The

clade conformed by *Bothrops sp. nov.*, *B. cf. sp. nov.*, *B. diporus*, *B. pauloensis*, *B. pubescens*, *B. aff. diporus* and *B. aff. pubescens* was supported by three morphological synapomorphies (number of anterior intercanthals, subfoveals, and length of capitulum/length of hemipenial lobe) and high jackknife and bootstrap values. Differences between the results of ML molecular and MP total evidence analyses involved the position of *Bothrops pauloensis* and the relationships among *B. mattogrossensis* and *B. aff. mattogrossensis* 1–3. The topology obtained for the *B. newwiedi* group in MP molecular-only analysis (Appendix 6) was the same than the one obtained in MP total evidence analyses. Morphology-only analyses, however, recovered different results (Appendix 7) in which (*Bothrops sp. nov.* + *B. cf. sp. nov.*) conformed a clade with *B. lutzi*, *B. marmoratus*, *B. mattogrossensis*, *B. aff. mattogrossensis* 1–3 and *B. pauloensis*. This relationship was supported by one morphological synapomorphy (venter heavily speckled) but unsupported by jackknife and bootstrap values.

Taxonomic decision. The new species was recovered in a distinct clade of geographically close specimens within the *Bothrops newwiedi* species group. This result supported the hypothesized membership of the new species to that group, and confirmed the distinctiveness of this population from Pampas del Heath, which we describe below as a new species.

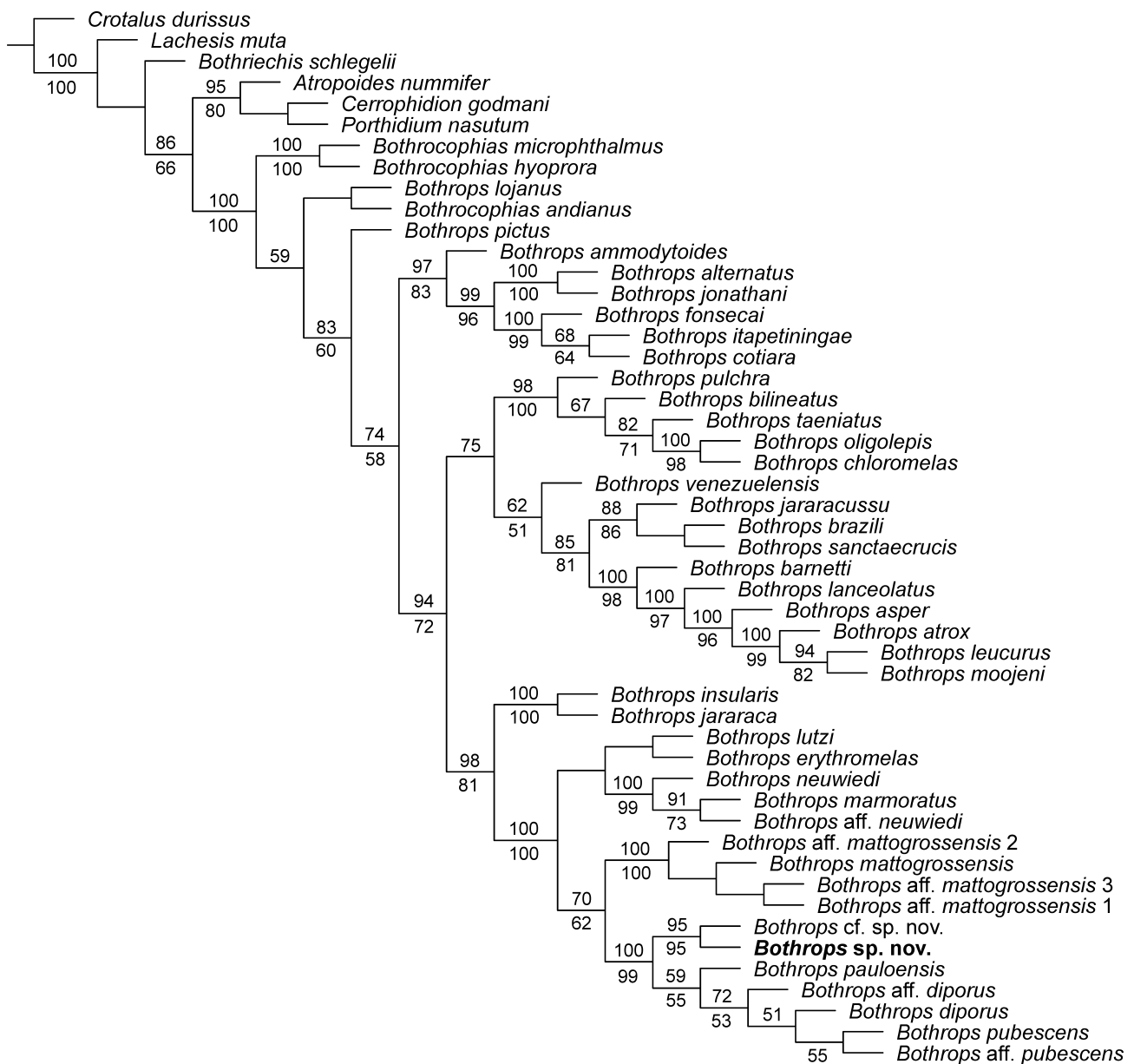


FIGURE 3. Maximum parsimony tree obtained in the analysis of total evidence under equal weights (single cladogram, length: 4153.215). Above and below nodes, jackknife and bootstrap proportions respectively. Values less than 50 % are not shown.

***Bothrops sonene* sp. nov.**

Figures 4–7, Table 1

Bothrops gr. *neuwiedi*: Cadle *et al.* 2002: 102 (MUSM 33453, from Pampa Juliaca, department of Madre de Dios, Peru).

Holotype. An adult female, MUBI 12319, collected on April 23, 2013 by J.A. Ochoa at Pampa Juliaca (12°57'46.79" S, 68°55'22.61" W; 206 m above sea level, asl hereafter), Pampas del Heath (Bahuaja-Sonene National Park), district of Tambopata, province of Tambopata, department of Madre de Dios, Peru (Figs. 5A, 5E, 6B).

Paratypes. Three specimens, all from Pampa Juliaca, Pampas del Heath (Bahuaja-Sonene National Park), district of Tambopata, province of Tambopata, department of Madre de Dios, Peru. An adult male, MUBI 14679, collected on August 14, 2015 by J.A. Ochoa, G. Sebastián & K. Huamani (12°57'47.47" S, 68°54'57.01" W; 210 m asl) (Figs. 5B, 5F, 6C). An immature male, MUSA 4350, collected on November 13, 2015 by R. Santa Cruz (12°57'2.47" S, 68°54'51.05" W; 209 m asl) (Figs. 4, 5C, 6A, D). An immature male, MUSM 33453, collected on between June 02–25, 1996 by J. Cadle (12°59'20.10" S, 68°55'48.45" W; 208 m asl) (Fig. 5D).

Diagnosis. *Bothrops sonene* can be distinguished from its congeners by the exclusive combination of the following morphological characters: dorsal color pattern of body consisting of small C-shaped blotches, apparently unique for the genus; postocular stripe originating posteriorly to the eye, two or two and a half scales width, covering three or four posterior supralabials; dorsum of the head with paired markings arranged symmetrically; venter cream heavily speckled with brown; lacunolabial scale absent, prelacunal discrete contacting second supralabial; prefoveals three to five; subfoveal single and usually present; postfoveals absent to two; canthals two; intersupraoculars seven; suboculars one or two; postoculars two or three; supralabials seven or eight; infralabials nine to eleven; interrials 26–27; middorsal scales 23–25; ventrals 172 in the female, 169–173 in males; subcaudals divided, 45 in the female, 50 in males.

Comparisons. *Bothrops sonene* is easily distinguished from its congeners by its unique dorsal color pattern of relatively small and C-shaped blotches. It is distinguished from *B. atrox*, *B. barnetti*, *B. jararaca*, *B. jararacussu*, *B. pictus* and *B. taeniatus* by the absence of a lacunolabial scale (= prelacunal fused with second supralabial), a condition shared with the species of the *B. alternatus* and *B. neuwiedi* groups. Similar C-shaped (or in the shape of a headphone) dorsal blotches are present in *B. alternatus*, *B. cotiara*, *B. fonsecai*, *B. itapetiningae* and *B. jonathani* (*B. alternatus* group excluding *B. ammodytoides*, whose dorsal blotches are quadrangular); however, the blotches of *B. sonene* are smaller and rounder than those species, the lower set of blotches are well separated from each other (except in *B. itapetiningae*), and blotches are bordered with black (vs. bordered with white). The new species can also be distinguished from some species of the *B. alternatus* group by the postocular stripe starting posteriorly to the eye (vs. below the eye in *B. alternatus*, *B. jonathani* and *B. ammodytoides*), number of prefoveals (3–5 vs. 3–15), subfoveals (0–1 vs. 1–6), supralabials (8 vs. 8–12) and infralabials (9–11 vs. 10–15), length of hemipenes (relatively short vs. long), and fusiform and divergent hemipenial lobes (vs. parallel and subcylindrical lobes). *Bothrops sonene* is more similar to the *B. neuwiedi* group of species than to the rest of the species of the genus. They all have a symmetrical pattern of blotches in the dorsum of the head, a brown and relatively wide postocular stripe that extends posterior to the rictal region, a discrete prelacunal scale contacting the second supralabial, and similar number of prefoveals, subfoveals, supralabials and infralabials. They also bear resemblance in hemipenial morphology; the overall morphology of the hemipenis of the new species is identical to that of the species of the *B. neuwiedi* group, which is characteristic of that group. Differences in coloration and scalation between *Bothrops sonene* and the species of the *B. neuwiedi* group are summarized in Table 2.

Description of the holotype. Adult female; snout-vent length 950 mm; tail length 123 mm; caudal spine length 8.9 mm; head length 44.8 mm; maximum head width 36.5 mm; interorbital space 7.2 mm; internasal space 2.4 mm; dorsal scale rows 23–24–19; ventral scales 172; subcaudal scales 45/45; rostral trapezoidal; canthus rostralis elevated forming a ridge; internasals 1/1; canthals 1/1, similar size than internasals; intercanthals, keeled, 4–5–5; intersupraoculars, keeled, 7; scales around supraoculars 11/10; supralabials 8/7; infralabials 10/11; postnasal in contact (left side) or not in contact (right side) with first supralabial; loreal subtriangular; prefoveals 5/5; subfoveal 1/0; postfoveals 1/1; prelacunal contacting the second supralabial; sublacunals 1/1; supralacunals 1/1; suboculars 2/1; postoculars 3/2; upper preoculars contributing to the canthus rostralis, right preocular divided; upper and medial preoculars in contact with the orbit; inferior preoculars not in contact with the orbit; scales between suboculars and 3rd–4th supralabials 2/2; scales between suboculars and 4th supralabial 2/2; scales between

suboculars and 4th–5th supralabials 2/2; interrials 26; gulars 4. Dorsal background color of body light brown; dorsum with dark brown, black-edged blotches, each blotch composed of dorsal and ventral C-shaped forms; ventral surface of body cream, heavily speckled with dark brown spots; dorsum of head with symmetrically arranged brown and dark-edged blotches, including a single blotch on the snout, a pair of blotches near supraoculars, a pair of parietal stripes that extend posterior-laterally, and a pair of occipital stripes that extend posteriorly; head with lateral, brown and black-bordered postocular stripes originating posterior to each eye.

TABLE 1. Morphometrics (in mm) and meristic variation of the types series of *Bothrops sonene*. Abbreviations: CaSp=caudal spine, G=gulars, ICa=anterior intercanthals, ICm=medial intercanthals, ICp=posterior intercanthals, IL=infralabials, IOL=interoculabials, IR=interrials, MD=middorsals, PC=postcanthals, PF=prefoveals, PTF=postfoveals, PTO=postoculars, SBF=subfoveals, SBO=suboculars, ScSPO=scales around supraoculars, SL=supralabials, SVL=snout-vent length, TaL=tail length, and V=ventrals.

	Holotype MUBI 12319 Adult female	Paratype MUBI 14679 Adult male	Paratype MUSA 4350 Subadult male	Paratype MUSM 33453 Subadult male
SVL	950	470	373	290
TaL	123	83	61.9	49
CaSp/TL	0.07	0.04	0.07	0.07
V	172	173	169	170
SC	45	50	50	50
MD	24	25	23	24
ScSPO	11/10	11/11	10/10	9/9
SL	8/7	8/8	8/8	8/7
IL	11/10	9/10	10/10	11/10
ICa	4	5	5	4
ICm	5	6	6	4
ICp	5	7	7	5
PF	5/5	4/4	4/4	3/4
SBF	1/0	1/1	1/0	1/0
PTF	1/1	2/1	0/0	0/0
SBO	2/1	1/1	2/1	1/1
PTO	3/2	2/2	2/2	2/2
IOL 3 rd -4 th SL	2/2	2/1	1/1	2/2
IOL 4 th SL	2/2	1/1	2/1	1/1
IOL 4 th -5 th SL	2/2	2/2	2/2	2/2
IR	26	26	27	26
G	4	4	5	5

Color pattern variation. Dorsal background color of head and body light brown with olive green tonality in live specimens; dorsum of body with 28–31 C-shaped, dark brown and black-edged, dorsal blotches; ventral portion of blotches formed by a pair of mostly-fused round blotches, forming a single curved blotch; ventral surface of body cream, speckled with dark brown spots, the spots forming a transversal stripe in some ventral scales; dorsum of head with brown, dark-edged, symmetrically arranged blotches; postocular stripes extend 1.5–3 scales posterior to rictal region, encroaching 3–4 supralabials and 1–2 infralabials; supralabials with dark brown spots, more conspicuous between 3rd–5th supralabials; infralabials mostly bordered with dark brown pigments. Gular region of males cream with irregular dark brown markings, absent in the female of the type-series suggesting a putative sexual dimorphism in this character; tip of tail pale ventrally in adult female and male (MUBI 14679); both dorsal and ventral surface of tip of tail pale in subadult males (MUSA 4350, MUSM 33453).



FIGURE 4. *Bothrops sonene* sp. nov. (MUSA 4350) from Pampas del Heath, Bahuaja-Sonene National Park, department of Madre de Dios, Peru.

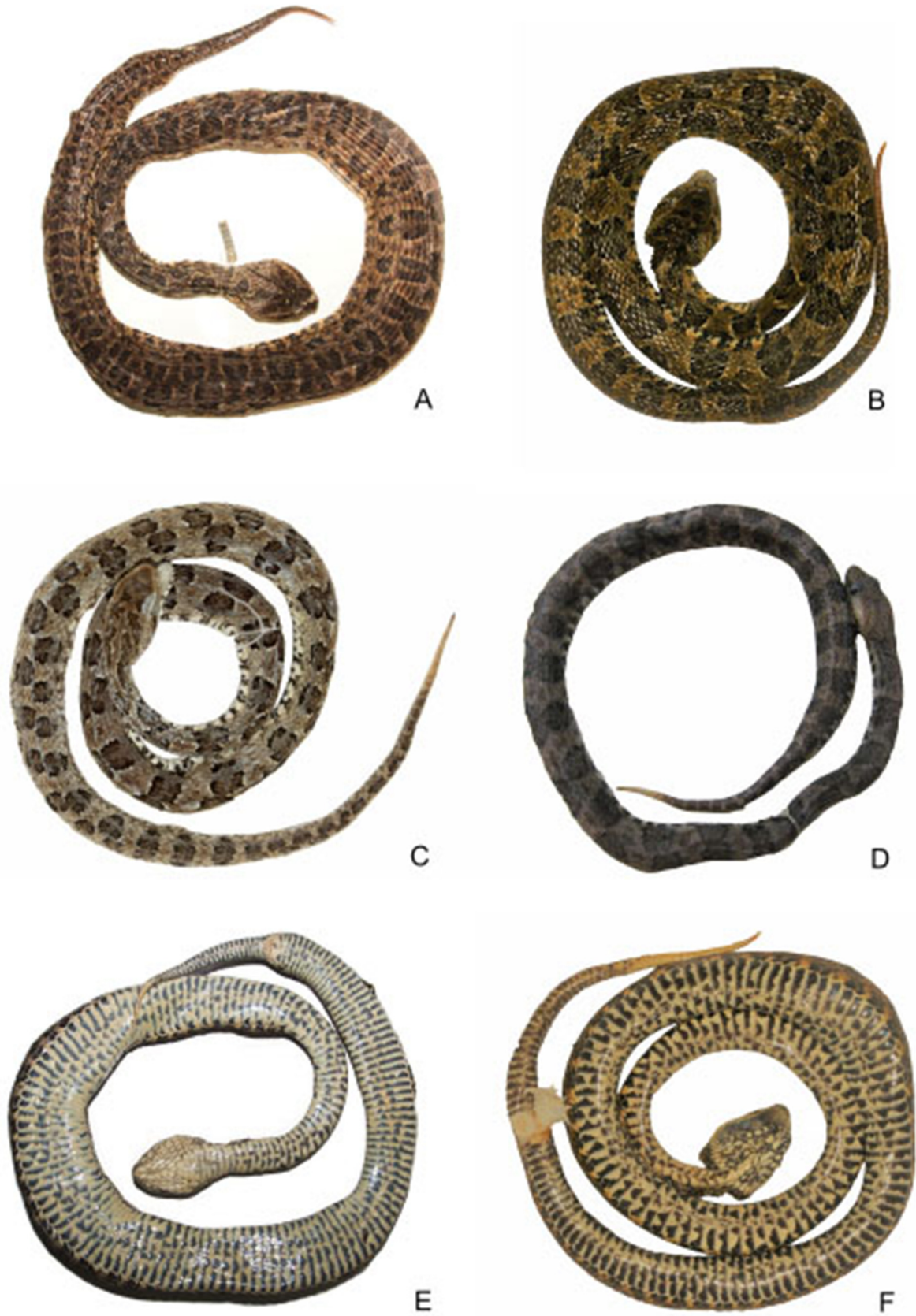


FIGURE 5. Dorsal view of the holotype (MUBI 12319—A, 1073 mm TL) and paratypes (MUBI 14679—B, 553 mm TL, MUSA 4350—C, 435 mm TL, MUSM 33453—D, 339 mm TL) of *Bothrops sonene*. Ventral view of holotype (MUBI 12319—E) and paratype (MUBI 14679—F). TL= total length.



FIGURE 6. Dorsolateral view of head of the paratype (MUSA 4350—A, D, 20 mm HL) and holotype (MUBI 12319—B, 44.8 mm HL), and dorsal view of head (MUBI 14679—C, 23 mm HL) of the paratype of *Bothrops sonene*. HL= head length.

Hemipenis morphology. Based on MUBI 14679 and MUSA 4350. Organ strongly bilobed; hemipenial lobes fusiform and divergent, 67% of total hemipenial length; hemipenial body 33% of total hemipenial length; capitulum longer on sulcate side, occupying 62% of lobe; base of capitulum covered with spinulate calyces; hook-shaped spines distributed asymmetrically on lobes, big sized spines located proximally; small, thin and curved spines present in the intralobular region, located distally on the lobes; hemipenial body covered with spinules; microornamentation on intrasulcar region absent; sulcus spermaticus bifurcating proximal to capitulum and ornamented with spinules (Fig. 7).

Distribution and natural history. Known to occur in Pampas del Heath, in the Bahuaja-Sonene National Park, province of Tambopata, department of Madre de Dios, Peru, at between 206–210 m asl (Fig. 2). The area is situated in southeastern Peru, near the border with Bolivia, between the Tambopata and Heath Rivers. *Bothrops sonene* is known from four close localities situated inside of the savannas of the Peruvian Pampas del Heath.

The specimens were collected at the end of the rainy season and during the dry season. They have diurnal activity; during the day they were observed on the ground and above grass at 500 mm above ground, sometimes slithering rapidly. They are associated with water bodies, which are widespread along the savannas of Pampas del Heath in rainy seasons (the soil is flooded up to 250 mm from the ground) and restricted to the “Aguajales” (Palm Tree areas) in dry seasons (Fig. 8).

Etymology. The specific epithet “sonene” is derived from the tacana language, and is the name used by the Ese’ejja for the Heath River. The Ese’ejja are an Amazonian community that inhabits the areas surrounding the Madre de Dios, Tambopata, and Heath Rivers, in the border between Peru and Bolivia. We named the new species “sonene” because its type locality, the Peruvian Pampas del Heath, is located near the Heath River.

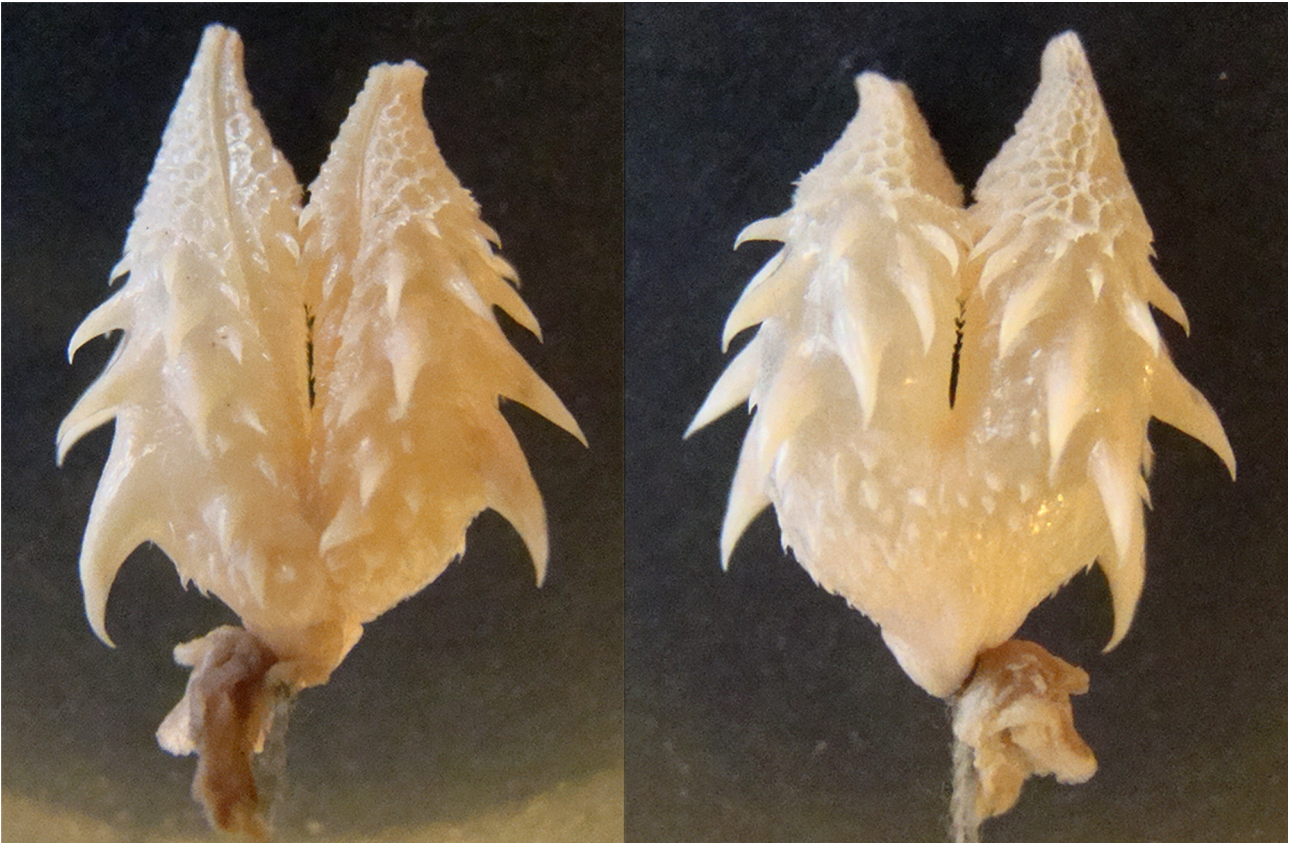


FIGURE 7. Sulcate (left) and asulcate (right) sides of the hemipenis of *Bothrops sonene* (MUBI 14670, 16.9 mm TL). TL = total length.



FIGURE 8. Landscape view of the type-locality of *Bothrops sonene* showing the “Aguajal” (Palm tree area) at Pampas del Heath (Bahuaja-Sonene National Park), department of Madre de Dios, Peru.

TABLE 2. Comparison of selected morphological characters between *Bothrops sonene* and the other species of the *B. neuwiedi* group (specimens examined in Appendix 1). Abbreviations: BP= body pattern, G=gulars, ICm=medial intercanthals, IL=infraorbital, IOL=interocular, ISPO=intersupraoculars, IR=interoculars, MD=mid-dorsals, PF=prefoveals, PM=parietal markings of the head, POSw= width of postocular stripe, PTF=postfoveals, PTO=postoculars, SBF=subfoveals, SBO=suboculars, SL=supralabials, SLPOS=supralabials encroached by postocular stripe, V= ventrals.

	<i>B. sonene</i>	<i>B. cf. sonene</i>	<i>B. neuwiedi</i>	<i>B. aff. neuwiedi</i>	<i>B. matogrossensis</i>	<i>B. aff. matogrossensis 1</i>	<i>B. aff. matogrossensis 2</i>	<i>B. aff. matogrossensis 3</i>
BP	C-shaped blotches	Trapezoidal or C-shaped blotches	Trapezoidal or subtriangular blotches	Trapezoidal or subtriangular blotches	Trapezoidal blotches	Trapezoidal blotches	Trapezoidal blotches	Trapezoidal blotches
PM	Stripes	Stripes or rounded blotches	Stripes	Stripes	Rounded blotches	Rounded blotches	Rounded blotches	Rounded blotches
POSw	2-2.5 x=2.2	2-3 x=2.4	2.5-3 x=2.8	1.5-3	2-2.5 x=2.3	2-3 x=2.2	1.75-3 x=2.2	2-2.5 x=2.2
SLPOS	3-4 x=3.2	3-4 x=3.3	2	2-3 x=2	3-4 x=3.8	2-4 x=3	2-4 x=3	2-3 x=2.7
V	164-172 x=169.5	173-178 x=175	166-172 x=169	172-184 x=177.9	183-190 x=186.5	163-173 x=167.5	155-165 x=160	167-177 x=173
MD	23-25 x=24	25-27 x=26	25-27 x=26.3	24-28 x=25.7	23-25 x=23.5	21-25 x=23	21-23 x=22.6	21-25 x=22.8
SL	8	8-9 x=8	8-11 x=9	8-9 x=8.4	7-10 x=8.3	7-8 x=7.6	7-8 x=7.6	7-9 x=8
IL	10-11 x=10.5	10-11 x=10	11-13 x=11.7	10-13 x=11	11-12 x=11.5	9-11 x=10	8-10 x=9.4	9-12 x=10.8
ICm	4-6 x=5.2	4-6 x=5.2	6	4-6 x=5	3-4 x=3.7	3-5 x=3.7	3-5 x=4.2	3-7 x=5
ISPO	7	7	8-11 x=9.3	7-11 x=8	5-8 x=7	5-7 x=6.3	4-7 x=5.6	6-8 x=7.3
PF	3-5 x=4	4	5-7 x=6.3	2-6 x=3.9	3-6 x=4.5	2-5 x=3.2	2-4 x=3.2	4-5 x=4.6
SBF	1	0-1 x=0.3	1-3 x=2	0-2 x=1	0-2 x=0.8	0-1 x=0.5	0-1 x=0.6	1-3 x=1.6

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TABLE 2. (Continued)

	<i>B. sonene</i>	<i>B. cf. sonene</i>	<i>B. newwiedi</i>	<i>B. aff. newwiedi</i>	<i>B. mattogrossensis</i>	<i>B. aff. mattogrossensis 1</i>	<i>B. aff. mattogrossensis 2</i>	<i>B. aff. mattogrossensis 3</i>
PTF	0-2 x=0.7	1	1-4 x=2	0-2 x=0.9	0-2 x=1	0-1 x=0.7	0-1 x=0.6	1-2 x=1.2
SBO	1-2 x=1.5	1	1	1	1-2 x=1.5	1	1-3 x=1.8	1-2 x=1.2
PTO	2-3 x=2.2	2	1-3 x=2	1-2 x=1.9	1-2 x=1.5	2	2	2
IOL at 4 th SL	1-2 x=1.5	1-2 x=1.3	1-2 x=1.7	1-2 x=1.9	1-2 x=1.5	1-2 x=1.5	2	1-2 x=1.8
IR	26-27 x=26.2	26-27 x=26.5	27-30 x=28.7	26-33 x=28.6	24-29 x=26.7	24-26 x=24.5	23-25 x=22.6	25-27 x=26.2
G	4-5 x=4.5	4-5 x=4.7	4-5 x=4.7	4-6 x=4.9	4-5 x=4.5	3-5 x=4	4-5 x=4.4	4

TABLE 2. Continues.

	<i>B. marmoratus</i>	<i>B. diporus</i>	<i>B. aff. diporus</i>	<i>B. pubescens</i>	<i>B. pubescens</i>	<i>B. aff. pubescens</i>	<i>B. pauloensis</i>	<i>B. lutzi</i>	<i>B. erythromelas</i>
BP	Trapezoidal blotches	Trapezoidal or subtriangular blotches	Trapezoidal or subtriangular blotches	Trapezoidal or subtriangular blotches	Trapezoidal or subtriangular blotches	Trapezoidal or subtriangular blotches	Trapezoidal blotches	Trapezoidal blotches	Trapezoidal blotches
PM	Rounded blotches	Stripes	Stripes	Stripes	Stripes	Stripes	Rounded blotches	Rounded blotches	Rounded blotches
POS _w	1.7-2 x=1.9	2-3 x=2.4	1.5-3 x=2.5	1.7	2-3 x=2.3	2-3 x=2.5	1.7-2 x=1.9	2.2-2.5 x=2.4	3.2-4.2 x=3.5
SLPOS	2-3 x=2.5	2-4 x=2.9	1-2 x=1.5	2	2-3 x=2.5	2-3 x=2.5	2-3 x=2.5	3-4 x=3.3	3-5 x=3.8
V	174-184 x=179	170-190 x=179.6	180-205 x=183.5	168	170-182 x=177.4	174-184 x=179	160-168 x=164.3	144-168 x=153.1	

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TABLE 2. (Continued)

	<i>B. marmoratus</i>	<i>B. diporus</i>	<i>B. aff. diporus</i>	<i>B. pubescens</i>	<i>B. aff. pubescens</i>	<i>B. pauloensis</i>	<i>B. lutzi</i>	<i>B. erythromelas</i>
MD	21-22 x=21.5	20-26 x=22.4	20-26 x=23.3	25	20-26 x=23	23-25 x=24	21-23 x=22.3	19-25 x=21
SL	8-9 x=8.5	8-9 x=8.2	8-11 x=8.7	8	8-9 x=8.3	8-9 x=8.5	7-8 x=7.5	8-9 x=8.2
IL	11-12 x=11.5	10-13 x=11.26	10-13 x=11.3	11	10-12 x=10.9	11-12 x=11.5	10-11 x=10.5	8-11 9.2
ICm	5	3-6 x=4.1	3-8 x=4.8	5	3-6 x=4	5	3-5 x=3.7	2-4 x=3.1
ISPO	7-9 x=8	6-10 x=7.5	7-11 x=7.9	8	6-10 x=7.8	7-9 x=8	4-7 x=5.2	4-5 x=4.2
PF	5	3-8 x=4.4	3-9 x=4.6	3	2-7 x=4.1	5	4-5 x=4.7	1-4 x=2.7
SBF	0-1 1.5	0-3 x=1.2	0-4 x=1.3	1	0-3 x=1.2	0-1 x=0.5	1-2 x=1.7	0-1 x=0.2
PTF	0-1	1-3 x=1.2	0-3 x=1.4	2	1-3 x=1.3	0-1 x=0.5	1	0-2 x=1
SBO	1	1-3 x=1.2	1-3 x=1.3	1	1-2 x=1.1	1	1	2-3 x=2.2
PTO	1-2 x=1.5	1-3 x=2	1-3 x=1.9	2	2-3 x=2	1-2 x=1.5	2	2-3 x=2.2
IOL at 4 th SL	1	1-2 x=1.6	1-2 x=1.8	2	1-2 x=1.7	1	1	1
IR	25-18 x=26.5	25-30 x=25.9	25-32 x=26.9	27	25-31 x=25.5	25-28 x=26.5	23-25 x=24.5	21-27 x=23.1
G	4-6 x=5	5-6 x=5.4	4-8 x=6	5	4-7 x=5.2	4-6 x=5	4	3-5 x=4.1

Discussion

Few species of *Bothrops* have been described in recent years, and most of them are from Brazil (Harvey 1994; Ferrarezi & Freire 2001; Silva & Rodrigues 2008; Barbo *et al.* 2012, 2016). The identification of *Bothrops sonene* in Pampas del Heath increases the number of known species of the genus, and also emphasizes the high diversity of South American open biomes (Myers *et al.* 2000; Colli *et al.* 2002; Nogueira *et al.* 2009; Werneck 2011; Werneck *et al.* 2011; Guedes *et al.* 2014, 2018). Another relevant aspect of the discovery of *Bothrops sonene* is that the species inhabits a protected area that is one of the most singular and lesser-known ecosystems in Peru. Pampas del Heath is the most northwestern incursion of the extensive grasslands at the east of the Heath River in Bolivia (Graham *et al.* 1980), and there is a high probability that *Bothrops sonene*, or a close relative, inhabit that area (see below). The Bolivian “pampas” are part of the ecoregion called the Beni savanna, which includes the La Paz Cerrado (in La Paz Department), the Llanos de Moxos (in Beni Department), and the Beni Cerrado (in Beni Department) (Olson *et al.* 2001; Larrea-Alcázar *et al.* 2010). Pampas del Heath and the Beni savannas may represent extensions of the Cerrado core (Werneck 2011; Werneck *et al.* 2012); however, they also share characteristics with Chaco, Chiquitania, and Pantanal. All of these ecoregions are inhabited by one or more species of the *Bothrops neuwiedi* group. *Bothrops sonene* is the westernmost representative of this group of species and may share some characteristics of its habitat with *B. matogrossensis*, which is commonly found in the seasonally flooded areas of the Pantanal (Monteiro *et al.* 2006).

In the re-description of *Bothrops matogrossensis*, Silva & Rodrigues (2008) mentioned that its distribution reaches Peru. The presence of representatives of the *Bothrops neuwiedi* species group in Peru was also suggested in a study of venoms of eleven pitviper species from the country (Guerra-Duarte *et al.* 2015, but see also Carrasco *et al.* 2016), which included a sample assigned to *B. neuwiedi*. Neither study provided precise localities, and voucher specimens were not available for examination in the course of the present study; but it seems likely that both studies have referred to *Bothrops sonene*. We examined three specimens from Beni Department, northern Bolivia, identified as *Bothrops neuwiedi* (ZMH 6678, from unknown locality) and *B. matogrossensis* (CBF 1554, 233, Santa Ana de Yacuma, at approximately 395 km southeast from Pampas del Heath) that resemble the new species in dorsal coloration. Molecular data from these or other specimens from Beni Department were not available; the geographically closest DNA samples available corresponded to (following identification in GenBank) three specimens of *Bothrops pauloensis* from Ñuflo de Chávez (SC, Bolivia, MJ 952, 957, 958) and one of *B. matogrossensis* from Serra da Borda (MT, Brazil, NORMAT Captivity), both localities at approximately 860 and 1010 km southeast from Pampas del Heath, respectively. As mentioned before, these haplotypes conformed a clade with the new species, but voucher specimens were not available for morphological examination. Interestingly, Machado *et al.* (2014), in their molecular study of the *Bothrops neuwiedi* group identified the *B. matogrossensis* haplotype from Serra da Borda as a new candidate species. This evidence strongly suggests that *Bothrops sonene* may have a wider distribution than reported here; however, we chose to refer to these specimens from Beni, Santa Cruz and Mato Grosso as *B. cf. sonene* until additional morphological and molecular evidence confirms their assignation to the new species.

Our results are conclusive regarding the membership of *Bothrops sonene* to the monophyletic *B. neuwiedi* species group. Most of the analyses we performed recovered the new species in a basal position to a clade that included *Bothrops diporus*, *B. pauloensis*, *B. pubescens*, *B. aff. diporus* and *B. aff. pubescens*, thus belonging to the southwest clade identified by Machado *et al.* (2014). This position for *Bothrops sonene* was supported by both molecular and morphological characters and high branch support values. The different position recovered by morphological-only analyses was apparently given by some homoplasy and was unsupported by jackknife and bootstrap values.

Giraud (2001) and Machado *et al.* (2014) highlighted on the necessity of a taxonomic revision of the *Bothrops neuwiedi* species group, and our results agree with their statement. Silva & Rodrigues (2008) performed a comprehensive taxonomic review of the group, recognizing the long-standing 12 subspecies as seven species and adding the new *Bothrops marmoratus*. However, that taxonomy presented a set of diagnostic characters, mostly from coloration, which does not allow unquestionable identifications. The recent extensive molecular study presented by Machado *et al.* (2014), besides bringing light to the complexity of the genetic structure within the *Bothrops neuwiedi* group, also indicated the incongruences between the taxonomy proposed by Silva & Rodrigues (2008) and the tree topology generated based on molecular evidence. Unfortunately, since much of the DNA

samples with doubtful identifications in GenBank were not evaluated morphologically in this study, or had their voucher specimens lost or even originally had no clear voucher information, the taxonomic and geographical limits of the species recognized in the current taxonomic arrangement of the *Bothrops neuwiedi* group are unclear and requires to be considered as tentative.

Populations from Argentina, Bolivia and Paraguay, a wide area within the distribution range of the *Bothrops neuwiedi* group which mostly correspond to the Chacoan region, should be assigned to *B. matogrossensis* and *B. diporus* according to Silva & Rodrigues (2008). In this study we observed that specimens of *Bothrops matogrossensis* from that region do not fit the morphotype described for that species, but resemble or are indistinguishable from specimens of *B. diporus* from the same region. We also observed that specimens of *Bothrops diporus* from Argentina showed significant differences in coloration with specimens of *B. diporus* from Brazil. Giraudo (2001) noted that populations from northeastern Argentina (province of Misiones and part of the province of Corrientes) differed in coloration from populations of the rest of the country, but were similar to populations from the states of Paraná and Rio Grande do Sul (Brazil). The lineages we identified in this study indicate that *Bothrops matogrossensis* and *B. diporus* represent each of them a putative complex of species. Results showed that specimens from center of Bolivia relates phylogenetically with the specimen from near the area of the type locality of *Bothrops diporus* in northern Argentina (“region of Rio Vermejo [Bermejo], border between Paraguay and Argentina”). Results also indicated that specimens of *Bothrops diporus* from the west of Argentina (probably from northwest to south) conform a distinct clade (*B. aff. diporus*), as was reflected in the position of the sample from Castro Barros (La Rioja Province) in our results and also those of Machado *et al.* (2014). Specimens of *Bothrops diporus* from Brazil conformed a distinct clade (*B. aff. pubescens*) related to *B. pubescens*. Although we did not include molecular samples from northeastern Argentina, our morphological comparisons (and the observations of Giraudo 2001) suggest that they may also be included in that clade. Based on the results of our combined analyses we also corroborate the suggestion of Machado *et al.* (2014) that the lineage *Bothrops aff. diporus* represent a candidate new species from western Argentina. We also conclude that the lineages *Bothrops aff. pubescens* and *B. aff. matogrossensis* 1–3 represent candidate new species. Although *Bothrops aff. neuwiedi* is morphologically and phylogenetically distinct from the nominal species *B. neuwiedi* (as considered here) from southeastern/southern Brazil, we prefer to be conservative about the taxonomic status of both lineages since the type locality of *B. neuwiedi* is southern Bahia state and we did not have access to any individual from such populations (Fig. 2).

The complexity of the diversification patterns within the *Bothrops neuwiedi* group is evident. Machado *et al.* (2014) mentioned introgressive hybridization, retention of ancestral polymorphism and morphological parallelism as possible historical processes involved in their current diversity; the great overlap of morphological characters among the species may be the result of those processes, challenging the attainment of clear diagnoses for the members of this group. However, the identification and description of *Bothrops sonene* and the preliminary morphological revision of the *B. neuwiedi* group of species we performed here also indicates that greater attention should be delivered to morphology and geographic distribution. Hopefully these results will contribute to clarify the systematics and the historical biogeography of this group of pitvipers inhabiting open biomes.

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Supplementary Material

APPENDIX 1. Specimens examined.

- Atropoides nummifer*—HONDURAS. Tela: MZUSP 2030. San Pedro Sula: MZUSP 8231.
- Bothriechis schlegelii*—PANAMA. Isthmus of Panamá: MZUSP 2072. Unknown locality: FML 99.
- Bothrocophias andianus*—BOLIVIA. COCHABAMBA. Chapare: MNK 3722. SANTA CRUZ. Florida: MNK 2715, 2955, 3327, 4319, 4542, 4543. LA PAZ. El Piñalito: CBF 221; Irupana: CBF 467. PERU. CUSCO. Machu Picchu: MUSM 2324, 2680, 3093, MUBI 5100; Quimbiri: MUSM 26492; Alto Shimá Native Community: CORBIDI 8355; Urusayhua: MUBI 13641, 13654; Monte Carmelo: MUBI 10501–02. Capire: CORBIDI 14690; Manu Cloud Forest Lodge: MUBI 5060; Alto Pilcomayo: MUBI 13376.
- Bothrocophias hyoprora*—BRAZIL. AMAZONAS. Prainha: MZUSP 5193. MATO GROSSO. Aripuanã: MZUSP 11152. COLOMBIA. META. La Macarena: MZUSP 6109. ECUADOR. NAPO. Cuyabeno: MZUSP 9540. PERU. LORETO. Bellavista: MUSM 2982.
- Bothrocophias microphthalmus*—BOLIVIA. COCHABAMBA. Carrasco National Park: CBF 1899; Leche River: MHNC 103, 104. LA PAZ. Pilón Lajas Biosphere Reserve: MNK 1801, 1814. BENI. Rurrenabaque: MNK 493. PERU. CUSCO. Timpia: MUSM 25673, 26443; La Convención: MUSM 26093; Bajo Puyantimari: CORBIDI 10712; Mabe: MUBI 7013; Nusinuscato: MUBI 7458. SAN MARTIN. Tarapoto: MUSM 3066; Paitoja: CORBIDI 1242; Bajo Naranjillo: CORBIDI 3384. JUNIN. Tarma: MUSM 2035. HUÁNUCO. Sira: MUSM 8605; Tingo María: CORBIDI 1464. CAJAMARCA. Cordillera del Cóndor: CORBIDI 1461–63. AMAZONAS. Cordillera del Colán: CORBIDI 2059. MADRE DE DIOS. Huasorocco: MUBI 10871; Amarakaeri Comunal Reserve: MUBI 14345, 14397.
- Bothrops alternatus*—ARGENTINA. FORMOSA. El Bagual Ecological Reserve: FML 1434. MISIONES. El Bonito: FML 67. CORRIENTES. Yacyretá: FML 2565. ENTRE RÍOS. Salto Grande: MLP 265. SANTA FE. Campo Garay: MACN (ex-CENAI) 285. SANTIAGO DEL ESTERO. Quimili: FML 2007. CÓRDOBA. Las Rabonas: CZA 138. BUENOS AIRES. Punta Lara: MLP 286; Sierra La Ventana: MLP 749. URUGUAY. Montevideo: MZUSP 1460. BRAZIL. SANTA CATARINA. Taunay: MZUSP 1966. MATO GROSSO DO SUL. Campo Grande: MZUSP 10235. RIO GRANDE DO SUL. Rosario do Sul: MZUSP 1968. GOIÁS. Parque Nacional das Emas: MZUSP 11727. DISTRITO FEDERAL. Brasília: MTKD 14649.
- Bothrops ammodytoides*—ARGENTINA. SALTA. Cafayate: FML 1802. CATAMARCA. Andalgalá: FML 1405. LA RIOJA. Castro Barros: FML 7804. TUCUMÁN. Tafí del Valle: FML 1592. CÓRDOBA. Calamuchita: CZA 29. SAN LUIS. Buena Esperanza: MACN 2329. Unknown locality: MTKD 28709. LA PAMPA. Bernasconi: MLP 789. BUENOS AIRES. Bahía Blanca: MLP 787. MENDOZA. Cerro Los Leones: MACN 29051. NEUQUEN. Zapala: FML 9215. RÍO NEGRO. Allen: MACN 3638. CHUBUT. Península de Valdés: MLP 563.
- Bothrops asper*—BELIZE. Orange Walk: MZUSP 6445.
- Bothrops atrox*—BOLIVIA. LA PAZ. Madidi: CBF 2347. PANDO. San Silvestre: MNKR 2269. PERU. MADRE DE DIOS. Manu: MUBI 10353. CUSCO. La Convención, Echarate: MUBI 9840. PASCO. Ciudad Constitución: MUSM 20070. LORETO. Loreto: MUSM 22293. BRAZIL. MARANHÃO. Baixada Maranhense: MZUSP 5543. RONDÔNIA. Nova Brasília: MZUSP 8764. MATO GROSSO. Apicás: MZUSP 11182. ACRE. Porto Walter: MZUSP 7376. RORAIMA. Rio Catrinami (km 137 Perimetral Norte): MZUSP 6969. PARA. Juruá, Rio Xingu: MZUSP 9413. COLOMBIA. BOLÍVAR. Santa Rosa: MZUSP 6288. CHOCÓ. 100 k S of Bogotá: MHNG 1329.042. CUNDINAMARCA. Bogotá: MHNG 1329.048. META. San Juan de Arama: MHNG 1050.023. TOLIMA. Ibagué: MHNG 2522.026, 2522.028. Armero: MHNG 2522.027.
- Bothrops barnetti*—PERU. LAMBAYEQUE. Lambayeque: CORBIDI 5618. Las Pampas: MUSM 23684; Corral de Arena: MUSM 23685; Chiclayo: MUSM 3019; Olmos: CORBIDI 1460, 10385-87, 14137.
- Bothrops bilineatus*—BOLIVIA. SANTA CRUZ. Mataracú: MNK 1429; Amboró National Park: MNK 328. BRAZIL. ESPIRITO SANTO. Santa Teresa: MZUSP 4456. RORAIMA. Serra dos Surucucus: MZUSP 10372. BAHIA. Olivená: MZUSP 13248. COLOMBIA. AMAZONAS. Leticia: MZUSP 3722. ECUADOR. NAPO. Añango: MZUSP 8253. PERU. AMAZONAS. Cordillera del Cóndor: MUSM 21295. LORETO. Pevás: MUSM 2148; Allpahuayo-Mishana National Reserve: MUSM 2611; Ampiyacu River: MZUSP 4398. SAN MARTIN. Tarapoto: MUSM 3084; MUSM 3260.
- Bothrops brazili*—PERU. AMAZONAS. Condorcanqui: MUSM 18586; Bagua: MUSM 2652. CUSCO. Echarate: MUSM 21748; Kinteroni: MUBI 9833. Porotobango: MUBI 9865, 9868. UCAYALI. Bolognesi: MUSM 20071. MADRE DE DIOS. Ponal: MUBI 10226.
- Bothrops chloromelas*—PERU. HUANUCO. Tingo María: MUSM 2088. JUNIN. Satipo: MUSM 2005; Huacapistana: MUSM 2003, 2252. PASCO. Oxapampa: MUSM 24747; Chontabamba: MUSM 23680, 24744; Manto: MUSM 18010, 19043; Santa Isabel: MUSM 19174.
- Bothrops cf. sonene*—BOLIVIA. BENI. Santa Ana de Yacuma: CBF 233, 1554. Unknown locality: ZMH 6678.
- Bothrops cotiara*—ARGENTINA. MISIONES. 2 de Mayo: MLP 882. Frontera Refugio: MACN 12712. BRAZIL. PARANA. Curitiba: MACN 31386; Porto União: MZUSP 2473; Inácio Martins: MZUSP 2467; Tangará: MZUSP 2470-71. SÃO PAULO. Presidente Epitácio: MZUSP 2468. SANTA CATARINA. Bom Retiro: MZUSP 2505.
- Bothrops diporus*—ARGENTINA. FORMOSA. Palmar Largo: MLP 5002, 5008; El Espinillo: CZA 128; María Cristina: MLP 5007, 5009; Estero Grande: MACN 44621; Las Lomitas: CZA 79, UNNEC 5677; Paraje Urbana Vieja: UNNEC 56,

496; Pirané: UNNEC 165; San Pedro: UNNEC 8927; Pozo del Tigre: MACN 44622. CHACO. Resistencia: MLP 5006; Roque Saenz Peña: FML 7397; La Sabana: MACN 44424; Fortín Chaja: MACN 44422; Colonia Benitez: MACN 44425; Las Palmas: MACN 44421; Colonia Las Mercedes: UNNEC 654, 1798–99; Pampa del Infierno: UNNEC 695, 697, 826–27; Tacú Pozo: UNNEC 671; Campo Largo: UNNEC 8152, 11328; Nueva Pompeya: UNNEC 9107; Fuerte Esperanza: MLP 5297, UNNEC 1805, 9109, 11033; Roque Sáenz Peña: FML 7397. SANTA FE. Villa Guillermina: UNNEC 11613; Santa Clara: MACN 44359, 44361; Esperanza: MACN 44360, 44367; La Gallareta: MACN 44362. BOLIVIA. TARIJA. Villa Montes: ZFMK 32622–23, 36500–01, 44747, NHM 28409; SANTA CRUZ. Samaipata: ZFMK 57522, 60173, 67052; Río Seco: ZFMK 67052; Pampagrande: ZFMK 67114–16, 75054–57; Mataral: ZFMK 75021; Ángel Sandóval: MNKR 1404; Algodonal: MNKR 4632; Cordillera: MHNC 315. CHUQUISACA. Tomina: CBF 482; Luis Calvo: FML 2388-I. PARAGUAY. BOQUERÓN. Filadelfia: ZFMK 61019, 62523, 37295–98, 37820–21, 38295, 39699–701, 45854, 66324–25, 73524–25, 72878; Loma Plata: ZFMK 46283–84, 46287–89, 50924–25, 53126–27. CAAGUAZÚ. Toledo: MTKD 43459–60. CORDILLERA. San Bernardino: MLP 290.

Bothrops aff. diporus—ARGENTINA. RÍO NEGRO. General Roca: FML 9439. NEUQUEN. Fortín de la Piedra: MLP 1797. MENDOZA. Dr. Gassul: MLP 881. SAN JUAN. Villa Media Agua: FML 782. LA PAMPA. Parque Nacional Lihué Calel: MACN 31383. SAN LUIS. Junín: FML 6952; Alto Pencoso: MLP 271, 272, 1897. CÓRDOBA. Cruz del Eje: CZA 2016; Río Ceballos: CZA 168; La Granja: CZA 205; La Posta: MLP 880; La Calera: CZA 204; Salsipuedes: CZA 173; Diquecito: CZA 170; Río Tercero: CZA 175; Molinari: CZA 180; Yocsina: CZA 208. SANTIAGO DEL ESTERO. Frias: FML 18489; Los Telares: MLP 1575; Sumapampa: MLP 268, 272–73; Pampa Pozo: FML 17893; Caspi Corral: FML 1262; Monte Quemado: FML 2027. TUCUMÁN. Bella Vista: FML 7348; Gobernador Garmendia: MACN 44623–24; Concepción: MACN 44625, FML 13711; La Cocha: MACN 44626, FML 1810; Tafi Viejo: MLP 1563; Buena Vista: FML 7348; Concepción: FML 13711; Trancas: FML 23882. CATAMARCA. Pomán: FML 16127; Tinogasta: FML 1675. LA RIOJA. Chilecito: MACN 1577; Anillaco: FML 9471; Famatina: MLP 108; Chamical: MACN 44357–58. SALTA. Aguas Blancas: FML 2611–12, 16128; General José de San Martín: FML 18065; Santa Victoria: FML 17939. Río Pescado: FML 627; Rosario de la Frontera: MLP 662, 664; Rivadavia: FML 15764; Urundel: FML 17915; General Güemes: FML 17444; JUJUY. Abra de Mortero: FML 25551; Caimancito: FML 17913; San Salvador de Jujuy: FML 18144; Yuto: FML 267; Unknown locality: NHM 28403/1–/3. BOLIVIA. TARIJA. Reserva Nacional de Flora y Fauna de Tariquía: CBF 2079; Arce: MHNC 310.

Bothrops erythromelas—BRAZIL. BAHIA. Santo Inácio: MZUSP 14047; Queimadas: MZUSP 10550; Sento Sé: MZUSP 15691; Alagoado: MZUSP 14048. Alagoas, Xingó: MZUSP 10881; Guanambi: IBSP 55541; Rio do Antônio: IBSP 79766. PARAÍBA. Patos: MZUSP 7282; Coremas: MZUSP 3550–51.

Bothrops fonsecai—BRAZIL. SÃO PAULO. Serra de Bocaina: MZUSP 1653; Parque Estadual Campos do Jordão: MZUSP 16802.

Bothrops insularis—BRAZIL. SÃO PAULO. Ilha Queimada Grande: MZUSP 1048, 1040.

Bothrops itapetiningae—BRAZIL. Brasília: MZUSP 13931. SÃO PAULO. Pirassununga: MZUSP 4006; Itapetininga: 1437.

Bothrops jararaca—ARGENTINA. MISIONES. Bernardo de Irigoyen: MACN 33030; Puerto Esperanza: MACN 38731; Montecarlo: MLP 1571; Aristóbulo del Valle: MLP 1812. BRAZIL. PARANÁ. Morretes: FML 2110; Ortigueira: MZUSP 13150. SÃO PAULO. Boracéia: MZUSP 4504. SANTA CATARINA. São Bento do Sul: MZUSP 7572. RIO DO JANEIRO. Rio do Janeiro: MZUSP 2727. MINAS GERAIS. Vargem Alegre: MZUSP 1426.

Bothrops jararacussu—ARGENTINA. MISIONES. San Pedro: FML 976; Libertad: MACN 35346; El Alcázar: MACN 38669. BRAZIL. SANTA CATARINA. Rio Vermelho: MZUSP 9533. SÃO PAULO. Estação Ecologica da Juréia: MZUSP 10560. RIO DE JANEIRO. Baixada Fluminense: MZUSP 2316.

Bothrops jonathani—ARGENTINA. JUJUY. Santa Bárbara: FML 1050. SALTA. La Caldera: FML 570; Santa Victoria: FML 1480. BOLIVIA. SANTA CRUZ. San Juan del Potrero: MNKR 1618, 2036; Valle Grande: MNKR 718. COCHABAMBA. Quillacollo: CBF 2673. TARIJA. Curqui: CBF 2318–20; Quebrada Loray: MHNC 206–07. Samaipata: ZFMK 57523, 58160, 60172.

Bothrops leucurus—BRAZIL. BAHIA. Salvador: MZUSP 2769, 2770; 30 km W Itaberaba: MZUSP 7800; Ilhéus: MZUSP 9623.

Bothrops lutzi—BRAZIL. PIAUI. Valença: MZUSP 5855, 5859; Parque Nacional Serra da Capivara: MZUSP 12842; Baixa Grande do Ribeiro: MZUSP 12536. TOCANTINS. Reserva Serra Geral do Tocantins: MZUSP 15005. BAHIA. Jaborandi: MZUSP 18703.

Bothrops marmoratus—BRAZIL. MINAS GERAIS. São Roque de Minas: MZUSP 15006, 15067–68; Serra do Salitre: BSP73286. GOIÁS. Ipameri: IBSP 55056; DF. Brasília: IBSP 21590.

Bothrops matogrossensis—BRAZIL. MATO GROSSO DO SUL. Miranda: IBSP 24489; Bodoquena: MZUSP 12872; Aquidauana: IBSP 13591; Corumbá: IBSP 19077.

Bothrops aff. matogrossensis 1—BRAZIL. MATO GROSSO. Porto Estrela: MZUSP 15048. Chapada dos Guimarães: MZUSP 11849, 11851; Jangada: IBSP 86481.

Bothrops aff. matogrossensis 2—BRAZIL. TOCANTINS. Peixe: MZUSP 15546, 15681; Guaraí: MZUSP 12679; Salvador do Tocantins: MZUSP 17871.

Bothrops aff. matogrossensis 3—BRAZIL. GOIÁS. Santa Rita do Araguaia: MZUSP 9914; Jataí: MZUSP 3781; Mineiros: MZUSP 11730, 12145–46, 15065.

Bothrops moojeni—ARGENTINA. MISIONES. Uruguái Hydroelectric Reserve: CZA 33; Libertad: MACN 35344. BOLIVIA.

SANTA CRUZ. Noel Kempff Mercado National Park: MNKR 156, 610. BRAZIL. SÃO PAULO. São Jose do Rio Preto: MZUSP 14213. MATO GROSSO. Claudia: MZUSP 11236. BAHIA. Maracujá, Santa Rita de Cássia: MZUSP 3665.

Bothrops neuwiedi—BRAZIL. SÃO PAULO. Angatuba: MZUSP 4917, IBSP 5555; Itu: IBSP 70337, 70723; Sorocaba: IBSP 71611; Santo André: IBSP 73292; Votorantin: IBSP 73637; Areias: IBSP 74218; Santana de Parnaíba: IBSP 75357. MINAS GERAIS. Baependi: IBSP 74563–66, IBSP 88757, MZUSP 17793; Três Corações: IBSP 71102; Machado: IBSP 74040; Aiuruoca: IBSP 71063; Munhoz: IBSP 72925, IBSP 73477; São Vicente de Minas: IBSP 73004–06; Andradas: IBSP 73007.

Bothrops aff. neuwiedi—BRAZIL. MINAS GERAIS. UHE Fumaça: MZUSP 15724; Serra do Cipó: MZUSP 7567, 17979; Santana do Riacho: IBSP 81110; Serra do Caraça: MZUSP 5249; Conselheiro Mata: MZUSP 8120; Pedra Menina: MZUSP 9044; Mariana: MZUSP 15724; Jabuticatubas: MZUSP 17979.

Bothrops oligolepis—PERU. PASCO. Chontabamba: MUSM 23415, 24748, 25317; Puerto Bermúdez: MUSM 25311–12.

Bothrops pauloensis—BRAZIL. MINAS GERAIS. Frutal: IBSP 58550, IBSP 71110–11; Águas de Santa Barbara: IBSP 89772; Prata: IBSP 71473–74.

Bothrops pictus—PERU. ICA. Hacienda Santiaguillo: MUSM 2139. LIMA. Lima: MUSM 2827, 2954; Cañete: CORBIDI 3650, 10384. La Molina: MUSM 27292; Jicamarca: MUSM 2433, 2365, 2536; Tornamesa: MUSM 25395; Lachay: MUSM 2705; Valle de Lurin: MZUSP 2755. ANCASH. Recuay: MUSM 25397. Huarmey: CORBIDI 425, 10382–83.

Bothrops pubescens—URUGUAY. TACUAREMBÓ. Pozo Hondo: MZUSP 5874.

Bothrops aff. pubescens—ARGENTINA. MISIONES. Isla Yacyertá: UNNEC 7089; San Javier: MACN 45849; Iguazú: MACN 44426; San Ignacio: MLP 1598; San Antonio: UNNEC 6023; General Belgrano: FML 26354–55; Puerto Leoni: UNNEC 11760. ENTRE RÍOS. Colonia Cerrito: MACN 44370. CORRIENTES. San Roque UNNEC 193; Mburucuyá: UNNEC 4830, 4994, 7335; Isla Apipé: UNNEC 10127, 10192, 10197, 10198–99, 10415, 10422–23, 10522–23, 10956, 10958; Ituzaingó: UNNEC 128, 459; San Luis de Palma: UNNEC 141; San Lorenzo: UNNEC 7030; Monte Grande: UNNEC 9793, 10133; Santa Ana: UNNEC 7940; Santa Teresa: UNNEC 4928; Laguna Cambá: UNNEC 10362; Garruchos: UNNEC 6177; Centinela: UNNEC 6748. BRAZIL. PARANA. Cascavel: IBSP 42474. SANTA CATARINA. Campo Erê: IBSP 29766; Chapecó IBSP 51448; Itapiranga: IBSP 27137. RIO GRANDE DO SUL. Tuparendi: IBSP 59629.

Bothrops pulchra—PERU. AMAZONAS. Bagua Grande: MUSM 2902.

Bothrops sanctaecrucis—BOLIVIA. BENI. Oromomo: CBF 1009, 1023. COCHABAMBA. Carrasco, Yuquis: CBF 776, 673; Chapare, Guacharos: MHNC 358. SANTA CRUZ. Potrerillo del Guenda: MNK 518, 633, 839; Yapacaní: MNK 248, 358; San Carlos: MNK 226; Amboró National Park: MNK 359, 619.

Bothrops taeniatus—PERU. CUSCO. Timpia: MUSM 25674. HUÁNUCO. Sira: MUSM 8606. JUNÍN. Satipo: MUSM 2006. BRAZIL. PARÁ. Serra de Kukoinhokren: MZUSP 11575. MATO GROSSO. Apiacás: MZUSP 11183; Claudia: MZUSP 111253–54.

Bothrops venezuelensis—VENEZUELA. YARACUY. Nirgua: MZUSP 7830.

Cerrophidion godmani—GUATEMALA. SANTIAGO SACATEPÉQUEZ. Santiago Sacatepéquez: MUSM 2574. CHIMALTENANGO. Quisaché: MZUSP 6477.

Crotalus durissus terrificus—ARGENTINA. CÓRDOBA. Cañada de Luque: CZA 170. MISIONES. San Vicente: MACN 36869. BOLIVIA. CHUQUISACA: CBF 02462. SANTA CRUZ. Mataral: MNK 2150.

Lachesis muta—BOLIVIA. LA PAZ. Alto Madidi: CBF 1935; Tumupasa: CBF 1723; San Ignacio: CBF 740, 2127. BENI. Oromomo: CBF 1024. PERU. CUSCO. Tambopata: MUSM 7376, 7377.

Porthidium nasutum—ECUADOR. ESMERALDAS. San Miguel: MZUSP 7480.

APPENDIX 2. GenBank accession numbers for each taxon used in phylogenetic analyses. *Voucher specimens of samples examined.

Previous identification	Re-identification (this study)	12S	16S	COI	Cyt-b	ND4
<i>Bothrops diporus</i>	<i>B. diporus</i>	-	-	this study	this study	this study
<i>Bothrops diporus</i>	<i>B. aff. pubescens</i>	-	-	-	KF801115	-
<i>Bothrops diporus</i>	<i>B. aff. pubescens</i>	-	-	-	KF801116	KF801248
<i>Bothrops diporus*</i>	<i>B. aff. pubescens</i>	-	-	-	AF292587	AF292625
<i>Bothrops diporus*</i>	<i>B. aff. pubescens</i> ¹	-	-	-	KF801111	KF801245
<i>Bothrops diporus*</i>	<i>B. aff. pubescens</i> ¹	-	-	-	KF801112	-
<i>Bothrops diporus</i>	<i>B. aff. pubescens</i>	-	-	-	KF801118	KF801250
<i>Bothrops diporus</i>	<i>B. aff. pubescens</i>	-	-	-	-	KF801252
<i>Bothrops diporus</i>	<i>B. aff. pubescens</i>	-	-	-	KF801120	KF801253
<i>Bothrops diporus</i>	<i>B. aff. pubescens</i>	-	-	-	KF801119	KF801254
<i>Bothrops diporus</i>	<i>B. aff. pubescens</i>	-	-	-	-	KF801249
<i>Bothrops diporus</i>	<i>B. aff. pubescens</i>	-	-	-	KF801121	KF801251
<i>Bothrops diporus</i>	<i>B. aff. pubescens</i>	-	-	-	KF801122	-
<i>Bothrops diporus</i>	<i>B. aff. pubescens</i>	-	-	-	KF801113	KF801246
<i>Bothrops diporus</i>	<i>B. aff. pubescens</i>	-	-	-	DQ305472	DQ305489
<i>Bothrops diporus</i>	<i>B. aff. diporus</i>	DQ305431	DQ305454	-	KF801114	KF801247
<i>Bothrops diporus</i>	<i>B. aff. pubescens</i>	-	-	-	KF801117	-
<i>Bothrops diporus</i>	<i>B. aff. pubescens</i>	-	-	-	KF801117	-
<i>Bothrops erythromelas</i>	<i>B. erythromelas</i>	-	-	-	AY865653	-
<i>Bothrops erythromelas*</i>	<i>B. erythromelas</i>	-	-	-	AF292588	AF292626
<i>Bothrops erythromelas*</i>	<i>B. erythromelas</i>	-	-	-	KF801126	KF801257
<i>Bothrops erythromelas*</i>	<i>B. erythromelas</i>	-	-	-	KF801128	KF801259
<i>Bothrops erythromelas*</i>	<i>B. erythromelas</i>	-	-	-	KF801123	KF801255
<i>Bothrops erythromelas</i>	<i>B. erythromelas</i>	-	-	-	KF801129	KF801260
<i>Bothrops erythromelas</i>	<i>B. erythromelas</i>	-	-	-	AY223600	-
<i>Bothrops erythromelas</i>	<i>B. erythromelas</i>	-	-	-	KF801124	-
<i>Bothrops erythromelas</i>	<i>Bothrops. sp.</i> ²	-	-	-	KF801125	KF801256
<i>Bothrops erythromelas</i>	<i>B. erythromelas</i>	-	-	-	KF801127	KF801258

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APPENDIX 2. (Continued)

Previous identification	Re-identification (this study)	12S	16S	COI	Cyt-b	ND4
<i>Bothrops lutzi</i> *	<i>B. lutzi</i> ³	-	-	-	KF801135	-
<i>Bothrops lutzi</i> *	<i>B. lutzi</i>	-	-	-	KF801136	KF801264
<i>Bothrops lutzi</i>	<i>B. lutzi</i>	-	-	-	KF801131	KF801261
<i>Bothrops lutzi</i>	<i>B. lutzi</i>	-	-	-	KF801132	-
<i>Bothrops lutzi</i>	<i>B. lutzi</i>	-	-	-	KF801130	-
<i>Bothrops lutzi</i>	<i>B. lutzi</i>	-	-	-	KF801133	KF801262
<i>Bothrops lutzi</i>	<i>B. lutzi</i>	-	-	-	KF801134	KF801263
<i>Bothrops marmoratus</i>	<i>B. aff. mattogrossensis</i> 2	-	-	-	KF801140	KF801268
<i>Bothrops marmoratus</i>	<i>B. aff. mattogrossensis</i> 2	-	-	-	KF801141	KF801269
<i>Bothrops marmoratus</i>	<i>B. marmoratus</i>	-	-	-	KF801142	KF801270
<i>Bothrops marmoratus</i>	<i>B. cf. pauloensis</i> ⁴	-	-	-	KF801139	KF801267
<i>Bothrops marmoratus</i>	<i>B. cf. pauloensis</i> ⁴	-	-	-	KF801143	KF801271
<i>Bothrops marmoratus</i>	<i>B. marmoratus</i>	-	-	-	KF801138	KF801266
<i>Bothrops marmoratus</i>	<i>B. marmoratus</i>	-	-	-	KF801144	KF801272
<i>Bothrops marmoratus</i>	<i>B. marmoratus</i>	-	-	-	KF801137	KF801265
<i>Bothrops marmoratus</i>	<i>B. marmoratus</i>	-	-	-	KF801145	KF801273
<i>Bothrops marmoratus</i> *	<i>B. marmoratus</i>	-	-	-	KF801147	KF801275
<i>Bothrops marmoratus</i> *	<i>B. marmoratus</i>	-	-	-	KF801146	KF801274
<i>Bothrops marmoratus</i> /mattogrossensis	<i>B. aff. mattogrossensis</i> 3	-	-	-	KF801108	KF801240
<i>Bothrops marmoratus</i> /pauloensis	<i>B. marmoratus</i>	-	-	-	KF801110	KF801241
<i>Bothrops marmoratus</i> /pauloensis	<i>B. marmoratus</i>	-	-	-	KF801109	KF801242
<i>Bothrops mattogrossensis</i>	<i>B. mattogrossensis</i>	-	-	-	KF801148	KF801276
<i>Bothrops mattogrossensis</i>	<i>B. diporus</i>	-	-	-	-	FN431768
<i>Bothrops mattogrossensis</i>	<i>B. diporus</i>	-	-	-	-	FN431771
<i>Bothrops mattogrossensis</i>	<i>B. diporus</i>	-	-	-	-	FN431770
<i>Bothrops mattogrossensis</i>	<i>B. diporus</i>	-	-	-	-	FN431769
<i>Bothrops mattogrossensis</i>	<i>B. diporus</i>	-	-	-	-	FN431767

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APPENDIX 2. (Continued)

Previous identification	Re-identification (this study)	12S	16S	COI	Cyt- <i>b</i>	ND4
<i>Bothrops mattogrossensis</i>	<i>B. cf. sonene</i> sp. nov.	-	-	-	KF801158	-
<i>Bothrops mattogrossensis</i>	<i>B. aff. mattogrossensis</i> 1	-	-	-	KF801151	KF801279
<i>Bothrops mattogrossensis</i>	<i>B. aff. mattogrossensis</i> 1	-	-	-	KF801149	KF801277
<i>Bothrops mattogrossensis</i>	<i>B. mattogrossensis</i>	-	-	-	KF801152	KF801280
<i>Bothrops mattogrossensis</i>	<i>B. mattogrossensis</i>	-	-	-	KF801157	KF801286
<i>Bothrops mattogrossensis</i>	<i>B. mattogrossensis</i>	-	-	-	KF801153	-
<i>Bothrops mattogrossensis</i>	<i>B. aff. mattogrossensis</i> 1	-	-	-	-	KF801281
<i>Bothrops mattogrossensis</i>	<i>B. mattogrossensis</i>	-	-	-	KF801154	KF801282
<i>Bothrops mattogrossensis</i>	<i>B. mattogrossensis</i>	-	-	-	-	KF801283
<i>Bothrops mattogrossensis</i>	<i>B. mattogrossensis</i>	-	-	-	KF801155	KF801284
<i>Bothrops mattogrossensis</i>	<i>B. mattogrossensis</i>	-	-	-	KF801150	KF801278
<i>Bothrops mattogrossensis</i>	<i>B. mattogrossensis</i>	-	-	-	KF801156	KF801285
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801160	KF801287
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801176	-
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801177	KF801299
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801184	-
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801195	KF801317
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801181	KF801301
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801107	KF801244
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801161	KF801288
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801172	-
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801163	-
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801189	KF801307
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801194	KF801312
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801182	-
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801106	KF801243
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801165	KF801291
<i>Bothrops neuwiedi</i>	<i>B. neuwiedi</i>	-	-	-	KF801180	KF801300

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APPENDIX 2. (Continued)

Previous identification	Re-identification (this study)	12S	16S	COI	Cyt-b	ND4
<i>Bothrops newwiedi</i>	<i>B. newwiedi</i>	-	-	-	KF801190	KF801313
<i>Bothrops newwiedi</i>	<i>B. aff. newwiedi</i>	-	-	-	KF801171	KF801295
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	AF292585	AF292623
<i>Bothrops newwiedi</i> *	<i>B. aff. matogrossensis</i> 2	-	-	-	AF292586	AF292624
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	-	KF801319
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	-	KF801302
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801178	-
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801179	-
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801159	-
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801196	KF801318
<i>Bothrops newwiedi</i> *	<i>B. aff. newwiedi</i> ⁵	-	-	-	KF801174	KF801297
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801192	KF801310
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801185	KF801305
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801198	KF801314
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801199	KF801315
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801200	KF801316
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801164	KF801290
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801197	KF801309
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801186	-
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801201	-
<i>Bothrops newwiedi</i> *	<i>B. aff. newwiedi</i>	-	-	-	KF801193	KF801311
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801166	KF801292
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801167	KF801293
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801168	-
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801169	KF801294
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801170	-
<i>Bothrops newwiedi</i> *	<i>B. newwiedi</i>	-	-	-	KF801191	KF801308
<i>Bothrops newwiedi</i>	<i>B. aff. newwiedi</i>	-	-	-	-	KF801304

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APPENDIX 2. (Continued)

Previous identification	Re-identification (this study)	12S	16S	COI	Cyt-b	ND4
<i>Bothrops newwiedi</i>	<i>B. aff. newwiedi</i>	-	-	-	KF801183	KF801303
<i>Bothrops newwiedi</i>	<i>B. aff. newwiedi</i>	-	-	-	KF801173	KF801296
<i>Bothrops newwiedi</i>	<i>B. aff. newwiedi</i>	-	-	-	KF801187	KF801306
<i>Bothrops newwiedi</i>	<i>B. marmoratus</i>	-	-	-	KF801188	-
<i>Bothrops newwiedi</i>	<i>B. aff. newwiedi</i>	-	-	-	KF801175	KF801298
<i>Bothrops newwiedi</i>	<i>B. newwiedi</i>	-	-	-	KF801162	KF801289
<i>Bothrops pauloensis</i>	<i>B. pauloensis</i>	-	-	-	KF801207	KF801323
<i>Bothrops pauloensis</i>	<i>B. cf. newwiedi</i>	-	-	-	KF801212	KF801328
<i>Bothrops pauloensis</i>	<i>B. pauloensis</i>	-	-	-	KF801204	KF801322
<i>Bothrops pauloensis</i>	<i>B. pauloensis</i>	-	-	-	KF801214	KF801330
<i>Bothrops pauloensis</i>	<i>B. pauloensis</i>	-	-	-	KF801223	KF801340
<i>Bothrops pauloensis</i>	<i>B. pauloensis</i>	-	-	-	KF801210	KF801326
<i>Bothrops pauloensis</i>	<i>B. pauloensis</i>	-	-	-	KF801203	KF801321
<i>Bothrops pauloensis</i>	<i>B. pauloensis</i>	-	-	-	KF801224	KF801341
<i>Bothrops pauloensis</i>	<i>B. marmoratus</i>	-	-	-	KF801211	KF801327
<i>Bothrops pauloensis</i>	<i>B. aff. mattogrossensis</i> 3	-	-	-	KF801215	KF801331
<i>Bothrops pauloensis</i>	<i>B. pauloensis</i>	EU867260	EU867272	-	EU867284	EU867296
<i>Bothrops pauloensis</i>	<i>B. pauloensis</i>	-	-	-	KF801205	KF801335
<i>Bothrops pauloensis</i>	<i>B. pauloensis</i>	-	-	-	KF801206	KF801336
<i>Bothrops pauloensis</i> *	<i>B. cf. newwiedi</i>	-	-	-	KF801225	KF801339
<i>Bothrops pauloensis</i> *	<i>B. pauloensis</i>	-	-	-	KF801208	KF801324
<i>Bothrops pauloensis</i> *	<i>B. pauloensis</i>	-	-	-	KF801209	KF801325
<i>Bothrops pauloensis</i> *	<i>B. pauloensis</i>	-	-	-	KF801221	KF801337
<i>Bothrops pauloensis</i> *	<i>B. pauloensis</i>	-	-	-	KF801222	KF801338
<i>Bothrops pauloensis</i> *	<i>B. cf. newwiedi</i>	-	-	-	KF801202	KF801320
<i>Bothrops pauloensis</i> *	<i>B. cf. newwiedi</i>	-	-	-	KF801218	KF801334
<i>Bothrops pauloensis</i>	<i>B. cf. newwiedi</i>	-	-	-	KF801213	KF801329
<i>Bothrops pauloensis</i>	<i>B. cf. sonene</i> sp. nov.	-	-	-	-	FN431773

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APPENDIX 2. (Continued)

Previous identification	Re-identification (this study)	12S	16S	COI	Cyt- <i>b</i>	ND4
<i>Bothrops pauloensis</i>	<i>B. cf. sonene</i> sp. nov.	-	-	-	-	FN431772
<i>Bothrops pauloensis</i>	<i>B. cf. sonene</i> sp. nov.	-	-	-	-	FN431774
<i>Bothrops pauloensis</i> *	<i>B. aff. mattogrossensis</i> 1	-	-	-	KF801216	KF801332
<i>Bothrops pauloensis</i> *	<i>B. aff. mattogrossensis</i> 1	-	-	-	KF801217	KF801333
<i>Bothrops pauloensis</i> *	<i>B. aff. mattogrossensis</i> 2	-	-	-	KF801219	KF801342
<i>Bothrops pauloensis</i> *	<i>B. aff. mattogrossensis</i> 2	-	-	-	KF801220	-
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801228	KF801345
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801230	KF801348
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801238	-
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801235	KF801353
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801226	KF801343
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801236	KF801354
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801232	KF801350
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801237	KF801355
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801239	KF801356
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	-	KF801346
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801229	KF801347
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801233	KF801351
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801231	KF801349
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801234	KF801352
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	KF801227	KF801344
<i>Bothrops pubescens</i>	<i>B. pubescens</i>	-	-	-	JN870200	JN870206
<i>Bothrops sonene</i> sp. nov.	<i>B. sonene</i> sp. nov.	this study	this study	this study	this study	this study
<i>Bothrops pauloensis</i>	<i>B. pauloensis</i>	this study	this study	this study	this study	this study
<i>Bothrops alcatraz</i>		this study	this study	this study	AY865820	this study
<i>Bothrops alternatus</i>		AY223660	AY223673	this study	AY223601	AY223642
<i>Bothrops amodytooides</i>		AY223658	AY223671	this study	AY223595	AY223639
<i>Bothrops asper</i>		EU624239	EU624273	KU986307	EU624301	EU624210

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APPENDIX 2. (Continued)

Previous identification	Re-identification (this study)	12S	16S	COI	Cyt-b	ND4
<i>Bothrops atrox</i>		KX694564	KX694620	KU986342	KX694835	GQ428485
<i>Bothrops barnetti</i>		-	-	KU986339	this study	this study
<i>Bothrops bilineatus</i>		AF057214	AF057261	-	AF292592	AF292630
<i>Bothrops brazili</i>		EU867252	EU867264	KU986320	EU867276	EU867288
<i>Bothrops caribbaeus</i>		-	-	-	AF292598	AF292636
<i>Bothrops chloromelas</i>		DQ305430	DQ305453	-	DQ305471	DQ305488
<i>Bothrops colombiensis</i>		-	-	-	MG265795	MG265817
<i>Bothrops cotiara</i>		AF057217	AF057264	-	AF292581	AF292619
<i>Bothrops fonsecai</i>		KX694552	KX694621	-	KX694832	AF292618
<i>Bothrops insularis</i>		AF057216	AF057263	-	AF292590	AF188705
<i>Bothrops isabelae</i>		-	-	-	MG265706	MG265807
<i>Bothrops itapetiningae</i>		EU867253	EU867265	-	EU867277	EU867289
<i>Bothrops jararaca</i>		NC030760	NC030760	NC030760	NC030760	NC030760
<i>Bothrops jararacussu</i>		KX694553	KX694622	this study	KX694834	AF292634
<i>Bothrops jonathani</i>		-	-	this study	-	-
<i>Bothrops lanceolatus</i>		-	-	-	AF292599	AF292637
<i>Bothrops leucurus</i>		EU867255	EU867267	KU986310	EU867279	EU867291
<i>Bothrops lojanus</i>		-	-	-	FR691566	FR691536
<i>Bothrops marajoensis</i>		-	-	-	AF292605	AF292643
<i>Bothrops moojeni</i>		EU867256	EU867268	this study	EU867280	EU867292
<i>Bothrops oligolepis</i>		-	KX660260	-	-	KX660646
<i>Bothrops osbornei</i>		KU999231	KU999160	-	KU999113	KU999120
<i>Bothrops otavioi</i>		this study	this study	this study	this study	this study
<i>Bothrops pictus</i>		-	-	-	AF292583	AF292621
<i>Bothrops pirajai</i>		this study	-	this study	this study	this study
<i>Bothrops pulchra</i>		JN870179	-	-	AF292593	AF292631
<i>Bothrops punctatus</i>		KU999235	KU999164	-	KU999116	KU999123
<i>Bothrops roedingeri</i>		-	-	this study	this study	-

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APPENDIX 2. (Continued)

Previous identification	Re-identification (this study)	12S	16S	COI	Cyt-b	ND4
<i>Bothrops taeniatus</i>		KX694566	KX694623	-	KX694836	AF292629
<i>Bothrops venezuelensis</i>		-	-	-	MG265697	MG265803
<i>Cerrophidion godmani</i>		DQ305419	DQ305442	-	AY220327	AY220350
<i>Crotalus durissus</i>		AF259248	AF259140	JQ627372	AF259178	AY704861
<i>Lachesis muta</i>		MF673200	MF673227	-	AY223604	AY223644
<i>Atropoides nummifer</i>		DQ305422	-	-	DQ061195	DQ061220
<i>Bothriechis schlegelii</i>		KC847270	KC847257	-	KC847272	KC847285
<i>Bothrocophias andianus</i>		-	-	this study	this study	-
<i>Bothrocophias campbelli</i>		-	-	-	AF292584	AF292622
<i>Bothrocophias hyoprora</i>		AF057206	AF057253	-	AF292576	AF292614
<i>Bothrocophias microphthalmus</i>		AY223657	AY223670	-	AY223594	AY223638
<i>Porthidium nasutum</i>		KX694567	KX694658	-	KX694838	DQ061235

¹These specimens of *Bothrops diporus* (IBSP60323 and IBSP60327) were geographically allocated as being from Blumenau (state of Santa Catarina, Brazil); but excepting these, there are no other records of *B. diporus* on the coastal region of Brazil in the Atlantic Forest. These two specimens were lost during the fire at the Butantan Institute in 2010, although information about them were kept in the collection notebooks. These specimens were sent to Butantan by the Fire Department of Blumenau, which does not mean that the specimens were collected in this municipality. It has been a common practice in Brazil that some institutions historically served as a hub for snakes sent to Butantan, and the Fire Department of Blumenau was one of them. According to phylogenetic results, we believe these specimens were probably from some locality within the estimated area of distribution of *B. aff. pubescens*.

² *Bothrops erythromelas* (UFBA4485) from Ibiraba (state of Bahia, Brazil) was recovered within the lineage of *B. lutzi*. It is probable that this specimen had been a polymorphism of *B. lutzi* misidentified as *B. erythromelas*. However, Ibiraba is a municipality that represents two ecoregions, the Atlantic dry Forest and the Caatinga, being the further the one that hold the habitat for *B. lutzi* (together with the Cerrado) and the latter the habitat of *B. erythromelas*. We preferred to keep this sample as *Bothrops* sp. until examination of the voucher specimen allows confirming its specific assignment.

³ *Bothrops lutzi* (IBSP73209) from Piracicaba (state of São Paulo, Brazil) probably represents a mistaken record. Information in collection notebooks of the Butantan Institute indicates that the specimen was previously identified as *B. newiedi* when it was admitted to the Institute in May of 2005. It was a male with approximately 500 mm of SVL that was lost during the fire at Butantan. Piracicaba is located at more than 600 km south of the southernmost confirmed record for *B. lutzi* (Paracatu, state of Minas Gerais, Brazil). The specimen was sent to Butantan by the Municipal Administration of Piracicaba, and there is no information about the specific site where it was collected or found.

⁴These specimens of *Bothrops marmoratus*, CEPB13422 from Hidrolândia and CEPB14067 from Luziânia (state of Goiás, Brazil) are phylogenetically apart from populations of *B. marmoratus* and nested within the diversity of *B. pauloensis*. We did not examine these two specimens, but according to their phylogenetic position we re-identified them as *B. cf. pauloensis* until further examination allows to confirm their specific assignment.

⁵*Bothrops newiedi* IBSP72734 from Ibiúna (state of São Paulo, Brazil) was not found in the collection of the Butantan Institute. The geographic location and the phylogenetic position of this specimen are discordant. It seems odd to us that all other specimens within the clade containing IBSP72734 are from the Espinhaço mountains (state of Minas Gerais) and only IBSP72734 is from Ibiúna, located at almost 500 km away from Espinhaço. We tend to consider the possibility that IBSP72734 is a mislabeled sample, and in reality the sequences deposited at Genbank are from IBSP72736 (from São Gonçalo do Rio Preto, MG, Brazil). According to phylogenetic position we identified this sample as *B. aff. newiedi*.

APPENDIX 3. Morphological characters used in phylogenetic analyses. * Characters adapted from Carrasco *et al.* (2012). ** Characters adapted from Silva & Rodrigues (2008). *** Characters defined in this study. Characters 52 and 58 were coded only for *Bothrocophias* and *Bothrops*. Characters 49–51 and 53–55 were coded only for the species of the *B. newwiedi* group. All characters were coded as non-additive. Characters 50–53 and 55 were coded as non-additive and additive, but results were the same under both coding. For those characters involving counts of both sides of the body, we included, when possible, counts from the left side.

- 0_ N° of scales between internasals ***
- 1_ N° of loreals ***
- 2_ N° of ventrals
- 3_ N° of intersupraoculars
- 4_ N° of scales contacting supraoculars
- 5_ N° of supralabials
- 6_ N° of infralabials
- 7_ N° of canthals
- 8_ N° of postcanthals
- 9_ N° of anterior intercanthals ***
- 10_ N° of medial intercanthals
- 11_ N° of posterior intercanthals ***
- 12_ N° of prefoveals
- 13_ N° of subfoveals
- 14_ N° of postfoveals
- 15_ N° of suboculars
- 16_ N° of postoculars
- 17_ N° of interoculabials between suboculars and 3rd–4th supralabials ***
- 18_ N° of interoculabials between suboculars and 4th supralabial
- 19_ N° of interoculabials between suboculars and 4th–5th supralabials ***
- 20_ N° of sublacunals ***
- 21_ N° of dorsocephalic scales from the snout to the neck, longitudinally ***
- 22_ N° of interrials
- 23_ N° of gulars
- 24_ N° of middorsal scales rows
- 25_ Distance between the eye and the anterior end of the head/length of the head
- 26_ Interorbital distance/maximum width of the head
- 27_ Internostril distance/width of the head
- 28_ Length of caudal spine/caudal length
- 29_ Postorbital stripe height (scored as n° of scales at the level of 5th–6th supralabials)
- 30_ Postorbital stripe length (scored as n° of scales posteriorly from the rictus) ***
- 31_ N° of supralabials encroached on by postorbital stripe
- 32_ N° of infralabials encroached on by postorbital stripe ***
- 33_ Inferior/superior margins of mid-dorsolateral blotch **
- 34_ Height of mid-dorsolateral blotch **
- 35_ Minimum space between mid-dorsolateral blotches **
- 36_ N° of dorsolateral blotches ***
- 37_ Width of the parietal/length of the parietal
- 38_ Minimum width across both frontals/length of the frontal
- 39_ Minimum width across both frontals/width of the parietal
- 40_ Length of the basisphenoid/length of the skull
- 41_ Length of the mandible/length of the skull
- 42_ Length of maxillary fang/height of maxilla
- 43_ Length of the ectopterygoid/length of base of the pterygoid
- 44_ N° of dentary teeth
- 45_ N° of pterygoid teeth
- 46_ N° of palatine teeth
- 47_ Length of hemipenial body (measured from the base of the organ to the bifurcation of the lobes)/total hemipenial length (measured from the base of the organ to the tips of the lobes) *
- 48_ Length of capitulum (measured ventrally on the lobes) /length of hemipenial lobe (measured from the bifurcation of the lobes to the tip of the lobes) *
- 49_ Shape of internasals (0) rounded, (1) oval ***
- 50_ Ventral markings (0) immaculate, (1) heavily speckled in all the ventral region, (2) heavily speckled in the lateroventral region, (3) slightly speckled in the lateroventral region, (4) very slightly speckled in the lateroventral region to almost

inmmaculate ***

- 51_ Gular coloration (0) strongly pigmented, (1) slightly pigmented, (2) immaculate ***
- 52_ Ventral margin of postorbital stripe starts (0) posterior, (1) below, (2) anterior to the orbit
- 53_ Pair of parietal blotches in the head (0) absent, (1) rounded, (2) elongated posteriorly ***
- 54_ Pair of occipital blotches (0) elongated posteriorly beyond the neck, (1) elongated posteriorly to the neck ***
- 55_ Superior portion of dorsolateral blotches (0) rectangular, (1) subtriangular, (2) rounded ***
- 56_ Markings on gulars (0) absent, (1) diffused markings, (2) conspicuous stripes
- 57_ Distinct white spots on posterior infralabials and gulars (0) absent, (1) present
- 58_ Dorsal color pattern (0) trapezoidal “*Bothrocophias microphthalmus*-type” markings, (1) rectangular “*Bothrops ammodytoides*-type” markings, (2) C-shaped “*B. alternatus*-type” markings, (3) trapezoidal “*B. newwiedi*-type” markings, (4) trapezoidal “*B. jararaca*-type” markings, (5) trapezoidal “*B. jararacussu*-type” markings, (6) banded “*B. taeniatus*-type” markings, (7) trapezoidal “*B. atrox*-type” markings *
- 59_ Green body coloration (0) absent, present (1)
- 60_ Supraoculars (0) oval, (1) rounded, (2) elongated *
- 61_ Middle preocular (0) not in contact with the orbit, (1) in contact with the orbit
- 62_ Upper preocular (0) does not contribute, (1) contributes to the canthus rostralis
- 63_ Lower preocular (0) not in contact, (1) in contact with the orbit ***
- 64_ Prelacunal (0) discrete, (1) fused with second supralabial *
- 65_ Prelacunal discrete (0) in contact with third supralabial, (1) in contact with second supralabial, (2) separated from second supralabial *
- 66_ Subfoveals (0) incomplete row, (1) complete row *
- 67_ Loreal (0) not elongated, (1) elongated
- 68_ Internasals (0) not elongated, (1) elongated
- 69_ Rostral (0) triangular or subtriangular, (1) quadrangular, (2) trapezoidal
- 70_ Rostral (0) not elevated, (1) elevated
- 71_ Canthus rostralis (0) not elevated, (1) elevated to form a distinct ridge
- 72_ Intersupraoculars (0) smooth or slightly keeled, (1) keeled
- 73_ Canthorostals (0) absent, (1) present
- 74_ Canthals (0) same size or slightly smaller than internasals, (1) bigger than internasals and rounded, (2) bigger than internasals and elongated *
- 75_ Intercanths (0) smooth or slightly keeled, (1) keeled ***
- 76_ Upper preocular (0) entire, (0) divided
- 77_ Tuberculate keels on mid-posterior dorsal scales (0) absent, (1) present
- 78_ Middle preocular and supralacunal (0) fused, (1) not fused
- 79_ Lowest preocular and sublacunal (0) not fused, (1) partially or totally fused
- 80_ Intersupraocular fusions in plate-like scales (0) absent, (1) usually present
- 81_ Subcaudals (0) entire, (1) entire and divided, (2) divided *
- 82_ Body compressed laterally (0) absent, (1) present ***
- 83_ Rattle (0) present, (1) absent
- 84_ Hemipenis (0) U-shaped and lobes well separated, (1) not U-shaped and lobes close to each other
- 85_ Lobes of the hemipenis (0) subcylindrical, (1) cylindrical, (2) fusiform *
- 86_ Orientation of hemipenial lobes (0) parallel, (1) divergent
- 87_ Distribution of hemipenial spines (0) heterogeneous, (1) homogeneous *
- 88_ Big sized spines in the hemipenis (0) absent, (1) positioned distally, (2) positioned proximally
- 89_ Hemipenial spines: (0) straight or curved; (1) hooked
- 90_ Intralobular spines (0) absent, (1) distally in the intralobular area, (2) along the intralobular area *
- 91_ Microornamentation in the capitulum (0) in all calyces, (1) in half or more of the calyces, (2) in the basal calyces, (3) absent
- 92_ Microornamentation in the ventral surface of the hemipenial body (0) in all the surface, (1) in part of the surface
- 93_ Microornamentation in the dorsal surface of the hemipenial body (0) in part of the surface, (1) in all the surface, (2) absent *
- 94_ Microornamentation in the intrasulcar area (0) absent, (1) present
- 95_ Swollen dorsal area in hemipenial body (0) absent, (1) present
- 96_ Swollen dorsal area in hemipenial body (0) well developed, (1) poorly developed
- 97_ Lateral depression in hemipenial body (0) absent, (1) present
- 98_ Lateral depression in hemipenial body (0) developed in both sides of the body, (1) developed in one side of the body, (2) poorly developed in one side of the body
- 99_ Swollen dorsal area in the intralobular area (0) absent, (1) present ***
- 100_ Posterolateral edges of dorsal surface of parietal: (0) with more or less pronounced shelf along the edges; (1) with poorly developed shelf in the posteriormost portion of the edge; (2) absent *
- 101_ Anterior end of the supratemporal: (0) in contact or close to parietal; (1) separated from parietal

- 102_ Internal lateral margin of the supratemporal: (0) straight; (1) curve
 103_ Posterior end of the supratemporal: (0) with internal and external lateral processes; (1) with external lateral process; (2) with internal lateral process *
 104_ Supraoccipital crests: (0) in contact or close to parietal; (1) separated from parietal
 105_ Anterior portion of the basisphenoid: (0) not sharpened; (1) sharpened
 106_ Crest of the basisphenoid: (0) uniformly elevated; (1) with a rounded differentiated elevation
 107_ Mandibular crest: (0) symmetric; (1) asymmetric
 108_ Medial wall of pit cavity in maxilla: (0) well developed; (1) weakly developed or absent
 109_ Choanal process of palatine: (0) positioned anteriorly; (1) positioned medially; (2) positioned posteriorly
 110_ Size of choanal process of palatine: (0) reduced; (1) moderate; (2) attenuate
 111_ Posterior lateral margin of ectopterygoid: (0) straight; (1) convex
 112_ Projection of ectopterygoid at point of articulation with pterygoid: (0) weakly differentiated; (1) well differentiated
 113_ Prootic foramina for nerves V2 and V3: (0) separated by bony partition; (1) not separated by bony partition
 114_ Foramen in ventral surface of lateral process of prootic: (0) absent; (1) present
 115_ Ventral process of basioccipital: (0) single; (1) bifurcates distally
 116_ Supratemporal: (0) rounded dorsal surface; (1) flat dorsal surface *
 117_ Angular and splenial: (0) partially or totally fused; (1) not fused *

APPENDIX 4. Morphological matrix.

&[cont]

<i>Crotalus durissus</i>	0.400	1.336	0.490-0.537	0.024-0.310	0.281-0.635	1.026-1.196	1.000-1.333	0.210	0.000	0.000	0.000	0.000	0.000	0.218-1.042	0.328-0.785	0.393-0.940	1.250	0.222-1.074	0.440-1.015	0.525-1.025	0.433-0.816	0.250	?	0.560-1.075	1.000-1.265	0.595	0.000	0.621	0.476	0.000	0.000	0.001	0.004	0.010	?	?	?	?	0.755	0.827	0.444	0.500	0.805	0.477	0.493	0.200				
<i>Lachesis muta</i>	0.800	1.119-2.537	0.973-1.045	1.288-1.570	0.802-0.975	1.127-0.476	0.816-1.183	0.000-1.920	1.000	1.000	1.000-1.400	1.000	0.232-1.128	0.208-0.487	0.850-2.483	0.775-1.325	0.718-1.318	0.895	1.150	1.070	0.250	1.000	0.666-1.000	0.893-1.156	0.870-1.254	0.003-0.004	0.405-0.702	0.095-0.904	0.333	0.479	1.000	0.200	0.000	?	?	?	?	0.355	0.988	1.185	0.055	0.800	0.672	0.392	0.400	0.000	0.310	0.302	0.000	
<i>Bothriechis schlegelii</i>	1.000	1.328	0.246	0.881	1.000	0.174	0.500	0.210	1.000	0.750	0.923	0.800	1.080	0.835	1.000	0.250	0.896	0.895	0.650	0.403	1.250	1.000	0.909	0.970	0.345	0.005	0.891	0.238	0.000	0.479	?	0.000	0.000	?	?	?	?	?	?	?	?	?	?	?	?	0.400				
<i>Porthidium nasutum</i>	0.400	0.336	0.038	0.595	0.833	0.322	0.500	0.210	0.500	0.500	0.769	0.700	0.680	0.557	0.333	1.250	0.896	0.895	1.150	0.736	0.250	0.352	0.363	0.000	0.345	0.005	0.675	0.285	?	0.599	0.533	0.800	0.333	?	?	?	?	?	?	?	?	?	?	?	?	0.133	0.367			
<i>Atropoides nummifer</i>	0.800	0.336	0.000-0.108	0.881	0.416	0.470	0.631-0.868	1.210	0.500	0.500	0.520-1.172	0.700	1.080	1.000-1.785	0.126-1.540	0.750	0.740-1.792	1.395	1.150	1.070	0.250	0.000	0.053-0.310	0.451-0.681	0.578-0.696	0.004	0.675	0.476	1.000	0.359	0.133	0.000	0.000	?	?	?	?	?	?	?	?	?	?	?	?	?	?			
<i>Cerrophidion godmani</i>	1.000	0.336	0.016	0.738	0.666	0.470	0.500	0.210	0.000	0.500	0.769	0.500	0.080	0.278	0.333	0.750	0.896	0.395	0.150	0.403	1.250	0.352	0.000	0.485	0.179	?	?	?	?	?	0.359	0.400	0.200	0.000	?	?	?	?	?	?	?	?	?	?	?	?				
<i>Bothrocophias hyoprora</i>	1.000	0.336	0.147	0.738	0.833	0.026	0.166	1.210	0.500	0.625	0.769	0.700	0.880	0.278	0.333	0.250	0.896	0.895	0.650	0.403	0.250	0.529	0.818	0.485	0.345	0.006	0.378	0.095	0.222	0.239	0.466	0.200	0.000	?	?	?	?	?	?	?	?	?	?	?	?	?	?			
<i>Bothrocophias microphthalmus</i>	0.278-0.622	0.118-0.812	0.142-0.224	0.517-0.874	0.750-0.916	0.026	0.048-0.081	0.210	0.390-0.720	0.421-0.613	0.563-0.783	0.500-0.717	0.482-1.144	0.175-0.548	0.333	0.100-0.800	0.733-1.355	0.385-0.905	0.040-0.445	0.203-0.546	0.100-1.070	0.500-0.754	0.500-0.812	0.231-0.597	0.080-0.371	0.004-0.008	0.459-0.783	0.285-0.523	0.111-0.222	0.251-0.467	0.525-0.624	0.258-0.442	0.333	?	?	?	?	?	0.688-0.844	0.264-1.068	0.277-0.592	0.166-0.944	0.702-0.862	0.247-0.274	0.392-0.696	0.579-0.721	0.264-0.735	0.810	0.186-0.395	0.375-0.453
<i>Bothrocophias andianus</i>	0.030-0.310	0.336	0.388-0.533	0.560-0.845	0.783-0.921	0.000-0.069	0.095-0.316	0.210	0.390-0.720	0.397-0.560	0.567-0.875	0.463-0.702	0.260-0.566	0.041-0.058	0.250-0.550	0.145-0.570	0.896	0.295-0.585	0.050-0.220	0.126-0.450	0.080-0.500	0.445-0.708	0.394-0.640	0.590-0.894	0.111-0.376	0.004-0.008	0.000-1.270	0.476-0.809	0.111-0.444	0.527-0.748	0.474-0.837	0.410-0.640	0.333	?	?	?	?	?	0.466-0.644	0.632-0.999	0.481-0.814	0.166-0.222	0.720-1.017	0.026-0.230	0.316-0.632	0.345-0.988	0.583-0.916	0.687-1.265	0.372	0.484
<i>Bothrops lojanus</i>	0.042-0.242	0.336	0.219-0.250	0.680-1.082	0.750	0.026	0.166	0.210	0.500	0.448-0.801	0.769	0.459-0.741	0.280	0.058-0.337	0.000	0.250	0.896	0.395	0.150	0.403	0.250	0.622-0.788	0.690-0.946	0.613-0.843	0.345	0.004-0.006	0.783-0.918	0.428-0.523	0.111-0.444	0.441-0.697	0.866	0.600	0.333	?	?	?	?	?	0.333	0.563	0.574	?	?	0.141	0.999	0.500	0.416	0.560	0.279	0.593
<i>Bothrops pictus</i>	0.050-0.150	0.336	0.307-0.461	0.431-0.760	0.750	0.140-0.652	0.431-0.901	0.130-1.290	0.460-1.040	0.345-0.488	0.426-0.958	0.302-0.764	0.330-0.530	0.000	0.530-1.470	0.625-1.125	0.896	0.395	0.150	0.403	0.250	0.492-0.742	0.662-																											

0.837 0.676-0.941 0.345 0.003 0.432-0.810 0.285-0.428 0.333-0.555 0.661-0.937 0.685-1.091 1.000 0.920-1.303 ? ? ?
? 0.400-0.555 0.218-0.287 0.240-0.277 0.333-0.388 0.754-0.811 0.292 0.253-0.379 0.379-0.521 0.382-0.617 0.507-0.862
0.139 0.562

Bothrops ammodytoides 0.038-0.064 0.336 0.239-0.365 0.860-1.188 0.675-0.818 0.329-0.502 0.556-0.833 0.930-1.740 0.450-
0.760 0.375 0.800-1.103 0.600 0.362-0.782 0.233-0.348 0.263-0.470 0.850-1.425 1.125-1.777 0.300-0.555 0.160-0.675 0.403
1.250 0.399-0.512 0.618-0.959 0.650-0.891 0.263-0.505 0.007-0.012 0.567-0.864 0.380-0.952 0.444-1.222 0.762-0.940 0.406-
0.553 0.870-1.050 0.333 ? ? ? ? 0.644-1.044 0.586-0.678 0.240-0.444 0.111-0.333 0.702-0.748 0.035 0.012-0.253
0.209-0.324 0.174-0.270 0.310 0.325-0.511 0.343-0.578

Bothrops jonathani 0.042-0.048 0.000-1.110 0.324-0.428 1.117-1.502 0.715-0.895 0.406-0.693 0.781-1.040 0.040-1.000
0.390-0.795 0.750 0.790-1.126 0.700 0.604-2.018 0.618-1.523 0.493-1.950 0.910-1.670 1.022-1.911 0.895-1.395 0.735-1.335
0.843-1.130 1.060-1.900 0.705 0.970-1.308 0.676-0.941 0.639-0.885 0.003-0.007 0.567-0.891 0.285-0.666 0.444-1.000 1.000-
1.297 0.389-0.544 0.492-0.642 0.333 ? ? ? ? 0.600-0.911 0.494-0.850 0.407-0.592 0.277-0.500 0.771-0.834 0.194-
0.371 0.240-0.569 0.245-0.383 0.083-0.250 0.000-0.190 0.860-1.069 0.218-0.468

Bothrops alternatus 0.000 0.336 0.426-0.538 0.000-1.102 0.715-0.902 0.278-0.393 0.678-1.051 0.210 0.395-0.765 0.437-
0.687 0.813-1.110 0.775-0.875 0.726-1.540 0.370-1.222 0.276-1.006 0.550-1.325 0.755-1.681 0.895 0.505-0.860 0.736 1.250
0.823 0.858-1.195 0.676-1.004 0.872-1.175 0.002-0.008 0.405-0.891 0.285-0.761 0.333-0.888 0.776-1.057 0.361-0.489 0.664-
0.836 1.000-1.333 ? ? ? ? 0.666-0.800 0.000-0.862 0.000-0.574 0.111-0.500 0.731-0.891 0.345-0.486 0.126-0.303
0.209-0.324 0.333 0.060 0.813-1.023 0.468-0.656

Bothrops cotiara 0.022-0.218 0.336 0.343-0.385 1.185-1.720 0.542-0.690 0.174 0.341-0.525 0.210 0.375-0.825 0.500 0.813-
1.032 0.800 0.372-0.828 0.139-0.863 0.430-1.070 0.125-0.575 1.155-1.822 0.895 0.650 0.736 1.250 0.705 0.766-1.306 0.478-
0.750 0.345-0.680 0.004-0.007 0.675-1.000 0.666-0.761 0.777-0.888 0.750-1.167 0.412-0.521 0.484-0.816 0.106-0.393 ? ?
? ? 0.711 0.919 0.574 0.444 0.851 0.362 0.126 0.300 0.333 0.310 0.627-0.813 0.328-0.390

Bothrops fonsecai 0.000 0.336 0.454 1.310 0.583 0.174 0.166 0.210 1.000 0.625 0.923 0.700 0.680 0.000 0.666 0.250 0.896
0.245-0.995 0.000-0.755 0.403 0.250 0.764 0.909 0.647 0.595 0.006 0.837 0.666 0.555 0.839 0.400 1.000 0.333 ? ? ? ?
0.933 0.586 0.351 0.333 0.811 0.336 0.291 0.300 0.500 0.310 0.558 0.375

Bothrops itapetiningae 0.000 0.336 0.101-0.222 0.704-0.868 0.646-0.742 0.174 0.333 0.210 0.500 0.625 0.769 0.700 0.280-
0.680 0.278 0.333 0.630-1.205 0.962-1.814 0.395 0.150 0.403 1.250 0.529 0.614-0.719 0.809 0.436-0.532 0.004-0.005 0.918-
1.108 1.000 0.333-0.666 0.719 0.533 1.000 1.000 ? ? ? ? 0.422 0.402 0.388 0.555 ? 0.477 0.873 0.000 0.000 ?
0.511 0.609

Bothrops sonene sp. nov. 0.000 0.336 0.445-0.486 0.738 0.737-0.929 0.174 0.196-0.470 0.210 0.125-0.375 0.490-0.635 0.660-
0.955 0.485-0.715 0.630-0.830 0.278 0.096-0.736 0.210-0.790 0.711-1.451 0.520-1.020 0.110-0.690 0.403 0.250 0.363-0.499
0.522-0.613 0.472-0.660 0.326-0.615 0.003-0.007 0.189-0.918 0.047-1.047 0.555-0.888 0.467-0.582 0.184-0.416 0.550-0.750
0.250-0.583 0.000-1.215 0.405-1.202 0.398-0.588 1.551-2.038 ? ? ? ? ? ? ? ? ? ? 0.255 0.515

Bothrops cf. *sonene* 0.000 0.336 0.475-0.552 0.668-0.998 0.732-0.850 0.139-0.309 0.293-0.485 0.210 0.045-0.620 0.500
0.629-0.806 0.600 0.432-0.662 0.066-0.253 0.333 0.250 0.896 0.440-1.015 0.030-0.605 0.403 0.250 0.537-0.717 0.526-0.655
0.500-0.686 0.478-0.713 0.999 0.513-1.135 0.380-0.571 0.666-0.777 0.455-0.705 0.386-0.525 0.552-0.782 0.363-0.746 0.907-
2.323 0.376-1.492 0.049-0.552 1.619-1.853 ? ? ? ? ? ? ? ? ? ? ? ? ? ?

Bothrops mattogrossensis 0.000 0.336 0.579-0.634 0.537-0.738 0.549-0.722 0.025-0.211 0.486-0.583 0.210 0.375-0.625
0.406-0.468 0.603-0.692 0.418-0.500 0.522-0.780 0.050-0.278 0.050-0.333 0.210-0.500 0.340-0.711 0.355-0.645 0.110-0.400
0.403 0.250 0.441-0.510 0.377-0.606 0.472-0.566 0.304-0.387 ? 0.594-0.810 0.380-0.523 0.666 0.470-0.539 0.280-0.366
0.650-0.750 0.333 0.676-1.430 1.256-1.560 0.256-0.842 0.375-0.624 ? ? ? ? ? ? ? ? ? ? ? ? ? ?

Bothrops aff. *mattogrossensis*_1 0.000 0.336 0.381-0.471 0.494-0.768 0.600-0.815 0.063-0.211 0.196-0.470 0.210 0.500
0.312-0.562 0.429-0.724 0.418-0.582 0.278-0.782 0.022-0.300 0.083-0.416 0.250 0.896 0.395 0.110-0.690 0.403 0.250 0.293-
0.441 0.318-0.500 0.352-0.618 0.210-0.481 0.001-0.009 0.405-0.945 0.142-0.809 0.333-0.666 0.419-0.659 0.366-0.500 0.370-
0.830 0.250-0.583 0.600-1.123 0.212-2.299 0.575-1.398 0.032-0.708 ? ? ? ? ? ? ? ? ? ? ? ? ? ?

Bothrops aff. *mattogrossensis*_2 0.000 0.336 0.305-0.382 0.375-0.701 0.570-0.662 0.034-0.196 0.085-0.381 0.210 0.500
0.343-0.456 0.476-0.815 0.435-0.525 0.352-0.688 0.013-0.041 0.016-0.383 0.230-1.070 0.896 0.395 0.150 0.403 0.250 0.217-
0.347 0.269-0.421 0.461-0.639 0.238-0.386 0.008-0.010 0.675-0.891 0.428-0.666 0.444-0.666 0.398-0.633 0.304-0.376 0.458-
0.742 0.333 0.753-1.261 0.632-1.492 0.059-0.335 0.551-1.048 ? ? ? ? ? ? ? ? ? ? ? 0.348 0.421

Bothrops aff. *mattogrossensis*_3 0.000 0.336 0.442-0.530 0.670-0.902 0.592-0.740 0.069-0.279 0.250-0.683 0.210 0.410-
0.925 0.385-0.573 0.575-0.963 0.466-0.634 0.690-0.910 0.197-0.693 0.250-0.550 0.125-0.575 0.896 0.275-0.680 0.365-0.770
0.343-0.686 0.250 0.380-0.600 0.487-0.640 0.485 0.220 0.005-0.010 0.729-0.810 0.476-0.666 0.333-0.555 0.470-0.609 0.358-
0.441 0.418-0.648 0.333 0.769-1.123 0.642-1.227 0.486-1.075 0.218-0.619 ? ? ? ? ? ? ? ? ? ? ? ? ? ?

Bothrops diporus 0.000 0.336 0.489-0.628 0.627-0.987 0.694-0.872 0.142-0.281 0.396-0.691 0.210 0.320-0.595 0.345-0.555
0.520-0.749 0.417-0.623 0.480-1.030 0.116-0.540 0.233-0.600 0.100-0.650 0.600-1.155 0.505-0.995 0.210-0.700 0.340-0.650
0.040-0.640 0.487-0.571 0.295-0.777 0.559-0.870 0.163-0.438 0.004 0.783 0.571 0.444 0.465-0.700 0.357-0.625 0.030-0.376
0.066-0.160 1.338-5.676 0.502-1.792 0.138-0.710 0.611-1.375 0.444-0.688 0.287-0.528 0.277-0.518 0.166-0.222 0.725-0.765
0.274-0.389 0.278-0.392 0.476-0.591 0.272-0.560 0.310 0.069-0.186 0.437-0.546

Bothrops aff. *diporus* 0.000 0.336 0.512-0.690 0.675-1.058 0.662-0.863 0.161-0.386 0.415-0.696 0.100-0.350 0.350-0.655
0.450-0.693 0.561-0.930 0.593-0.836 0.496-1.124 0.122-0.615 0.233-0.700 0.120-0.695 0.681-1.059 0.570-1.035 0.310-0.765
0.390-0.893 0.000-1.000 0.580-0.865 0.410-0.850 0.621-0.991 0.198-0.538 0.171 0.027 0.000 0.666 0.434-0.760 0.282-0.614

0.044-0.234 0.053-0.080 1.030-4.876 0.531-1.869 0.016-0.618 0.768-1.590 0.577 0.252 0.444 0.222 0.760 0.274 0.392 0.500
0.583 0.560 0.116-0.325 0.468
Bothrops aff. pubescens 0.000 0.336 0.466-0.602 0.671-1.048 0.622-0.795 0.116-0.321 0.356-0.615 0.210 0.385-0.640 0.536-
0.713 0.478-0.764 0.551-0.729 0.440-0.962 0.103-0.562 0.236-0.626 0.160-0.405 0.659-1.192 0.510-1.005 0.240-0.720 0.353-
0.720 0.000-0.900 0.464-0.782 0.278-0.718 0.525-0.823 0.175-0.525 0.004-0.007 0.513-0.837 0.238-0.619 0.444-0.666 0.429-
0.688 0.373-0.609 0.040-0.308 0.060-0.160 1.507-6.784 0.512-1.618 0.082-0.773 0.406-1.208 ? ? ? ? ? ? ? ?
? ? ? ?
Bothrops pubescens 0.000 0.336 0.432 0.881 0.750 0.174 0.500 0.210 0.500 0.500 0.769 0.500 0.480 0.278 0.666 0.250 0.896
0.395 0.650 0.736 0.250 0.764 0.636 0.647 0.512 ? 0.621 0.571 0.666 0.419 0.400 0.400 0.333 1.599 1.739 0.000 0.799
0.400 0.528 0.425 ? ? 0.592 0.240 0.400 0.166 0.310 0.255 0.640
Bothrops neuwiedi 0.000 0.336 0.396-0.489 0.854-1.290 0.565-0.684 0.066-0.578 0.418-0.803 0.210 0.380-0.955 0.500-0.750
0.923 0.676-0.791 0.916-1.378 0.278-0.835 0.090-1.243 0.250 0.155-1.637 0.895 0.195-0.770 0.240-1.010 0.250 0.752-0.835
0.649-0.926 0.500-0.686 0.527-0.720 ? 0.648 0.523 0.444 0.609-0.748 0.392-0.430 0.400 0.080-0.303 1.446-2.492 1.212-
2.425 0.184-1.187 0.193-0.782 0.444 0.379 0.407 0.333 0.000 0.442 0.848 0.600 0.333 0.310 0.232-0.558 0.343-0.359
Bothrops aff. neuwiedi 0.000 0.336 0.482-0.596 0.840-1.208 0.561-0.771 0.158-0.316 0.308-0.691 0.210 0.380-0.760 0.470
0.680-0.858 0.517-0.740 0.382-0.920 0.050-0.506 0.056-0.516 0.250 0.511-1.074 0.415-0.950 0.390-0.770 0.366-0.723 0.010-
0.770 0.564-0.695 0.512-1.046 0.512-0.736 0.456-0.686 0.002-0.008 0.540-0.864 0.428-0.714 0.333-0.555 0.446-0.683 0.333-
0.457 0.408-0.622 0.333 0.323-3.769 1.338-2.415 0.542-0.937 0.329-1.103 ? ? ? ? ? ? ? ? ? ? ? ?
Bothrops marmoratus 0.000 0.336 0.309-0.542 0.738 0.565-0.684 0.174 0.131-0.368 0.210 0.395-1.105 0.323-0.676 0.366-
1.018 0.359-0.641 0.598-1.162 0.278 0.000 0.250 0.896 0.895 0.150 0.403 0.250 0.399-0.482 0.435-0.564 0.485 0.161-0.280
0.006-0.007 0.567-1.081 0.571-0.857 0.444-0.555 0.407-0.491 0.172-0.361 0.600 0.333 0.215-1.923 0.724-1.062 0.542-0.773
0.234-0.896 ? ? ? ? ? ? ? ? ? ? ? ? ? ?
Bothrops pauloensis 0.000 0.336 0.474-0.629 0.680-1.082 0.666 0.353 0.465-0.701 0.210 0.395-1.105 0.500 0.769 0.500
0.880 0.058-0.337 0.070-0.736 0.250 0.000-1.051 0.395 0.150 0.000-0.473 0.250 0.529 0.398-0.783 0.419-0.875 0.311-0.546
0.003-0.005 0.702-0.918 0.523-0.666 0.555-0.666 0.407-0.491 0.320-0.413 0.358-0.642 0.333 1.015-1.200 1.178-1.695 1.000-
1.467 0.000-0.662 ? ? ? ? ? ? ? ? ? ? ? ? ? ?
Bothrops erythromelas 0.000 0.336 0.206-0.331 0.310 0.565-0.725 0.137-0.285 0.035-0.465 0.210 0.500 0.218-0.343 0.244-
0.678 0.159-0.441 0.116-0.444 0.069-0.208 0.083-0.416 0.625-1.125 0.807-1.918 0.020-0.520 0.150 0.043-0.430 0.250 0.164-
0.277 0.008-0.112 0.289-0.600 0.000-0.191 ? 0.648-0.972 0.619-0.761 0.888 0.824-1.033 0.368-0.521 0.800 0.666 0.799-
1.476 0.000-0.739 0.144-0.240 0.537-0.672 0.822 0.344 0.222 0.222 0.748 0.362 0.392 0.300 0.250 0.310 ? ?
Bothrops lutzi 0.000 0.336 0.332-0.410 0.274-0.702 0.299-0.534 0.174 0.298-0.535 0.210 0.500 0.323-0.676 0.583-0.801
0.479-0.621 0.638-0.922 0.220-0.615 0.333 0.250 0.896 0.000-0.500 0.150 0.000-0.473 0.010-1.160 0.340-0.424 0.235-0.491
0.485 0.145-0.380 ? 0.864-1.351 0.523-1.190 0.666 0.527-0.611 0.333 0.558-0.842 0.333 2.123-2.661 1.207-1.545 0.049-
0.279 0.961-1.457 ? ? ? ? ? ? ? ? ? ? ? ? ? ?
Bothrops jararaca 0.400 0.336 0.613-0.702 0.828-1.124 0.687-1.034 0.174 0.235-0.486 0.210 0.500 0.375 0.563-0.821 0.700
0.480-0.880 0.000 0.333 0.250 0.474-1.074 0.395 0.150 0.403 0.250 0.529 0.700-1.390 0.611-1.006 0.332-0.692 0.002-0.006
0.594-0.891 0.380-0.619 0.333-0.555 0.455-0.623 0.400 0.410-0.630 0.083-0.416 ? ? ? ? 0.600 0.597 0.499 0.388
0.788 0.637 0.544 0.800 0.666 0.560 1.046 0.281
Bothrops insularis 0.400 0.336 0.563-0.640 0.565-0.768 0.732-0.850 0.174 0.035-0.298 0.210 0.395-1.105 0.375 0.461 0.500
0.238-0.522 0.000 0.333 0.250 0.000-1.051 0.395 0.150 0.403 0.250 0.588 0.508-0.764 0.613-0.843 0.311-0.546 0.005 0.837
0.666 0.333 0.479 0.066 0.400 0.333 ? ? ? ? 0.577 0.413 0.314 1.055 1.000 0.389 0.607 0.200 0.666 0.810 0.139 0.296
Bothrops taeniatus 0.400 0.336 1.016-1.121 0.512-0.964 0.875-1.041 0.026 0.226-0.506 0.210 0.145-0.855 0.373-0.688 0.506-
0.724 0.375-0.475 0.086-0.954 0.069-0.181 0.170-0.763 0.125-0.575 0.711-1.377 0.270-0.720 0.150 0.320-0.653 0.250 0.740-
0.906 0.411-0.788 0.451-0.762 0.390-0.735 0.002-0.005 0.081-1.162 0.142-0.714 0.111-0.444 0.338-0.491 0.066-0.533 0.400
0.333 ? ? ? ? 0.000 0.183 0.499 ? ? 0.999 0.481 0.700 0.583 0.810 0.255-0.465 0.515-0.531
Bothrops pulchra 0.400 0.336 0.486 0.595 0.833 0.026 0.000 0.210 0.500 0.375 0.461 0.300 0.680 0.000 0.333 0.250 0.155
0.395 0.150 0.403 0.250 0.470 0.363 0.647 0.012 0.006 1.027 0.714 0.111 0.719 0.400 0.000 0.000 ? ? ? ? ? ? ? ?
? ? ? ? ? ? ? ? ? ?
Bothrops chloromelas 0.400 0.336 0.541-0.727 0.554-0.851 0.780-0.920 0.026 0.130-0.276 0.210 0.500 0.375 0.358-0.598
0.500 0.214-0.568 0.000 0.333 0.140-0.470 0.177-0.955 0.395 0.150 0.403 0.250 0.647 0.334-0.635 0.593-0.917 0.183-0.434
0.002-0.014 0.486-0.972 0.142-0.523 0.111 0.599 0.333 0.400 0.000 ? ? ? ? 0.466-0.933 0.275-0.804 0.222-0.888
0.333-0.388 0.754-0.811 0.256-0.513 0.253-0.379 0.538-0.962 0.430-0.902 0.757-1.112 0.069 0.640
Bothrops oligolepis 0.400 0.336 0.695-0.760 0.727-0.892 0.982-1.100 0.026 0.208-0.375 0.210 0.500 0.250 0.335-0.587 0.400
0.116-0.444 0.000 0.196-1.136 0.000-1.000 0.837-1.696 0.395 0.150 0.070 0.250 0.529 0.480-0.655 0.634-0.822 0.328-0.488
0.004-0.005 0.702-0.891 0.380-0.666 0.000 0.479 0.133 ? 0.000 ? ? ? ? 0.577 0.655 0.703 0.444 0.788 0.309 0.177
0.600 0.750 1.060 ? ?
Bothrops bilineatus 0.400 0.336 0.734-0.827 0.525-0.910 0.842-1.130 0.032-0.190 0.363-0.636 0.210 0.575-1.090 0.375
0.489-0.741 0.500 0.334-1.026 0.220-0.615 0.250-0.550 0.250 0.748-1.466 0.395 0.150 0.403 0.250 0.764 0.668-1.097 0.461-
0.833 0.610-0.890 0.003-0.007 0.729-1.108 0.190-0.571 0.000-0.222 0.119 0.000 0.200 0.333 ? ? ? ? 0.711 0.678
0.259 0.333 0.697 0.477 0.000 1.000 0.833 0.810 0.395 0.281
Bothrops sanctaerucis 0.358-0.534 0.336 0.532-0.642 0.601-0.808 0.647-0.837 0.091-0.207 0.216-0.366 0.210 0.375-0.875

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Bothrops lanceolatus ???0??007001101??00201100000101200111000200000?0?00000001101200??0??

APPENDIX 5. Genetic distances among the lineages of the *Bothrops newwiedi* group of species.

Lineages	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
<i>B. lutzi</i>	1	0.0030	0.0415	0.0694	0.0762	0.0578	0.0830	0.0868	0.0877	0.0947	0.0829	0.0791	0.0755	0.0820	0.0804	0.0799
<i>B. erythromelas</i>	2	0.0473	0.0027	0.0819	0.0887	0.0703	0.0956	0.0994	0.1003	0.1073	0.0954	0.0917	0.0880	0.0945	0.0930	0.0925
<i>B. aff. newwiedi</i>	3	0.0737	0.0859	0.0013	0.0292	0.0331	0.1052	0.1090	0.1099	0.1169	0.1050	0.1013	0.0976	0.1041	0.1026	0.1021
<i>B. marmoratus</i>	4	0.0798	0.0920	0.0311	0.0006	0.0399	0.1120	0.1158	0.1167	0.1237	0.1119	0.1081	0.1045	0.1110	0.1094	0.1089
<i>B. newwiedi</i>	5	0.0617	0.0739	0.0354	0.0415	0.0009	0.0936	0.0974	0.0983	0.1053	0.0934	0.0897	0.0860	0.0925	0.0910	0.0905
<i>B. mattogrossensis</i>	6	0.0878	0.1000	0.1083	0.1144	0.0963	0.0018	0.0139	0.0148	0.0218	0.0750	0.0712	0.0676	0.0741	0.0725	0.0720
<i>B. aff. mattogrossensis</i> 3	7	0.0925	0.1047	0.1129	0.1191	0.1009	0.0183	0.0026	0.0152	0.0222	0.0788	0.0750	0.0714	0.0779	0.0763	0.0758
<i>B. aff. mattogrossensis</i> 2	8	0.0923	0.1045	0.1128	0.1189	0.1007	0.0181	0.0194	0.0015	0.0113	0.0797	0.0759	0.0723	0.0788	0.0772	0.0767
<i>B. aff. mattogrossensis</i> 1	9	0.0986	0.1108	0.1191	0.1252	0.1070	0.0244	0.0257	0.0137	0.0008	0.0867	0.0829	0.0792	0.0858	0.0842	0.0837
<i>B. cf. sonene</i>	10	0.0871	0.0993	0.1076	0.1137	0.0956	0.0780	0.0826	0.0824	0.0887	0.0012	0.0303	0.0266	0.0331	0.0315	0.0310
<i>B. aff. diporus</i>	11	0.0822	0.0944	0.1026	0.1088	0.0906	0.0730	0.0777	0.0775	0.0838	0.0315	--	0.0199	0.0264	0.0248	0.0243
<i>B. diporus</i>	12	0.0817	0.0940	0.1022	0.1083	0.0902	0.0726	0.0772	0.0770	0.0833	0.0310	0.0231	0.0032	0.0168	0.0089	0.0084
<i>B. pauloensis</i>	13	0.0859	0.0981	0.1064	0.1125	0.0944	0.0768	0.0814	0.0812	0.0875	0.0352	0.0273	0.0209	0.0009	0.0217	0.0212
<i>B. aff. pubescens</i>	14	0.0849	0.0971	0.1054	0.1115	0.0933	0.0757	0.0804	0.0802	0.0865	0.0342	0.0263	0.0136	0.0241	0.0014	0.0060
<i>B. pubescens</i>	15	0.0839	0.0961	0.1043	0.1104	0.0923	0.0747	0.0794	0.0792	0.0855	0.0332	0.0252	0.0125	0.0231	0.0084	0.0009

