



## Molecular and phenotypic data reveal a new Amazonian species of pit vipers (Serpentes: Viperidae: *Bothrops*)

Francisco Dal Vechio, Ivan Prates, Felipe G. Grazziotin, Roberta Graboski & Miguel Trefaut Rodrigues

To cite this article: Francisco Dal Vechio, Ivan Prates, Felipe G. Grazziotin, Roberta Graboski & Miguel Trefaut Rodrigues (2020) Molecular and phenotypic data reveal a new Amazonian species of pit vipers (Serpentes: Viperidae: *Bothrops*), *Journal of Natural History*, 54:37-38, 2415-2437, DOI: [10.1080/00222933.2020.1845835](https://doi.org/10.1080/00222933.2020.1845835)

To link to this article: <https://doi.org/10.1080/00222933.2020.1845835>



Published online: 11 Mar 2021.



Submit your article to this journal [↗](#)



View related articles [↗](#)



View Crossmark data [↗](#)



## Molecular and phenotypic data reveal a new Amazonian species of pit vipers (Serpentes: Viperidae: *Bothrops*)

Francisco Dal Vechio <sup>a</sup>, Ivan Prates <sup>b</sup>, Felipe G. Grazziotin <sup>c</sup>, Roberta Graboski <sup>d</sup> and Miguel Trefaut Rodrigues <sup>a</sup>

<sup>a</sup>Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil;

<sup>b</sup>Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; <sup>c</sup>Laboratório de Coleções Zoológicas, Instituto Butantan, São Paulo, Brazil; <sup>d</sup>Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil

### ABSTRACT

Recent genetic studies have found unclear species boundaries and evidence of undescribed diversity in the poorly studied *jararacussu* species group within *Bothrops*. In this contribution, we investigate phenotypic and genetic diversity in the Amazonian snake *Bothrops brazili* to test previous assertions of unrecognised species diversity within this taxon. Our phylogenetic results and inferences of independently evolving lineages based on molecular data recover two divergent clades within *B. brazili*, one restricted to areas north and another to areas south of the Amazon River. Phylogenetic relationships between these lineages and other species in the *jararacussu* species group reveal *B. brazili* to be paraphyletic, with the northern clade inferred as sister to a clade composed of Atlantic Forest taxa (*B. jararacussu*, *B. muriciensis*, *B. pirajai*). External morphology (number of ventral and subcaudal scales) and colouration patterns (lateral trapezoidal marks) consistently separate the two lineages of *B. brazili*. We therefore recognise and describe the northern lineage as a new species of *Bothrops*, improving our knowledge of species diversity within a medically important clade of venomous South American snakes.

### ARTICLE HISTORY

Received 7 July 2020

Accepted 29 October 2020

### KEYWORDS

Amazonia; Amazon River; *Bothrops brazili*; *B. jararacussu*; *B. pirajai*; *B. muriciensis*; species delimitation

## Introduction

Venomous snakes in the genus *Bothrops* Wagler 1824 (family Viperidae) have been the focus of several phylogenetic and diversification studies over recent years (Wüster et al. 2008; Fenwick et al. 2009; Carrasco et al. 2012; Alencar et al. 2016), with an equally active taxonomy revealing unrecognised diversity at the species level (Ferrarezzi and Freire 2001; Marques et al. 2002; Da Silva and Rodrigues 2008; Folleco-Fernández 2010; Barbo et al. 2012, 2016; Carrasco et al. 2019; Timms et al. 2019). Despite these efforts, conflicting topologies, taxonomic uncertainty, and underestimation of species diversity persist (Machado et al. 2014; Dal Vechio et al. 2018, 2020). Among the *Bothrops* species whose taxonomy is poorly known are the members of the *jararacussu* species group. In this group, the phylogenetic relationships of *B. sanctaerucis* Hoge 1966, phylogenetic

relationships and taxonomic status of populations from the coastal Atlantic Forest (corresponding to three taxa, *B. jararacussu* Lacerda 1884, *B. muriciensis* Ferrarrezzi & Freire 2001, and *B. pirajai*; Amaral 1923), as well as of phenotypically distinct populations in Amazonia (under the name *B. brazili* Hoge 1953), remain unclear (Cunha and Nascimento 1975, 1978, 1993; Campbell and Lamar 2004; Dal Vechio et al. 2020).

Similar to the other members of the *jararacussu* group, *Bothrops brazili*, the Brazil's lancehead, is characterised by a robust body and terrestrial habit. Despite its broad distribution in Amazonian lowlands from Brazil, Peru, Bolivia, Colombia, Ecuador, Venezuela, Guyana, French Guiana and Surinam, these reddish pit vipers went unnoticed by 19<sup>th</sup> century naturalists, possibly as a consequence of their rarity in most localities. Only in 1953 Hoge recognised and described the species, named in honour of Dr. Vital Brazil, the founder of the Butantan Institute in São Paulo. This description was performed on the basis of two specimens, a male and a female, from a single locality that lies south of the Amazon River: 'Tomé Assu, Acará Mirim River, state of Pará, Brazil'. Cunha and Nascimento (1975, 1978, 1993) recognised pronounced phenotypic variation between populations of *B. brazili* that occur north and south of the Amazon region, suggesting well defined 'geographic races'.

Recently, Dal Vechio et al. (2020) studied the demographic history of the *Bothrops jararacussu* species group on the basis of molecular data, recovering two allopatric lineages under the taxon name of *B. brazili*. One such lineage is restricted to areas north of the Amazon River, while the other occurs south to the river on its right bank. Surprisingly, that study recovered northern and southern lineages to be non-sister, with samples from the northern population clustering with a clade composed of specimens from the Atlantic Forest. This Atlantic Forest clade included populations recognised as three distinct taxa: *B. muriciensis*, *B. pirajai* and *B. jararacussu* (Dal Vechio et al. 2020).

Evidence of *B. brazili* as a paraphyletic species, coupled with previously suggested morphological differences between populations north and south of the Amazon River, indicate the need of an evaluation of unrecognised species diversity under this taxon name. In this contribution, we perform a comprehensive assessment of phenotypic variation across the distribution of *B. brazili* under the light of patterns of spatial genetic structure. This assessment reveals a new species of Amazonian pit viper, which we formally describe.

## Materials and methods

### External morphological data

We examined 41 specimens of *Bothrops brazili* spanning the taxon's entire geographic distribution (Appendix A). We complemented this examination with data available in the literature (Amaral 1923; Hoge 1953, 1966; Hoge & Lancini 1962; Roze 1966; Hoge et al. 1972; Cunha & Nascimento 1975, 1978, 1993; Gasc & Rodrigues 1980; Chippaux 1986; Dixon & Soini 1986; Campbell & Lamar 1989, 2004; Ferrarrezzi & Freire 2001; Duellman 2005; Da Silva & Rodrigues, 2008; Carrasco et al. 2019; Timms et al. 2019).

We follow Campbell (1985) in the description of scale attributes and counts. Morphometric measurements were taken using a solid metallic ruler to the nearest 10 mm or a caliper to the nearest 0.1 mm. Measurements were: body length (SVL), from

the tip of the snout to the cloaca; tail length (TL), from the posterior border of the cloaca to the tip of the tail; head length (HL), from the snout to the posterior edge of the lower jaw. Observation of scale attributes and scale counts were performed under a Zeiss stereomicroscope. Traits were: number of ventrals (V), subcaudals (SC), supra (SL) and infralabials (IL), prefoveals, postfoveals, state of prelacunar (fused or not with the second supralabial), preocular, postocular, interoculabials, circumorbitals, gulars, canthals, posterior intercanthals, intersupraoculars, dorsals scales and number of lateral trapezoidal marks (LTM) from the neck to the cloaca region (blotches on the tail were not considered).

### **Hemipenial preparation**

We prepared the left hemipenis of the holotype of the new species (MZUSP 23282) following Manzani and Abe (1988) and Zaher (1999). The retractor muscle was manually separated and the everted organ filled with stained petroleum jelly. The organ was immersed in an alcoholic solution of Alizarin Red for 24 hours to stain possible calcified structures as proposed by Nunes et al. (2012). The terminology of hemipenial structures follows Dowling and Savage (1960) and Zaher (1999).

### **Molecular data**

For phylogenetic inference, we used sequences from 18 samples from the *jararacussu* species group generated by Dal Vechio et al. (2020), as follows: nine specimens of *Bothrops brazili*, six *B. jararacussu*, two *B. muriciensis*, and one *B. pirajai*. We also included in our alignments two additional samples of *B. brazili* and two of *B. jararacussu* available in the Genbank. Lastly, we added previously published sequences from 49 samples representing species from other *Bothrops* groups, composing a matrix with 23 terminal taxa (Appendix B). Our final molecular dataset was a concatenated matrix including nine molecular markers, four of which mitochondrial and five nuclear (total alignment length 6,009 bp), as follows: the mitochondrial genes *16s ribosomal RNA* (*16s*, 534 bp; Palumbi 1996), *12s ribosomal RNA* (*12s*, 394 bp; Palumbi 1996), *cytochrome b* (*cytb*, 737 bp; Pook et al. 2000), and *NADH dehydrogenase subunit 4* (*nd4*, 798 bp; Arévalo et al. 1994), and the nuclear genes *megakaryoblastic leukaemia 1* (*mkl1*, 787 bp; Townsend et al. 2008), *recombination-activating gene 1* (*rag1*, 995 bp; Wiens et al. 2008), *prolactin receptor* (*prlr*, 551 bp; Townsend et al. 2008), *oocyte maturation factor* (*c-mos*, 575 bp; Godinho et al. 2006), and *brain-derived neurotrophic factor* (*bdnf*, 639 bp; Townsend et al. 2008).

We performed Bayesian inference for phylogenetic reconstruction using MrBayes v. 3.2 (Ronquist et al. 2012), through the Cipres Science Gateway (Miller et al. 2010). Sequences were aligned using MUSCLE (Edgar 2004) as implemented in MEGA v. 6 (Tamura et al. 2013) under default settings. The best-fit model of evolution for each aligned marker was determined using jModelTest v. 2.1.3 (Darriba et al. 2012) under the Akaike Information Criterion (AIC). Best-fit models were as follows: GTR+G + I for all mitochondrial markers, HKY for *bdnf* and *c-mos*, GTR for *prlr*, GTR+I for *rag1*, and HKY+I for *mkl1*. For the Bayesian analyses, two independent runs were performed with four Markov chains each, starting from a random tree; each run consisted of 10,000,000 generations, with trees sampled every 1,000 generations. We discarded the first 25% of trees as burn-in; convergence of independent runs and stationarity of model parameters (effective sampling sizes > 200)

were verified using Tracer v. 1.6 (Rambaut and Drummond 2009). A 50% majority consensus tree was summarised from MrBayes analyses. Resulting phylogenies were visualised using FigTree 1.4.0 (<http://tree.bio.ed.ac.uk/>). Uncorrected genetic distances (p-distances) were calculated using MEGA v. 6 (Tamura et al. 2013).

### **Inferring independently evolving lineages**

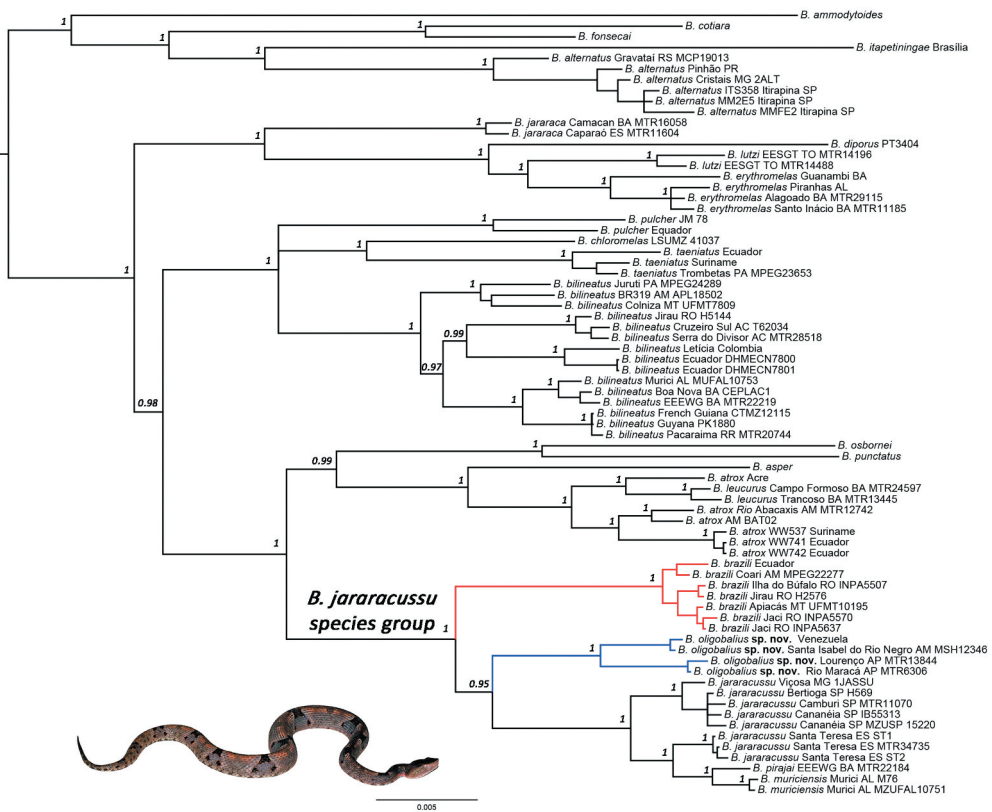
To support an evaluation of unrecognised species diversity in the *B. jararacussu* species group, we estimated independently evolving lineages using the molecular species delimitation algorithm *Generalised Mixed Yule-Coalescent* (GMYC; Pons et al. 2006), using its Bayesian implementation (bGMYC; Reid and Carstens 2012). This method interprets splits on a phylogeny as either species divergences or intra-specific coalescent events. As GMYC assumes that the frequency of coalescent events within species is higher than the rate of divergences between species, attempting to find a threshold that distinguishes these two branching types on a target phylogeny, it is important that the dataset bears samples representative of intra and inter specific splits. An ultrametric target tree was inferred based on the mitochondrial dataset using BEAST 1.8.4 (Drummond et al. 2012), with two independent runs of 20 million generations each sampling every 2,000 generations. We used as parameters and priors to the analysis: uncorrelated relaxed clock and lognormal for relaxed distribution; random starting tree; and Yule process for speciation model. The best-fit model of evolution for each mitochondrial marker was identified using jModelTest v. 2.1.3 (Darriba et al. 2012) and the Akaike Information Criterion (AIC), resulting GTR+G + I for all of them. bGMYC was applied based on 500 posterior trees from BEAST runs using the *bgmyc.multiphyll()* function in R 3.3.2, with 50,000 MCMC steps (40,000 steps as burn-in) sampling every 100 steps. We used a probability threshold of 0.7 because the default value (0.5) failed to recognise well established species based on morphological and genetic data within the *neuwiedii*, *alternatus*, and *taeniatus* species groups of *Bothrops*.

## **Results**

### **Phylogenetic relationships**

Our molecular phylogenetic analysis inferred the *Bothrops jararacussu* species group as monophyletic (PP = 1) and sister to the *Bothrops atrox* group (Figure 1). We found *B. brazili* to be paraphyletic, forming two highly supported non-sister clades. One such clade occurs north of the Amazon River; this clade is sister to a clade composed of the Atlantic Forest species *B. jararacussu*, *B. pirajai*, and *B. muriciensis* (PP = 1). A second *B. brazili* clade included samples from south of the Amazon River; this clade was inferred as the sister of the ancestor of all other samples in the *jararacussu* species group (PP = 1) (Figure 1).

Like *B. brazili*, we inferred *B. jararacussu* as paraphyletic. Samples from the coastal state of Espírito Santo in Brazil form a clade that is sister to the ancestor of *B. pirajai* and *B. muriciensis* (PP = 1). In turn, samples of *B. jararacussu* from the states of São Paulo and Minas Gerais in Brazil's southeast compose a clade that is sister to the ancestor of all remaining Atlantic Forest samples (PP = 1) (Figure 1).



**Figure 1.** Phylogenetic relationships within *Bothrops* highlighting the placement of *B. oligobalius* sp. nov. (blue) and *B. brazili* (red) in the *jararacussu* species group. Posterior probabilities are shown above nodes. Scale bar indicates substitutions per site. Photo: *B. oligobalius* sp. nov. from French Guiana, by Maël Dewynter.

Uncorrected pairwise genetic distances between the northern clade of *B. brazili* and species in the Atlantic Forest clade ranged from 3.9 to 4.9% for the mitochondrial markers *nd4*. Genetic distances between the two non-sister clades of *B. brazili* ranged from 5.6 to 7.1%.

### Inferences of independently evolving lineages

Our molecular species delimitation analysis based on bGMYC identified three independently evolving lineages within the *B. jararacussu* species group. One of these lineages is composed of samples of *B. brazili* from south of the Amazon River; one is composed of samples of *B. brazili* from north of the Amazon River; and another one is composed of samples from the Atlantic Forest species *B. jararacussu*, *B. pirajai* and *B. muriciensis* (the Atlantic clade) (Figure 1).

Morphological examination of specimens demonstrates that the non-sister northern and southern clades of *B. brazili* have consistent phenotypic differences (see below). Based on these differences, coupled with the phylogenetic and molecular delimitation results, we describe the northern clade as a new species, as follows.

## *Bothrops oligobalius* sp. nov.

(Figures 2–7)

*Bothrops neglecta*, Amaral 1923: 100–102; in part;  
*Bothrops neglecta*, Hoge & Lancini 1962: 16;  
*Bothrops brazili*, Hoge 1962: 63;  
*Bothrops neglecta*, Roze 1966: 286;  
*Bothrops brazili*, Hoge et al. 1972: 231; in part;  
*Bothrops brazili*, Cunha & Nascimento 1975: 12–17; in part;  
*Bothrops brazili*, Gasc & Rodrigues 1980: 591;  
*Bothrops brazili*, Chippaux 1986: 50;  
*Bothrops brazili*, Campbell & Lamar 1989;  
*Bothrops brazili*, Campbell & Lamar 2004.  
*Bothrops brazili* 'northern clade', Dal Vechio et al. 2020

### Holotype

A male, MZUSP 23282 (MTR 13844) collected by Miguel Trefaut Rodrigues and Antoine Fouquet on 22 April 2007, under a leaf of *Cecropia* sp. in *terra firme* (non-flooded) Amazonian lowland forests, at the Lourenço municipality (00°41'48.2"S, 57°42'45.1"W), state of Amapá, Brazil (Figure 3). ZooBank ID number: 8E9124E2-94E6-434B-B086-5043CE02893C

### Paratypes

Ten specimens: **Brazil**: MNRJ 10050–51 (females) from São Gabriel da Cachoeira municipality, state of Amazonas; MPEG 3274 (female) from Almeirim municipality, state of Pará; MZUSP 11719 (male) from Rio Maracá, Mazagão municipality, state of Amapá; **Colombia**: ICN 2155 (male) from Puerto Santander, Caquetá river, Araracuara, Amazonas; ICN 10000 (female), ICN 10001–02 (male) from Puré river, Letícia, Amazonas (Figure 5); ICN 8176 (female) from Caparú, Taraira lake, Vaupés; ICN 10404 (female) from Mosiro Itajura biological research, Taraira lake, Vaupés.

### Diagnosis

(1) A robust body species, SVL = 245–805 mm, (2) short tail, TL = 35–113 mm; (3) 23–27 dorsals on the first third of the body; (4) 23–25 dorsals at midbody; (5) 18–21 dorsals on the last third of the body; (6) 156–164 ventrals in females (median = 160) and 154–159 in males (median = 156); (7) anal scale not divided; (8) 42–48 paired subcaudals in females (median = 44.5) and 47–52 in males (median = 48.8); (9) 9–13 lateral trapezoidal marks in each side (median = 11,01 and 11,7 on the right and left side, respectively); (10) post-orbital stripe absent or poorly marked; (11) eight supralabials; (12) 10–12 infralabials; (13) second supralabial fused to the prelacunal; (14) belly checkered; (15) absence of vertebral stripe.

### Comparisons with other species

Comparisons for meristic and qualitative characters are presented in Table 1. Attributes from other species are presented in parenthesis. The new species can be promptly assigned to the *jararacussu* group due its morphological similarities with

**Table 1.** Meristic and qualitative features within the *B. jararacussu* species group. LTM: lateral trapezoidal marks; POS: posocular stripe; SC: subcaudals V: ventrals; VS: vertebral stripe.

|                                       | LTM   | V                          | SC                     | POS             | VS      | Distribution        |
|---------------------------------------|-------|----------------------------|------------------------|-----------------|---------|---------------------|
| <i>B. oligobalius</i> <b>sp. nov.</b> | 9–13  | 156–164 (F)<br>154–159 (M) | 42–48 (F)<br>47–52 (M) | Absent or faded | Absent  | Amazonia            |
| <i>B. brazili</i>                     | 13–21 | 174–186 (F)<br>175–184 (M) | 45–56 (F)<br>54–61 (M) | Absent or faded | Present | Amazonia            |
| <i>B. muriciensis</i>                 | 12–15 | 151–155 (F)<br>148–150 (M) | 45–51 (F)<br>51–55 (M) | Absent or faded | Absent  | Atlantic Forest     |
| <i>B. pirajai</i>                     | 12–18 | 157–168 (F)<br>155–167 (M) | 45–58 (F)<br>43–54 (M) | Present         | Absent  | Atlantic Forest     |
| <i>B. jararacussu</i>                 | 16–22 | 170–186 (F)<br>166–181 (M) | 44–55 (F)<br>50–68 (M) | Present         | Absent  | Atlantic Forest     |
| <i>B. sactaegrucis</i>                | 18–21 | 171–185                    | 56–57                  | Absent or faded | Absent  | Montains in Bolivia |

*B. brazili*, with whom the new species shared its specific epithet for over 60 years. Like other members in the *jararacussu* group, the new species presents the second labial scale fused to the prelacunal (separate in the *alternatus* and *neuwiedi* groups), postorbital stripe absent or faint (present in the *alternatus*, *neuwiedi*, *atrox* and *jararaca* groups), dorsal skin of the head with homogeneous colouration (blotched or patterned in the *alternatus* and *neuwiedi* groups and generally in the *atrox* group as well), stout and cylindrical bodies and terrestrial habit (slender, laterally compressed and arboreal habit in the *taeniatus* group), reddish and greyish background skin colouration and reddish eye (greenish background colour in the *taeniatus* group, skin generally brownish in the *atrox*, *jararaca*, *alternatus* and *neuwiedi* groups), eight supralabials and lower number of ventrals (generally 7 supralabials and higher number of ventrals in *atrox* group). Assignment of *B. oligobalius* to the *jararacussu* group is further supported by our molecular approach (Figure 1).

Within the *jararacussu* species group, *Bothrops oligobalius* can be distinguished from *B. jararacussu* and *B. pirajai* by the absence or a faint postorbital stripe (present in both species), greyish and reddish background skin colouration (yellowish or brownish background with black trapezoidal marks in both species), reddish eye (yellowish or brownish in both species). The number of ventrals in *B. oligobalius* (156–164 in females, 154–159 in males) distinguishes it from *B. muriciensis* (151–155 in females, 148–150 in males) and *B. jararacussu* (170–186 in females, 166–181 in males) (Table 1). The phylogenetic position of *B. oligobalius* sister to a clade assembling *B. jararacussu*, *B. pirajai* and *B. muriciensis* (which correspond to the Atlantic Forest lineage in the *jararacussu* group) is further supported by high genetic divergence and highly disjunct distributions (Figure 1).

*Bothrops oligobalius* is similar to *B. brazili* in background skin colouration and general external morphology features. However, the species can be promptly distinguished from *B. brazili* due to a smaller number of lateral trapezoidal marks (9–13; mean = 11.01 and 11.7 on the right and left side, respectively; in *B. brazili*, 13–21 mean = 17.1 and 16.3 on the right and left side, respectively) and absence of a vertebral stripe (present in *B. brazili*) (Table 1, Figures 2, Figures 3, Figures 5 and Figures 8). Moreover, the low number of ventrals (156–164 in females and 154–159 in males), and subcaudals 42–48 in females and 47–52 in males in *B. oligobalius* also distinguish it from *B. brazili* (ventrals 174–186 in females and 175–184 in males, and subcaudals 45–56 in females and 54–61 in males) (Table 1). Molecular analysis





**Figure 2.** *Bothrops oligobalius* **sp. nov.** from French Guiana. Photo by Maël Dewynter.

recovered these two species as non-sister lineage, with pronounced genetic distances between them. Moreover, the new species occurs north of the Amazon River, while *B. brazili* is restricted to areas south of the river, with no reported sympatry (Figures 6, Figures 8).

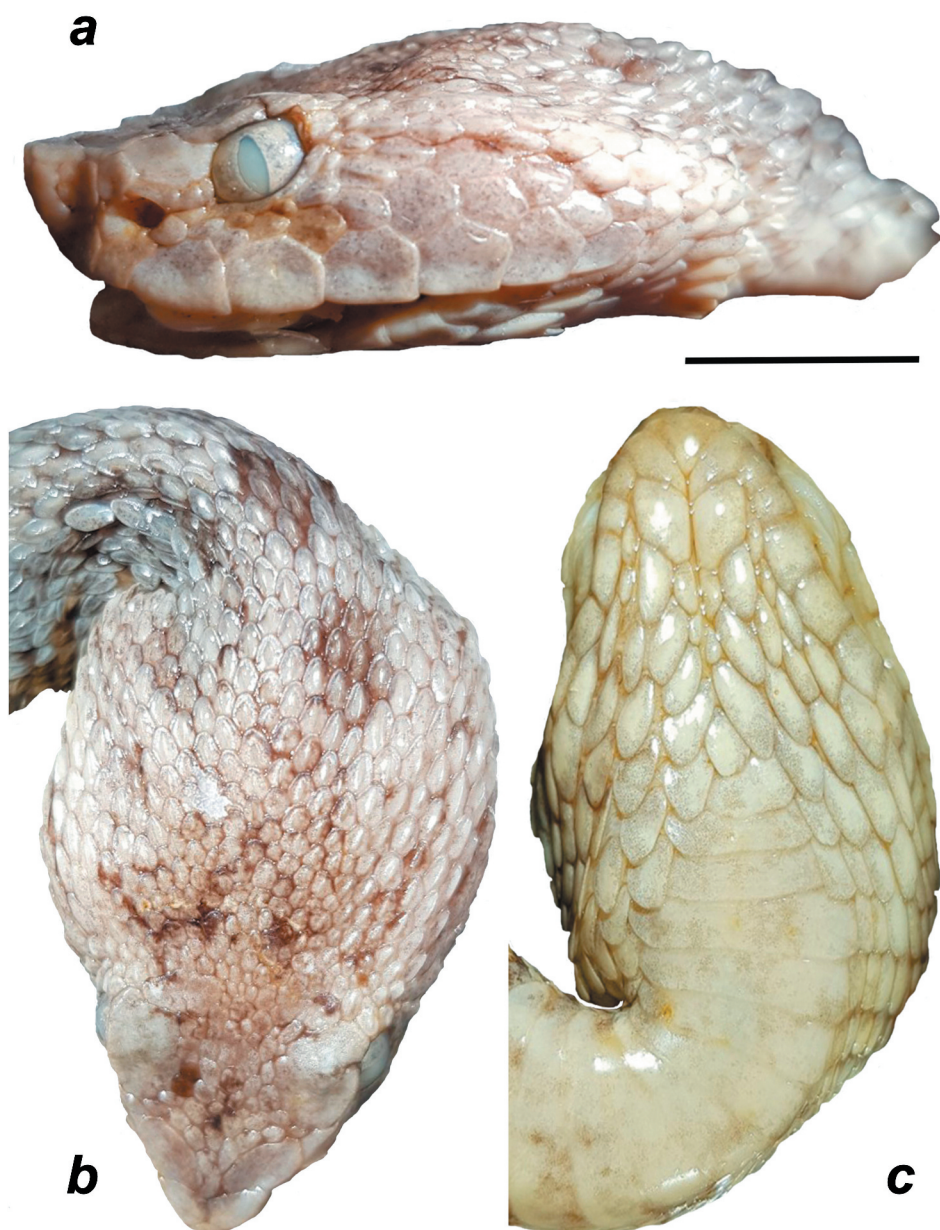
Lastly, the new species is distinguished from *B. sanctaerucis*, a poorly known member of the *jararacussu* group from Bolivia, by the lower number of ventrals and caudals (171–185 and 56–57 respectively in *B. sanctaerucis*), lower number of lateral trapezoidal marks (18–21), and greyish to reddish background skin colouration (brownish to yellowish).

### **Description of the holotype**

Subadult male (Figures 3, Figures 4), preserved in ethanol with the left hemipenis prepared; SVL 594 mm; TL 95 mm (13.7% of total length); head length 32.1 mm; head width 20.7 mm; rostral 4.6 mm high; nasals divided; 2/1 prefoveals; 2/2 postfoveals; prelacunal fused with second supralabial in both sides of head (lacunolabial present); 1/1 preocular; 2/2 postoculars; 8/8 supralabials; 7/7 interoculars; 5/5 circumorbitals; 10/11 infralabial; mental longer than broad, contacting the first two infralabials anteriorly (on each side); five gulars between the chin shield and the first ventral scale; 4/5 rows of gulars separating the first ventral scales from the infralabials; 2/1 canthals; 8 posterior intercanthals; 4 intersupraoculars; cephalic scales with no or weak keels; 25/24/19 dorsals; 159 ventrals; anal scale not divided; 48 divided subcaudals. Posterior cephalic scales longer than wide and strongly keeled; temporal scales keeled; internasals, canthals and supraoculars smooth. At midbody, eight rows of paravertebral scales between two adjacent lateral trapezoidal marks. In preservation, the background skin colouration of the holotype is greyish, with 12/11 brownish/greyish lateral trapezoidal marks lighter on the



**Figure 3.** Holotype of *Bothrops oligobalius* sp. nov. (MZUSP 23282, field number MTR 13844), from Lourenço Municipality, State of Amapá, Brazil. A: Dorsal view; B: Ventral view. Scale bar = 1 cm.



**Figure 4.** Head of the holotype (MZUSP 23282) of *Bothrops oligobalius* **sp. nov.** in (a) lateral, (b) dorsal and (c) ventral views. Scale bar 1 cm.

central part; marks usually meet on the dorsum. Dorsal skin of the head homogeneously greyish, without distinctive blotches. Ventral skin of the head light grey; Postorbital stripe absent to faint. Tail greyish/brownish dorsally with small dark brown lateral blotches; posterior part of tail dark brown;



**Figure 5.** Paratype of *Bothrops oligobalius* **sp. nov.** (ICN10002) from Leticia, Amazonas, Colombia. A: Dorsal view; B: Ventral view. Scale bar = 1 cm.

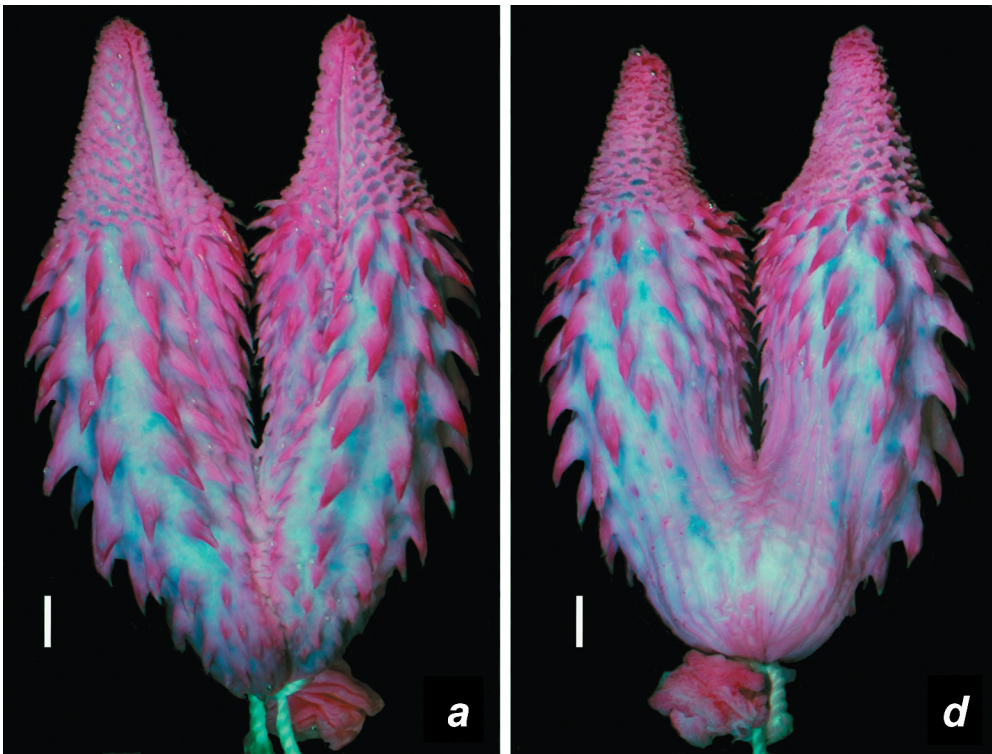


**Figure 6.** Geographic map of South America showing the distribution of *Bothrops oligobalius* **sp. nov.** (yellow) and *B. brazili* (red). Outlined diamonds and circles represent sites with genetic samples available. Diamonds with an inner dot indicate the type locality of each both species. The Amazonian biome is highlighted in green; the Amazon River is also indicated. Photos: *B. oligobalius* **sp. nov.** from French Guiana, by Maël Dewynter; *B. brazili* from Porto Velho, RO, Brazil, by FDV.

subcaudals speckled on anterior portion and brownish posteriorly. Venter irregularly speckled with cream and greyish; speckles reaching the middle of the subcaudal scales.

**Hemipenial morphology**

Hemipenis bilobed, bicalyculate, and bicapitate (with thinning towards the tip of the capitulum); sulcus spermaticus extending to the tip of each lobe (Figure 7). Large calcified spines present on the body of the hemipenis on the sulcate and asulcate faces, reaching the capitulum. On the asulcate face, region from the base of the hemipenis to the fork



**Figure 7.** Left Hemipenis from the holotype of *Bothrops oligobalius* **sp. nov.** (MZUSP 23282, field number MTR 13844). A: Sulcated face; B: Asulcated face. Scale bar = 1 mm.

smooth and without spines. Intrasulcar region with many ossified spines; sulcus spermaticus bordered by small calcified spines up to the calyce (Figure 7).

### **Variation**

The type series is fairly homogeneous in colouration pattern and pholidosis. The specimen ICN10001 shows nine supralabials on the right side, probably as a result of split of the fifth supralabial into two. Chin colouration can vary from totally cream to presenting the central part of scales cream with a greyish border. One specimen (MZUSP 11719) shows 13 lateral trapezoidal marks on each side of the body; one (ICN8176) has 13 marks on the left side; all other specimens have less than 13 dorsal trapezoidal marks.

### **Distribution and natural history**

The new species is known from areas north of the Amazon River in the Guiana Shield region extending into western Amazonia, an area that includes Brazil (states of Amapá, Roraima, and the northern parts of Pará and Amazonas), French Guiana, Surinam, Guyana, Venezuela and Colombia. Its currently known range limit to the west lies around the Putumayo River, although the species may also occur in the Putumayo-Napo interfluvium. The new species presents stout body and terrestrial habit, being found mainly in *terra*



**Figure 8.** *Bothrops brazili* in dorsal view. A- DHMECN3035 from Ecuador B- QCAZ4827 from Pastaza, Ecuador. Scale bar = 1 cm.

*firme* (non-flooded) Amazonian lowland forests. The species has rarely been sampled in the *várzea* (flooded) forests.

### Etymology

The specific name derives from the Greek *oligos* (few) and *balios* (spotted, dappled). The name is a reference to the smaller number of lateral trapezoidal marks on the body compared to *Bothrops brazili*, a name now restricted to the populations south of the Amazon River (see below).

### Discussion

#### **A new Amazonian species of *Bothrops***

The systematics and taxonomy of *Bothrops* snakes have been highly active over the last two decades, with several new species descriptions (e.g. *B. marmoratus*, Da Silva & Rodrigues 2008; *B. ayerbeii*, Folleco-Fernandez 2010; *B. otavioi* and *B. sazimai*, Barbo et al. 2012, 2016; Carrasco et al. 2019; *B. monsignifer* Timms et al. 2019) and phylogenetic studies failing to fully resolve relationships within the *neuwiedi*, *atrox*, *taeniatus* and *jararacussu* species groups (Machado et al. 2014; Saldarriaga-Córdoba et al. 2017; Dal Vechio et al. 2018, 2020). These studies have revealed persistent knowledge gaps about species diversity and evolutionary relationships in *Bothrops*, in spite of the medical significance of these ubiquitous venomous snakes.

These knowledge gaps involve broadly distributed taxa, which is the case of the two lineages that we recovered under the name *Bothrops brazili*. These snakes are easily distinguished from the other *Bothrops* species but since its description by Hoge (1953), *B. brazili* has been treated as a single taxon, despite previous suggestions of differences in scale traits between populations north and south of the Amazon River (Cunha and Nascimento 1975, 1978, 1993). In agreement with these previous morphological evidences, we found consistent differences in the number of ventral and subcaudal scales between populations of *B. brazili* that occur north and south to the Amazon River. Moreover, we found that the number of lateral trapezoidal marks is a distinguishing feature between these two geographical groups. Lastly, our molecular analyses inferred high genetic divergence between the northern and southern lineage of *B. brazili*, which were also found to be non-sister. Based on these results, we describe the northern lineage as a new taxon, which we name as *B. oligobalius*. The name *B. brazili* is now restricted to those populations that occur south of the Amazon River, given the original type locality of *B. brazili* in 'Tomé Assú' (Tomé Açu municipality), Brazilian state of Pará, south of the Amazon River.

Specimens of *B. oligobalius* may have been previously treated under the name *B. neglecta*, presently a synonym of *B. pirajai*. Amaral (1923) described *B. neglecta* based on two specimens: one from 'southeastern Bahia', the holotype, and one paratype from 'British Guyana', a specimen reported in Boulenger's snake catalogue (1896). These specimens caught the attention of Amaral because of their low number of ventral and caudal scales. While we did not have access to the specimen from 'British Guyana', the locality and morphological features described by Amaral (1923) suggest that it corresponds to *B. oligobalius*. The name *B. neglecta*, however, is tied to the holotype, a specimen from an Atlantic Forest population referred earlier to *B. pirajai*, and is therefore not available.

### **Evolutionary relationships in the *Bothrops jararacussu* species group**

Our phylogenetic results agree with previous suggestions based on morphological attributes that *B. pirajai* and *B. muriciensis* are nested within the *jararacussu* species group of *Bothrops*. These two species clustered with *B. jararacussu*, forming a clade that occurs in the coastal Atlantic Forest. In turn, we inferred this Atlantic Forest clade as sister to an Amazonian lineage that occurs north of the Amazon River, previously referred to as *B. brazili*. We inferred northern and southern Amazonian lineages of *B. brazili* as not sister, revealing that *B. brazili* is a paraphyletic taxon, a finding that supports the recognition of *B. oligobalius*. Like *B. brazili*, we inferred *B. jararacussu* as a paraphyletic species, with samples from the Brazilian state of Espírito Santo clustering with *B. pirajai* and *B. muriciensis*, while samples from São Paulo and Minas Gerais composed a clade that is sister to the ancestor of all remaining Atlantic Forest samples.

These phylogenetic results recapitulate the biogeographic investigation of Dal Vechio et al. (2020). Based on inference of historical demography, that study found support for a history of vicariant separation between the ancestor of *B. oligobalius* and *B. brazili* during the Late Miocene, presumably as a result of the establishment of the modern Amazon River drainage (Dal Vechio et al. 2020). This vicariant event was followed by the colonisation of the Atlantic Forest during the Late Pleistocene as a result of a rainforest expansion event that would have connected eastern Amazonia and the Atlantic Forest through the



Caatinga in present-day Brazil's northeast. Our phylogenetic results support that this proposed colonisation event triggered a new radiation of snakes in the Atlantic Forest region, a radiation that now includes *B. muriciensis*, *B. pirajai*, and the two non-sister lineages within *B. jararacussu*.

### Concluding remarks

On the basis of morphological and genetic data, we found unrecognised species diversity and paraphyletic taxa within the *jararacussu* species group of *Bothrops*. We therefore assign a new taxon name, *B. oligobalius*, to the populations that occur north of the Amazon River. The new species occurs in Amazonian lowlands in Colombia, Venezuela, French Guiana, Surinam, Guyana, and Brazil. We retrieved *B. oligobalius* as sister to a clade composed of Atlantic Forest species, supporting the hypothesis that climate-driven changes in habitat distribution contributed to the diversification of South American lanceheads. Our results also reveal additional taxonomic issues that require further evaluation, particularly the paraphyletic nature of the Atlantic Forest taxon *Bothrops jararacussu*. Resolving these issues will rely on comprehensive sampling of phenotypic and genetic variation in this medically important clade of venomous snakes.

### Acknowledgements

We are grateful to all researchers, institutions and curators of herpetological collections that contributed with tissue samples and specimens to this work: Omar Torres-Carvajal and Fernando Ayala, from Museum of Zoology (QCAZ), Pontificia Universidad Católica del Ecuador; Jorge Valencia, from Fundación Herpetológica Gustavo Orcés, Ecuador; Mario Yánes, from Instituto Nacional de Biodiversidad, Ecuador; Martha L. Calderón Espinosa and Maria Alejandra Erazo, from Colección de Reptiles Instituto de Ciencias Naturales, Universidad Nacional, Colombia; John D. Lynch, from Colección del Instituto de Ciencias Naturales, Universidad Nacional, Colombia; Addison Wynn and Kenneth Tighe from Smithsonian National Museum of Natural History, Washington D.C., US; Felipe F. Curcio, from Universidade Federal do Mato Grosso, Brazil; Ana Prudente, from Museu Paraense Emílio Goeldi, Brazil; Vinícius Carvalho, Albertina Lima, and Richard Vogt, from the Instituto de Pesquisas da Amazônia, Brazil; Selma Torquato and Tami Mott, from Museu de Zoologia da Universidade Federal do Alagoas, Brazil; Hussam Zaher from Museu de Zoologia da Universidade de São Paulo and Renato Feio and Henrique Costa, from Universidade Federal de Minas Gerais, Brazil. We are also grateful to Beatriz Freire for helping in the generation of molecular data and Antoine Fouquet and Secretaria de Ciência e Tecnologia do Amapá for helping during field work in state of Amapá. Maël Dewynter provided pictures of the new species in life. Instituto Chico Mendes de Conservação da Biodiversidade issued collection permits (SISBIO 36753-1, 36753-4 and 27290-3).

### Author contributions

F.D.V. gathered the molecular data, performed analyses, and wrote the manuscript; I.P. contributed to the molecular analyses and wrote the manuscript; M.T.R. funded the research, supervised and contributed intellectually to the project, and revised the text; F.G.G. and R.G. contributed intellectually to the project and revised the text.

### Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This work was co-funded by Fundação de Amparo à Pesquisa do Estado de São Paulo [FAPESP - 2003/10335-8, 2011/50146-6, 2013/50297-0]; NSF [DEB 1343578], and NASA through the Dimensions of Biodiversity Program, with additional support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes).

## ORCID

Francisco Dal Vechio  <http://orcid.org/0000-0003-1328-0243>

Ivan Prates  <http://orcid.org/0000-0001-6314-8852>

Felipe G. Grazziotin  <http://orcid.org/0000-0001-9896-9722>

Roberta Graboski  <http://orcid.org/0000-0002-9123-4819>

Miguel Trefaut Rodrigues  <http://orcid.org/0000-0003-3958-9919>

## Data accessibility

All sequence data were deposited in the GenBank.

## References

- Alencar LRV, Quental T, Grazziotin F, Alfaro M, Martins M, Venzon M, Zaher H. 2016. Diversification in vipers: phylogenetic relationships, time of divergence, and shifts in speciation rates. *Mol Phylogenet Evol.* 105:50–62. doi:10.1016/j.ympev.2016.07.029.
- Amaral A. 1923. New genera and species of snakes. *Proc New England Zool Club.* 8:85–105. doi:10.5962/bhl.part.4002.
- Arévalo ES, Davis SK, Sites JW. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. *Syst Biol.* 43:387–418. doi:10.1093/sysbio/43.3.387.
- Barbo FE, Gasparini JL, Almeida AP, Zaher H, Grazziotin FG, Gusmão RB, Ferrarini JMG, Sawaya RJ. 2016. Another new and threatened species of lancehead genus *Bothrops* (Serpentes, Viperidae) from Ilha dos Franceses, Southeastern Brazil. *Zootaxa.* 4097(4):511–529. doi:10.11646/zootaxa.4097.4.4.
- Barbo FE, Grazziotin FG, Sazima I, Martins M, Sawaya RJ. 2012. A new and threatened insular species of lancehead from southeastern Brazil. *Herpetologica.* 68:418–429. doi:10.1655/HERPETOLOGICA-D-12-00059.1.
- Boulenger GA. 1896. *Catalogue of the snakes in the British Museum (Natural History)*. Vol. 3. London: Trustees of the British Museum; p. 727. doi:10.5962/bhl.title.29427.
- Campbell JA. 1985. A new species of highland pitviper of the genus *Bothrops* from southern Mexico. *Journal of Herpetology.* 19:48–54.
- Campbell JA, Lamar WW. 1989. *The venomous reptiles of Latin America*. Ithaca: Cornell University Press, New York; p. 425.
- Campbell JA, Lamar WW. 2004. *The venomous reptiles of the western hemisphere*. Ithaca: Cornell University Press, New York; p. 870.
- Carrasco PA, Grazziotin FG, Farfán RSC, Koch C, Ochoa JA, Scrocchi GJ, Leynaud GC, Chaparro JC. 2019. 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. *Zootaxa.* 4565(3):301–344. doi:10.11646/zootaxa.4565.3.1.
- Carrasco PA, Manttoni CI, Leynaud GC, Scrocchi GJ. 2012. Morphology, phylogeny and taxonomy of South American bothropoid pitvipers (Serpentes, Viperidae). *Zool Scr.* 41:109–124. doi:10.1111/j.1463-6409.2011.00511.x.

- Chippaux JP. 1986. Les serpentes de la Guyane Française, Coll. fauna tropicale XXVII. Paris: Ed. de L'Orstom; p. 165.
- Cunha OR, Nascimento FP. 1975. Ofídios da Amazônia. VII. As serpentes do gênero *Bothrops* (jararaca) e *Lachesis* (surucucu) da região leste do Pará. (Ophidia, Viperidae). Bol Mus Para Emílio Goeldi. 83:1–42.
- Cunha OR, Nascimento FP. 1978. Ofídios da Amazônia. X. As cobras da região leste do Pará. Publicações Avulsas Do Museu Paraense Emílio Goeldi, Belém. 31:1–218.
- Cunha OR, Nascimento FP. 1993. Ofídios da Amazônia. 10. As cobras da região leste do Pará. Bol Mus Para Emílio Goeldi. 9(l):1–191.
- Da Silva VX, Rodrigues MT. 2008. Taxonomic revision of the *Bothrops neuwiedi* complex (Serpentes, Viperidae) with description of a new species. Phyllomedusa. 7:45–90. doi:10.11606/.2316-9079.v7i1p45-90.
- Dal Vechio F, Prates I, Grazziotin FG, Zaher H, Graboski R, Rodrigues MT. 2020. Rainforest shifts through time and riverine barriers shaped the diversification of the terrestrial South American pit vipers in the *Bothrops jararacussu* species group. J Biogeogr. 47:516–526. doi:10.1111/jbi.13736.
- Dal Vechio F, Prates I, Grazziotin FG, Zaher H, Rodrigues MT. 2018. Phylogeography and historical demography of the arboreal pit viper *Bothrops bilineatus* (Serpentes, Crotalinae) reveal multiple connections between Amazonian and Atlantic rain forests. J Biogeogr. 2018:1–12. doi:10.1111/jbi.13421.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 9:772–772. doi:10.1038/nmeth.2109.
- Dixon JR, Soini P. 1986. The reptiles of the upper Amazon basin, Iquitos region, Peru, I-VII. USA: Milwaukee Public Museum; p. 154.
- Dowling HG, Savage JM. 1960. A guide to the snake hemi-penis: a survey of basic structure and systematic characteristics. Zoologica. 45:17–28.
- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol. 29:1969–1973. doi:10.1093/molbev/mss075.
- Duellman WE. 2005. Cusco Amazónico. The lives of amphibians and reptiles in an Amazonian rainforest. Ithaca (New York, U.S.A): Comstock Publishing Associates; p. 433.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797. doi:10.1093/Nar/Gkh340.
- Fenwick AM, Gutberlet RL Jr, Evans JA, Parkinson CL. 2009. Morphological and molecular evidence for phylogeny and classification of South American pitvipers, genera *Bothrops*, *Bothriopsis*, and *Bothrocophias* (Serpentes: Viperidae). Zool J Linn Soc. 156:617–640. doi:10.1111/j.1096-3642.2008.00495.x.
- Ferrarezi H, Freire EMX. 2001. New species of *Bothrops* Wagler, 1824, from the Atlantic forest of northeastern Brazil (Serpentes, Viperidae, Crotalinae). Bol Mus Nacional, Rio De Janeiro. 440:1–10.
- Folleco-Fernández AJ. 2010. Taxonomía del complejo *Bothrops asper* (Serpentes: Viperidae) en el sudoeste de Colombia. revalidación de la especie *Bothrops rhombeatus* (García 1896) y descripción de una nueva especie. Rev Novedades Colombianas. 10(1):33–70.
- Gasc JP, Rodrigues MT. 1980. Lista préliminaire des serpents de Guyane Française. Bull Mus Hist Nat, Paris. 2(2):559–598.
- Godinho R, Crespo EG, Ferrand N, Harris DJ. 2006. Phylogeny and evolution of the green lizards, *Lacerta* spp. (Squamata: Lacertidae) based on mitochondrial and nuclear DNA sequences. Amphibia-Reptilia. 26:271–285.
- Hoge AR. 1953. A new *Bothrops* from Brazil - *Bothrops brazili*, sp. nov. Memórias Do Instituto Butantan. 25:15–21.
- Hoge AR. 1966. Preliminary account on Neotropical *Crotalinae* (Serpentes: Viperidae). Memórias Do Instituto Butantan. 32(1965):109–184.
- Hoge AR, Lancini AR. 1962. Sinopsis de las Serpientes Venenosas de Venezuela. Publicaciones Ocasionales del Museo de Ciencias Naturales Caracas, Venezuela. 1:1–24.
- Hoge AR, Santos NP, Heitor C, Lopes LA, de Souza IM. 1972. Serpentes coletadas pelo projeto Rondon VII em Iauareté, Brasil. Mem Inst Butantan. 36:221–232.

- Lacerda JB. 1884. Leçons sur le venin des serpents du Brésil et sur la méthode de traitement des morsures venimeuses par le permanganate de potasse. Rio de Janeiro: Lombaerts & C.; p. 194.
- Machado T, Silva VX, Silva MJJ. 2014. Phylogenetic relationships within *Bothrops neuwiedi* group (Serpentes, Squamata): geographically highly-structured lineages, evidence of introgressive hybridization and Neogene/Quaternary diversification. *Mol Phylogenet Evol.* 71:1–14. doi:10.1016/j.ympev.2013.10.003.
- Manzani PR, Abe AS. 1988. Sobre dois novos métodos de preparo do hemipênis de serpentes. *Mem Inst Butantan.* 50:15–20.
- Marques OAV, Martins M, Sazima I. 2002. A new species of pitviper from Brazil, with comments on evolutionary biology and conservation of the *Bothrops jararaca* group. *Herpetologica.* 58:303–312. doi:10.1655/0018-0831(2002)058[0303:ANISOP]2.0.CO;2.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010. New Orleans, LA; pp. 1–8.
- Nunes PMS, Fouquet A, Curcio FF, Kok PJR, Rodrigues MT. 2012. Cryptic species in *Iphisa elegans* Gray, 1851 (Squamata: Gymnophthalmidae) revealed by hemipenial morphology and molecular data. *Zool J Linn Soc.* 166:361–376. doi:10.1111/j.1096-3642.2012.00846.x.
- Palumbi SR. 1996. Nucleic acids. II. The polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK, editors. *Molecular systematics*. Sunderland (MA): Sinauer & Associates; p. 205–247.
- Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell, S. et al. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Syst. Biol.* 55:595–609.
- Pook CE, Wüster W, Thorpe RS. 2000. Historical biogeography of the western rattlesnake (Serpentes: Viperidae: *Crotalus viridis*), inferred from mitochondrial DNA sequence information. *Mol Phylogenet Evol.* 15:269–282. doi:10.1006/mpev.1999.0756.
- Rambaut A, Drummond AJ. 2009. MCMC trace analysis tool version v1.5.0. Institute of Evolutionary Biology, University of Edinburgh and Department of Computer Science, University of Auckland. [accessed 2020 October 11]. <http://beast.bio.ed.ac.uk/>.
- Reid N, Carstens B. 2012. Phylogenetic estimation error can decrease the accuracy of species delimitation: a Bayesian implementation of the general mixed Yule-coalescent model. *BMC Evol. Biol.* 12:196.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol.* 61:539–542. doi:10.1093/sysbio/sys029.
- Roze JA. 1966. La taxonomía y zoogeografía de los ofidios en Venezuela. Caracas: Universidad Central de Venezuela; p. 362.
- Saldarriaga-Córdoba M, Parkinson CL, Daza JM, Wüster W, Sasa M. 2017. Phylogeography of the Central American lancehead *Bothrops asper* (Serpentes: Viperidae). *PLoS One.* 12(11):e0187969. doi:10.1371/journal.pone.0187969.
- Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol.* 30:2725–2729. doi:10.1093/molbev/mst197.
- Timms J, Chaparro JC, Venegas PJ, Salazar-Valenzuela D, Scrocchi G, Cuevas J, Leynaud G, Carrasco PA. 2019. A new species of pitviper of the genus *Bothrops* (Serpentes: Viperidae: Crotalinae) from the Central Andes of South America. *Zootaxa.* 4656(1):099–120. doi:10.11646/zootaxa.4656.1.4.
- Townsend TM, Alegre RE, Kelley ST, Wiens JJ, Reeder TW. 2008. Rapid development of multiple nuclear loci for phylogenetic analysis using genomic resources: an example from squamate reptiles. *Mol Phylogenet Evol.* 47:129–142. doi:10.1016/j.ympev.2008.01.008.
- Wagler J. 1824. *Serpentum Brasiliensium species novae, ou histoire naturelle des espèces nouvelles de serpens*. In: de Spix J, editor. *Animalia nova sive species novae. Natrix bahiensis*: 27. Hübschmanni: Monaco Typis Franc. Seraph; p. vii + 75.
- Wiens JJ, Kuczynski CA, Smith SA, Mulcahy D, Sites JW Jr, Townsend TM, Reeder TW. 2008. Branch lengths, support, and congruence: testing the phylogenomic approach with 20 nuclear loci in snakes. *Syst Biol.* 57:420–431. doi:10.1080/10635150802166053.

- Wüster W, Peppin L, Pook CE, Walker DE. 2008. A nesting of vipers: phylogeny and historical biogeography of the Viperidae (Squamata: Serpentes). *Mol Phylogenet Evol.* 49:445–459. doi:10.1016/j.ympev.2008.08.019.
- Zaher H. 1999. Hemipenial morphology of the South American xenodontine snakes with a proposal for a monophyletic Xenodontinae and a reappraisal of colubrid hemipenes. *Bull Am Mus Nat Hist.* 240:1–168.

## Appendix A. Examined specimens

**MTR:** Field numbers acronyms of specimens housed at Laboratório de Herpetologia do Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil, under the care of Miguel Trefaut Rodrigues; **MZUSP:** Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil; **IB:** Instituto Butantan, São Paulo, São Paulo, Brazil; **MPEG:** Museu Paraense Emílio Göeldi, Belém, Pará, Brazil; **MZUFAL:** Museu de Zoologia, Universidade Federal de Alagoas, Maceió, Alagoas, Brazil; **MNRJ:** Museu Nacional, Rio de Janeiro, Rio de Janeiro, Brazil; **MZUESC:** Museu de Zoologia, da Universidade Estadual de Santa Cruz; **CEPLAC:** Comissão Executiva de Planejamento da Lavoura Cacaueira, Brazil; **INPA:** Instituto Nacional de Pesquisas da Amazônia, Amazonas, Brazil, **MZML:** Museu de Zoologia Melo Leitão, Santa Tereza, Espírito Santo, Brazil, **MECN:** Museo Equatoriano de Ciencias Naturales, Quito, Equador; **QCAZ:** Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito, Equador; **FHGO:** Fundación Herpetologica Gustavo Orcés, Quito, Equador; **ICN:** Museo de Historia Natural, Universidad Nacional de Colombia, Bogotá, Colombia, **USNM:** Smithsonian National Museum of Natural History, Washington D.C., United States.

*Bothrops atrox*: ( $n = 7$ ) BRAZIL: **Acre:** Cruzeiro do Sul: MTR 28211, MTR 28473; **Amazonas:** Beruri: MTR 18742, MTR 18745, MTR 18849, MTR 18979–80, MTR 19304; Japurá: MTR33835; **Rondônia:** Guajará Mirim: MTR 25508, MTR 25759.

*Bothrops brazili*: ( $n = 36$ ) BRAZIL: **Amazonas:** Coari: MZUSP 10534 (INPA326); **Pará:** Belém: MPEG 3274; Benevides: MPEG 6854; Canindé: MZUSP 4260; Melgaço: MPEG 20914; Portel: MPEG 22703; Santo Antônio do Tauá: MPEG 5450, Tailândia: MZUSP 18128; **Rondônia:** Porto Velho: MPEG 17824–25, MZUSP 20563 (H2576); Mutum Paraná: MZUSP 4895. ECUADOR: DHMECN 3035, FHGO 96, FHGO 232, FHGO 6501, FHGO 8666, FHGO 9155, FHGO 10753, FHGO 10760, FHGO 11512, FHGO 12031, FHGO 12509, FHGO 12513–14, FHGO 12555; **Morona Santiago:** QCAZ 5169; **Pastaza:** QCAZ 4827, QCAZ 4992, QCAZ 11766, USNM 165339. PERU: **Amazonas:** USNM 316671, USNM 560445, USNM 566700, USNM 566705. **Madre de Dios:** USNM 345184.

*Bothrops oligobalius* **sp. nov.**: ( $n = 12$ ) BRAZIL: **Amapá:** Lourenço: MZUSP 23282 (Holotype), Mazagão: MZUSP 11719; **Amazonas:** São Gabriel da Cachoeira: MNRJ 10050–51; **Pará:** Almerim: MPEG 3274. COLOMBIA: **Amazonas:** Aracuara: ICN 2155; Leticia: ICN 10000–02; **Vaupés:** Taraira: ICN 81761, CN10404. VENEZUELA: **Amazonas:** USNM 562703

*Bothrops jararacussu*: ( $n = 25$ ) BRAZIL: **Espírito Santo:** MBML 625, MBML 1398; Cariacica: MBML 1715; Domingos Martins MBML 2725; Santa Leopoldina: MBML 447, MBML 466, MBML 1116; Santa Maria de Jetibá: MBML 90, MBML 515; Santa Teresa: MBML 19, MBML 21, MBML 32, MBML 268, MBML 342, MBML 398, MBML 400, MBML 436, MBML 490, MBML 501, MBML 515, MBML 532, MBML 645, MBML 1222, MBML 1265, MBML 2730.

*Bothrops leucurus*: ( $n = 2$ ) BRAZIL: **Alagoas:** Marechal Deodoro: MZUFAL 5562; Rio Largo: MZUFAL 10764.

*Bothrops muriciensis*: ( $n = 5$ ) BRAZIL: **Alagoas:** Murici: MZUFAL 379, MZUFAL 10751, MZUFAL 10958, MZUFAL 11094, MZUESC 509.

*Bothrops pirajai*: ( $n = 6$ ) BRAZIL: **Bahia:** CEPLAC 1171, CEPLAC 4316, CEPLAC 5345, CEPLAC 5471, CEPLAC 5125; Wenceslau Guimarães: MTR 22184.

**Appendix B. Genbank accession number for each taxon used in this study, with voucher and locality data. “-”: without information**

| Voucher     | Species                     | Locality                              | 12s       | 16s       | cytb      | nd4       | pir1     | c-mos    | bdnf     | rag1     | mk11     |
|-------------|-----------------------------|---------------------------------------|-----------|-----------|-----------|-----------|----------|----------|----------|----------|----------|
| MCP19013    | <i>Bathrops alternatus</i>  | Gravataí, Rio Grande do Sul, Brazil   | MH697889  | MH697948  | MH698145  | -         | -        | -        | MH698008 | MH698277 | -        |
| 2ALT        | <i>Bathrops alternatus</i>  | Cristais, Minas Gerais, Brazil        | -         | MH697947  | MH698146  | MH698184  | -        | MH698061 | -        | -        | MH698147 |
| MMFE2       | <i>Bathrops alternatus</i>  | Itirapina, São Paulo, Brazil          | EU867250  | EU867262  | EU867274  | EU867286  | -        | -        | -        | -        | -        |
| ITS358      | <i>Bathrops alternatus</i>  | Itirapina, São Paulo, Brazil          | EU867251  | EU867263  | EU867275  | EU867287  | -        | -        | -        | -        | -        |
| MMZES       | <i>Bathrops alternatus</i>  | Itirapina, São Paulo, Brazil          | EU867249  | EU867261  | EU867273  | EU867285  | -        | -        | -        | -        | -        |
| MVZ 223514  | <i>Bathrops alternatus</i>  | Pinhão, Paraná, Brazil                | -         | -         | AF292579  | AF292617  | -        | -        | -        | -        | -        |
|             | <i>Bathrops</i>             | Neuquen, Argentina                    | AY223658  | AY223671  | AY223595  | AY223639  | -        | -        | -        | -        | -        |
|             | <i>ammodytoides</i>         |                                       |           |           |           |           |          |          |          |          |          |
| MZUCR 11152 | <i>Bathrops asper</i>       | Costa Rica                            | AF057218  | AF057265  | AY223599  | U41876    | -        | -        | -        | -        | -        |
| -           | <i>Bathrops atrox</i>       | Acre, Brazil                          | -         | -         | AF246268  | AF246277  | -        | -        | -        | -        | -        |
| MTR12742    | <i>Bathrops atrox</i>       | Rio Abacaxis, Amazonas, Brazil        | MH697890  | MH697949  | MH698144  | MH698185  | MH698242 | MH698062 | -        | -        | -        |
| BAT02       | <i>Bathrops atrox</i>       | Amazonas, Brazil                      | MH697891  | MH697950  | MH698143  | MH698186  | MH698243 | MH698063 | MH698009 | MH698278 | MH698148 |
| -           | <i>Bathrops atrox</i>       | Suriname                              | -         | -         | AF246267  | AF246278  | -        | -        | -        | -        | -        |
| WW741       | <i>Bathrops atrox</i>       | Ecuador                               | GQ428495  | GQ428470  | GQ428476  | GQ428485  | -        | -        | -        | -        | -        |
| WW742       | <i>Bathrops atrox</i>       | Ecuador                               | GQ428496  | GQ428471  | GQ428477  | GQ428486  | -        | -        | -        | -        | -        |
| MTR22219    | <i>Bathrops bilineatus</i>  | EEEWG, Bahia, Brazil                  | MH697899  | MH697959  | MH698135  | MH698194  | MH698251 | MH698068 | MH698018 | MH698284 | MH698156 |
| MUFAL10753  | <i>Bathrops bilineatus</i>  | Murici, Alagoas, Brazil               | MH697913  | MH697979  | MH698115  | MH698214  | MH698259 | MH698074 | MH698037 | MH698301 | MH698167 |
| CEPLAC1     | <i>Bathrops bilineatus</i>  | Boa Nova, Bahia, Brazil               | MH697897  | MH697956  | MH698138  | MH698191  | MH698248 | MH698065 | MH698015 | MH698281 | MH698153 |
| MTR20744    | <i>Bathrops bilineatus</i>  | Pacaraima, Roraima, Brazil            | MH697921  | MH697981  | MH698113  | MH698216  | MH698261 | MH698076 | -        | MH698303 | MH698168 |
| PK1880      | <i>Bathrops bilineatus</i>  | Lower slope of Maringma-tepui, Guyana | MH697908  | MH697968  | MH698126  | MH698203  | MH698255 | MH698071 | MH698026 | MH698290 | MH698159 |
| CTMZ12115   | <i>Bathrops bilineatus</i>  | Potaro-Siparuni, Guyana               | MH697907  | MH697967  | MH698127  | MH698202  | -        | -        | MH698025 | -        | -        |
| MPEG24289   | <i>Bathrops bilineatus</i>  | Juruti, Pará, Brazil                  | MH697913  | MH697973  | MH698121  | MH698208  | MH698258 | MH698073 | MH698031 | MH698295 | MH698163 |
| UFMT-R7809  | <i>Bathrops bilineatus</i>  | Cohniza, Mato Grosso, Brazil          | MH697898  | MH697957  | MH698137  | MH698192  | MH698249 | MH698066 | MH698016 | MH698282 | MH698154 |
| APL18502    | <i>Bathrops bilineatus</i>  | Br-319, Amazonas, Brazil              | MH697896  | MH697955  | MH698139  | MH698190  | MH698247 | -        | MH698014 | MH698280 | MH698152 |
| T62034      | <i>Bathrops bilineatus</i>  | Cruzeiro do Sul, Acre, Brazil         | MH697892  | MH697951  | MH698142  | MH698187  | MH698244 | MH698064 | MH698010 | MH698279 | MH698149 |
| MTR28518    | <i>Bathrops bilineatus</i>  | Serra do Divisor, Acre, Brazil        | MH697929  | MH697989  | MH698105  | MH698223  | MH698264 | -        | MH698045 | MH698309 | MH698173 |
| H5144       | <i>Bathrops bilineatus</i>  | UHE Jirau, Rondônia, Brazil           | MH697910  | MH697970  | MH698124  | MH698205  | MH698256 | -        | MH698028 | MH698292 | MH698161 |
| DHMECN7800  | <i>Bathrops bilineatus</i>  | Ecuador                               | MH697900  | MH697960  | MH698134  | MH698195  | MH698252 | MH698069 | MH698019 | MH698285 | MH698157 |
| DHMECN7801  | <i>Bathrops bilineatus</i>  | Ecuador                               | MH697901  | MH697961  | MH698133  | MH698196  | MH698253 | MH698070 | MH698020 | MH698286 | MH698158 |
| -           | <i>Bathrops bilineatus</i>  | Leticia, Colombia                     | AF057214  | AF057261  | AY223591  | U41875    | -        | -        | -        | -        | -        |
| -           | <i>Bathrops oligoballus</i> | Venezuela                             | EU867252* | EU867264* | EU867276* | EU867288* | -        | -        | -        | -        | -        |

(Continued)



(Continued).

| Voucher                      | Species                       | Locality  | 12s       | 16s       | cytb      | nd4       | pirI     | c-mos    | bdnf     | rag1     | mk11     |
|------------------------------|-------------------------------|---|-----------|-----------|-----------|-----------|----------|----------|----------|----------|----------|
| MSH1 2346                    | <i>Bathrops oligoballus</i>   | Santa Isabel do Rio Negro, Amazonas, Brazil     | MN296327  | MN296341  | MN296439  | MN296451  | MN296468 | -        |          |          |          |
| MTR6306                      | <i>Bathrops oligoballus</i>   | Igarapé Camaipi, Rio Maracá, Amapá, Brazil      | MH697936  | MH697996  | MTR6306   | MH698230  | MH698267 | MH698079 | MH698051 | MH698314 | MH698177 |
| MTR13844<br>(MZUSP<br>23282) | <i>Bathrops oligoballus</i>   | Lourenço, Calçoene, Amapá, Brazil               | MH697935  | MH697995  | MH698099  | MH698229  | MH698266 | MH698078 | MH698050 | MH698313 | MH698176 |
| H2576                        | <i>Bathrops brazili</i>       | UHE Jirau, Abunã, Porto Velho, Rondônia, Brazil | MN296324  | MN296338  | MN296436  | MN296448  | MN296467 | MN296421 | MN296424 | MN296473 | MN296459 |
| UFMT10195                    | <i>Bathrops brazili</i>       | Apiacás, Mato Grosso, Brazil                    | MN296321  | MN296335  | MN296434  | MN296447  | MN296466 | MN296420 | MN296422 | MN296471 | MN296458 |
| -                            | <i>Bathrops brazili</i>       | Ecuador   | -         | -         | AF292597  | AF292635  | -        | -        | MN296427 | MN296476 | MN296460 |
| MPEG22277                    | <i>Bathrops brazili</i>       | Coari, Amazonas, Brazil                         | MN296322  | MN296336  | MN296435  | -         | -        | -        | -        | -        | -        |
| INPA5507                     | <i>Bathrops brazili</i>       | Rio Madeira, Ilha do Bufalo, Rondônia, Brazil   | MN296323  | MN296337  | -         | -         | -        | -        | MN296423 | MN296472 | -        |
| INPA5570                     | <i>Bathrops brazili</i>       | Rio Madeira, Jaci, Rondônia, Brazil             | MN296325  | MN296339  | MN296437  | MN296449  | -        | -        | MN296425 | MN296474 | -        |
| INPA5637                     | <i>Bathrops brazili</i>       | Rio Madeira, Jaci, Rondônia, Brazil             | MN296326  | MN296340  | MN296438  | MN296450  | -        | -        | MN296426 | MN296475 | -        |
| LSJUMZ41037                  | <i>Bathrops chloromelas</i>   | Pasco, Peru                                     | DQ305430  | DQ305453  | DQ305471  | DQ305488  | -        | -        | -        | -        | -        |
| WW                           | <i>Bathrops cotiara</i>       | Brazil  | AF057217  | AF057264  | AY223597  | AY223640  | -        | -        | -        | -        | -        |
| PT3404                       | <i>Bathrops diporus</i>       | Argentina                                       | DQ305431  | DQ305454  | DQ305472  | DQ305489  | -        | -        | -        | -        | -        |
| MTR11185                     | <i>Bathrops erythromelas</i>  | Santo Inácio, Bahia, Brazil                     | MH697937  | MH697998  | MH698096  | MH698232  | MH698268 | MH698080 | MH698053 | -        | MH698178 |
| MTR29115                     | <i>Bathrops erythromelas</i>  | Alagoado, Bahia, Brazil                         | -         | MH697997  | MH698097  | MH698231  | -        | -        | MH698052 | MH698315 | -        |
| IB55541                      | <i>Bathrops erythromelas</i>  | Guanambi, Bahia, Brazil                         | -         | -         | AF292588  | AF292626  | -        | -        | -        | -        | -        |
| RG829                        | <i>Bathrops erythromelas</i>  | Piranhas, Alagoas, Brazil                       | AF057219  | AF057266  | AY223600  | U48177    | -        | -        | -        | -        | -        |
| IB 55543                     | <i>Bathrops fonsecai</i>      | Campos do Jordão, São Paulo, Brazil             | -         | -         | AF292580  | AF292618  | -        | -        | -        | -        | -        |
| ITS 427                      | <i>Bathrops itapetingaiae</i> | Itirapina, São Paulo, Brazil                    | EU867253* | EU867265* | EU867277* | EU867289* | -        | -        | -        | -        | -        |
| MTR16058                     | <i>Bathrops jararaca</i>      | Serra Bonita, Camacan, Bahia, Brazil            | MH697938  | MH697999  | MH698095  | MH698233  | MH698269 | MH698081 | MH698054 | -        | -        |
| MTR11604                     | <i>Bathrops jararaca</i>      | Caparaó, Espírito Santo, Brazil                 | MH697939  | MH698000  | MH698094  | MH698234  | MH698270 | MH698082 | MH698055 | MH698316 | MH698179 |

(Continued)

