

Range extension of the critically endangered true poison-dart frog, *Phyllobates terribilis* (Anura: Dendrobatidae), in western Colombia

ROBERTO MÁRQUEZ^{1,*}, GERMÁN CORREDOR², CARLOS GALVIS³ DANIEL GÓEZ², ADOLFO AMÉZQUITA¹

¹ Department of Biological Sciences, Universidad de los Andes, AA 4976. Bogotá D.C., Colombia. *Corresponding author. E-mail: roberto.marquez33@gmail.com

² Biology Department, Universidad del Valle, AA 25360, Cali, Colombia

³ Cali Zoological Foundation, Carrera 2 oeste Calle 14 esquina, Barrio Santa Teresita. Cali, Colombia

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Abstract. The poison-dart frog *Phyllobates terribilis* is currently classified as endangered or critically endangered due to its extremely restricted geographic distribution and intensive smuggling by pet traffickers. Based on molecular data, we here report two new localities representing a 60 km northward extension of its previously recognized range. The identity of other *Phyllobates* populations in western Colombia is discussed, as well as the current morphological criteria used to distinguish *P. terribilis* and the similar *P. bicolor*.

Keywords. *Phyllobates terribilis*, geographic distribution, DNA barcoding, conservation status.

The Golden Poison-Dart Frog, *Phyllobates terribilis*, is one of the three species that was used by the Emberá people of western Colombia to poison darts, and is by far the most toxic among them. The species was described by Myers et al. (1978), based on specimens collected at Quebrada Guangüí (type locality) and La Brea, about 8 km west of the type locality, in the Departamento del Cauca, Colombia, which remained the only known localities for almost 20 years. While topotypic specimens are yellow to orange, a mint-colored morph was later reported from La Sirpa near the mouth of the Saijá River, about 15 km west of Quebrada Guangüí (Lötters et al., 1997). In the same paper, two series of *Phyllobates* frogs collected about 50 km south of Buenaventura (Departamento del Valle del Cauca), and 70 km north of the type locality, were reported to exist at the herpetological collection of the Universidad del Valle, Cali (UVC 7208-7210, 7135-7137). These were tentatively diagnosed as *Phyllobates bicolor*, based on their snout-vent length (SVL) and their blackish distal limb and throat coloration. Moreover, it was suggested that *P. terribilis*

could represent an extreme of clinal variation in *P. bicolor* (Lötters et al., 1997). Recent molecular analyses have, however, unambiguously supported *P. bicolor* and *P. terribilis* as two separate and well differentiated species (Grant et al., 2006).

Because it was unclear whether specimens of *Phyllobates* occurring south of Buenaventura, belonged to either *P. bicolor*, or *P. terribilis*, we used DNA barcoding to diagnose frogs from two localities in southern Departamento del Valle del Cauca: (1) Río Naya ($3^{\circ}17'N$, $77^{\circ}24'W$) and (2) Boca Yurumanguí ($3^{\circ}23'N$, $77^{\circ}18'W$), in the Naya and Yurumanguí river drainages, respectively (Fig. 1). We refrain from providing more detailed coordinates due to the intensive smuggling suffered by this frog species and its very restricted distribution range, which led Castro (2004) to declare *P. terribilis* as one of the few critically endangered amphibian species in Colombia.

We captured four individuals at Río Naya and two at Boca Yurumanguí. We measured their SVL and collected tissue samples by mouth-swabbing. Two frogs from each locality were maintained as vouchers (field numbers GECO H 355, GECO H 434, GECO H 643, and GECO H 1163; Fig. 2) and the other ones released. Specimens from Río Naya ranged 38-41 mm (mean 39.75, standard deviation 1.22) and those from Yurumanguí were 37 and 40 mm in SVL.

DNA was extracted from the collected epithelial tissue using DNeasy Animal Blood and Tissue Kits (Qiagen), and the 5' fragment of the Cytochrome c oxidase I (COI) mito-

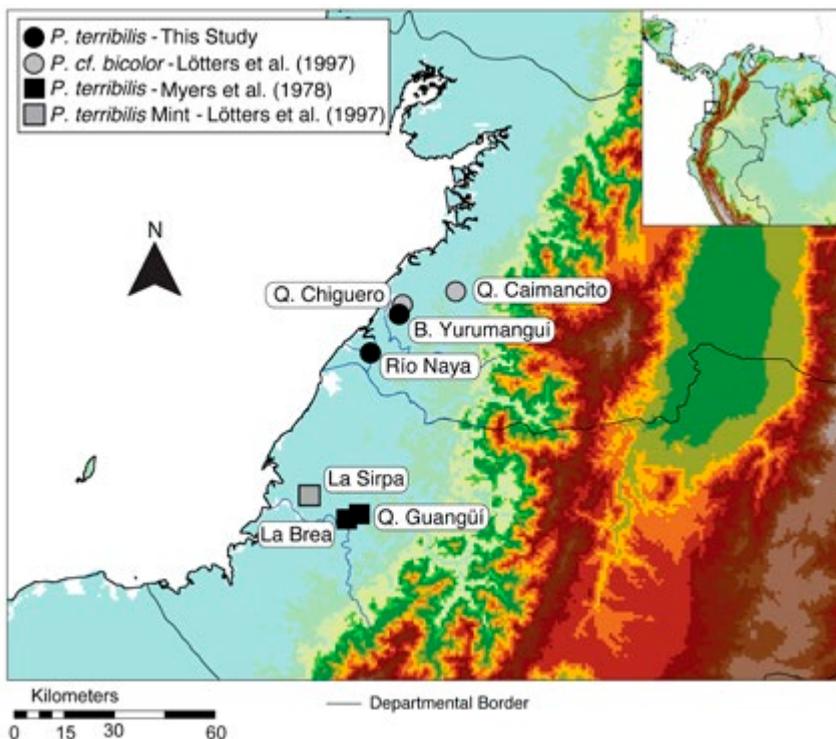


Fig. 1. Reported localities of *Phyllobates terribilis*, including those reported in this paper.

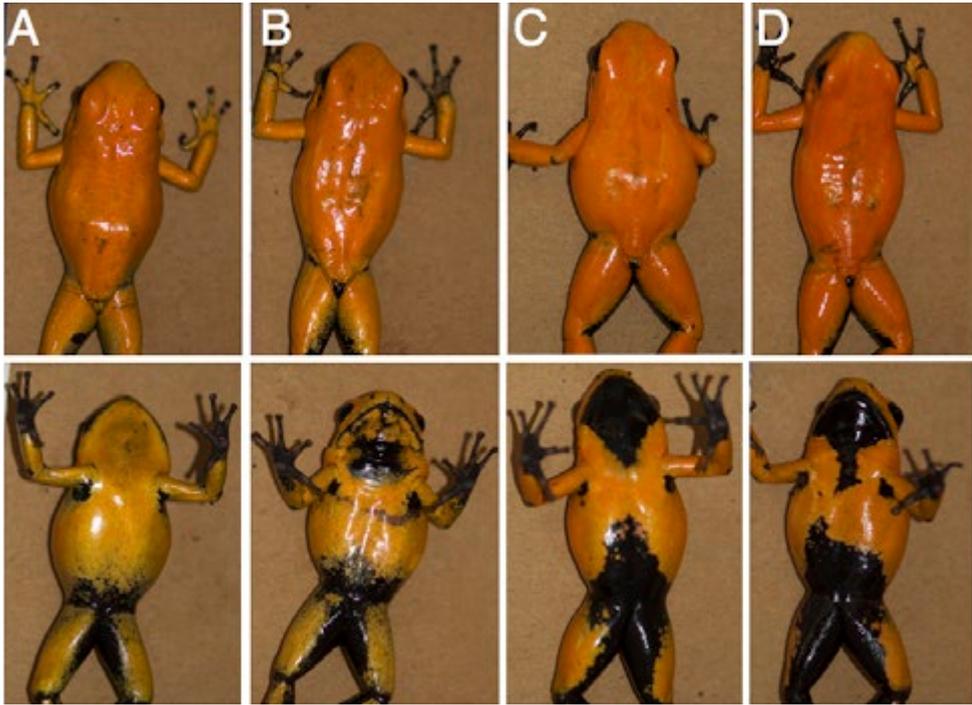


Fig. 2. Ventral and dorsal views of *P. terribilis* from A-B. Río Naya and C-D. Boca Yurumanguí.

chondrial gene was sequenced by using the dgLCO1490 and dgHCO2198 primers (Meyer et al., 2005). The obtained sequences were deposited in GenBank under accession numbers JX887784–JX887787. We then used the Barcode of Life Database Identification System (BOLD IDS; <http://www.boldsystems.org/>) to identify the sequences that best matched the query sequences from our specimens. To further corroborate our results, we inferred Bayesian and maximum likelihood gene trees using MrBayes (Ronquist and Huelsenbeck, 2003) and RAxML (Stamatakis, 2006), respectively, from our sequences and all CO1 sequences from *Phyllobates* species available in GenBank (Fig. 3; Grant et al., 2006).

The two sequences from Río Naya were 99.8% similar among them, and those from Boca Yurumanguí were identical. The BOLD IDS yielded a 0.992 probability of assigning the two Boca Yurumanguí sequences to *P. terribilis*, and 0.987–0.988 probabilities of assigning the Río Naya individuals to the same species, which was the best match for the four sequences. Assignment probabilities to *P. bicolor* (0.940, 0.935–0.936, respectively) were lower in all cases. Regarding the gene tree, the four query sequences were nested within a very well supported clade that included all the other *P. terribilis* sequences (Fig. 3). Hence, both the barcoding and phylogenetic analyses strongly support the frogs from Boca Yurumanguí and Río Naya as part of *P. terribilis*.

Given the short geographic distance (Fig. 1) between the localities reported here and the other two localities referred to by Lötters et al. (1997) and the similar body size (all the collected adults range from 38.2–41.2 mm) among all these frogs, as well as their

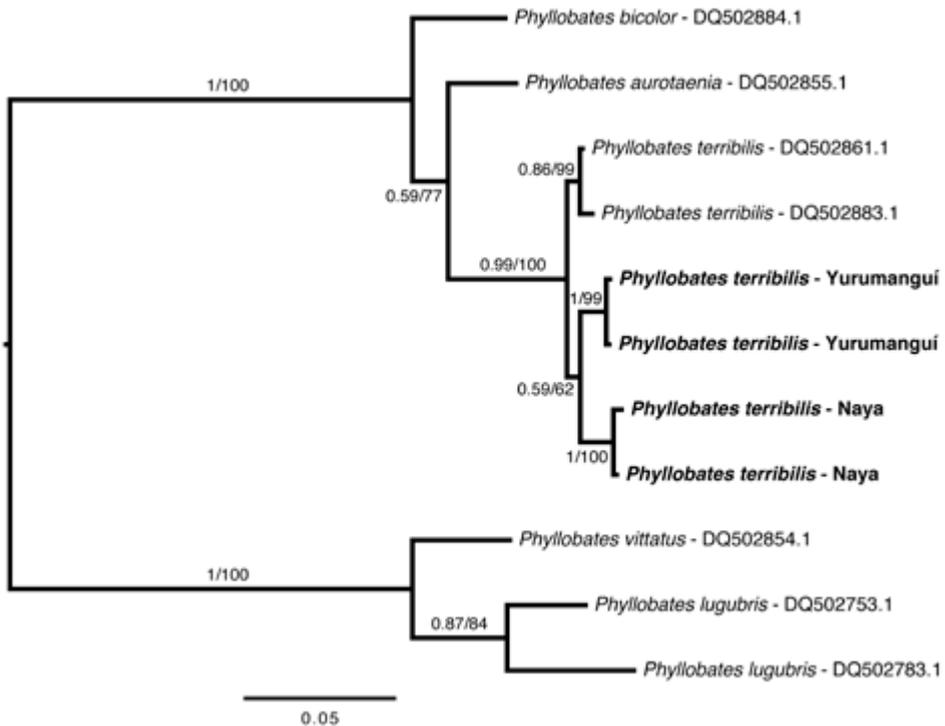


Fig. 3. Bayesian gene tree inferred from CO1 sequences of *Phyllobates* spp. available in GenBank and those of frogs collected in two new localities (in bold). The numbers on internodes represent Bayesian posterior probabilities followed by ML bootstrap values (1000 pseudoreplicates).

blackish distal limb and throat coloration, it is most likely that frogs referred by them (including those tentatively assigned to *P. bicolor*) are indeed *P. terribilis*.

Myers et al. (1978) suggested that the only useful morphological characters at distinguishing *bicolor* and *terribilis* were color pattern and body size. Our data suggest otherwise, posing a potential conflict between genetic and morphologic (traditionally based on body size and color) diagnosis of both species. Further taxonomic and phylogenetic studies with much wider sampling are urgently needed to clarify this issue.

Our results expand the distribution of *P. terribilis* about 60 km north of its previously known range, with the proposed distribution now including localities in two departments of western Colombia (Cauca and Valle del Cauca). Since one of the motives leading to the classification of *P. terribilis* as critically endangered (Rueda-Almonacid et al., 2004) or endangered (Bolívar and Lötters, 2004) was its very restricted extension of range, our findings could improve the conservation status of the species. However, further field censuses and studies are necessary to conduct a thorough reevaluation of the conservation status of *P. terribilis*.

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