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Molecular phylogenetics of the *Pristimantis lacrimosus* species group (Anura: Craugastoridae) with the description of a new species from Colombia

Mauricio Rivera-Correa, Juan M. Daza*

*Grupo Herpetológico de Antioquia, Instituto de Biología, Universidad de Antioquia, Calle 67 # 53–108, Bloque 7–121, A.A. 1226, Medellín, Colombia. *Correspondig author.* E-mail: jumadaza@gmail.com

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Abstract. The *Pristimantis lacrimosus* species group, with 24 species distributed in the Neotropics, is a group of arboreal frogs commonly inhabiting bromeliads. Previous studies have claimed the group to be monophyletic but few species have been included in phylogenetic analyses. In this paper, we included five additional species from the northern Andes in Colombia and tested the monophyly of this phenetic group using genetic data under a Bayesian approach. Our results show that the *P. lacrimosus* group represents two distant and unrelated clades. Clade "A" is endemic to Colombia while Clade "B" encompasses species distributed in Central America, Ecuador and Peru. For the first time, we reveal the phylogenetic position of *P. boulengeri* and a new species is described. The new taxon is most closely related to *P. brevifrons* from southwestern Colombia with a genetic distance of 4.3% for 16S and 10.6% for COI. Our results suggest, one more time, that morphological similarity among species in the most diverse vertebrate genus not necessarily agree with its evolutionary history and that more effort in alpha taxonomy needs to be done in order to understand the tremendous radiation of this lineage in the Neotropics.

Keywords. Cordillera Occidental, diversity, external morphology, molecular phylogenetics, taxonomy, systematics.

INTRODUCTION

The frog genus *Pristimantis*, one of the largest lineages of terrestrial vertebrates, harbors more than 480 species distributed in the Neotropics (Frost, 2015). Because of its high species diversity and taxonomic complexity, the genus has been divided in several phenetic species groups (Lynch, 1976; Lynch and Duellman, 1980). Recent phylogenetic studies, using exclusively molecular data, have corroborated the monophyly of some groups and rejected some others (Hedges et al., 2008; Padial et al., 2014). Currently, 11 species groups are recognized but more than 300 species are not yet assigned to any named group (Padial et al., 2014).

The *Pristimantis lacrimosus* species group was recognized as an assembly in the *Eleutherodactylus marti-nicensis* series within the *P. unistrigatus* species group (Lynch and Duellman, 1980). Hedges et al. (2008), in a molecular phylogenetic study, recovered the group as monophyletic (its analysis incorporated only 3 species). Recently, Padial et al. (2014), in a molecular phylogenetic revision where no additional species from this group were included, maintained the monophyly and, based on morphological characters, assigned several species to the group. Currently, the *P. lacrimosus* species group is composed by 24 species: *P. acuminatus* (Shreve, 1935), *P. apiculatus* (Lynch and Burrowes, 1990), *P. aureolineatus* (Guayasamin, Ron, Cisneros-Heredia, Lamar and McCracken, 2006), *P. boulengeri* (Lynch, 1981), *P. brevifrons* (Lynch, 1981), *P. bromeliaceus* (Lynch, 1979), *P. dorsopictus* (Rivero and Serna, 1988 "1987"), *P. eremitus*

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(Lynch, 1980), *P. geyi* Lehr, Gregory and Catenazzi, 2013, *P. lacrimosus* (Jiménez de la Espada, 1875), *P. latericius* Batallas and Brito, 2014, *P. mendax* (Duellman, 1978), *P. mindo* Arteaga, Yanez-Munoz and Guayasamin, 2013, *P. olivaceus* (Köhler, Morales, Lötters, Reichle and Aparicio, 1998), *P. padiali* Moravec, Lehr, Pérez-Peña, López, Gagliardi-Urrutia and Arista-Tuanama, 2011, *P. pardalinus* (Lehr, Lundberg, Aguilar and von May, 2006), *P. petersi* (Lynch and Duellman, 1980), *P. royi* (Morales, 2007), *P. pseudoacuminatus* (Shreve, 1935), *P. schultei* (Duellman, 1990), *P. tantanti* (Lehr, Torres-Gastello and Suárez-Segovia, 2007), *P. tayrona* (Lynch and Ruiz-Carranza, 1985), *P. waoranii* (McCracken, Forstner and Dixon, 2007), and *P. zimmermanae* (Heyer and Hardy, 1991).

The *Pristimantis lacrimosus* species group ranges from the upper Amazon Basin and adjacent slopes of the Andes from Colombia to Bolivia with several species inhabiting humid forests on the Pacific versant of Ecuador and Colombia and the Sierra Nevada de Santa Marta in northern Colombia (Frost, 2015). According to Hedges et al. (2008), this group is defined by having the body moderately robust with a broad, flat head and acuminate, round, or truncate snout; moderately long limbs; dorsal skin shagreen or smooth; belly areolate; finger I shorter than finger II; toe V much longer than toe III, extending to the distal edge of the distal subarticular tubercle on toe IV; digital discs expanded; tympanic annulus present; tympanic membrane usually differentiated; cranial crests absent; vocal slits and vomerine teeth present. The species placed into the group by Padial et al. (2014) share the presence of some putatively synapomorphic character-states of the group (i.e., acuminate snout, smooth dorsal skin, round and ovate finger and toe discs).

Herein, we test the monophyly of the *P. lacrimosus* species group, including five additional species from the northern Andes, and describe a new species from the cloud forests in north-western Andes of Colombia.

MATERIALS AND METHODS

Morphological analysis

Specimens were euthanized with 2% lidocaine, fixed in 10% formalin and stored in 70% ethanol. Before, a piece of tissue was removed from some specimens and preserved in 95% ethanol for genetic studies. Sex and maturity was determined by examination of secondary sexual characters (presence of vocal slits and expansion of vocal sac in adult males). The description follows Lynch and Duellman (1997) and diagnostic characters follow Duellman and Lehr (2009). Measurements, taken with digital calipers and rounded to the nearest 0.1 mm, are: snout–vent length (SVL), head length (HL, obliquely from angle of jaw to tip of snout), head width (HW, at level of angle of jaw), eye diameter (ED), eye to nostril distance (END, straight line distance between anterior corner of orbit and posterior margin of nares), nostril to tip of snout distance (NSD), internarial distance (IND), distance between the anterior margins of eyes (AMD), tympanum diameter (TD), forearm length (FAL), forearm breadth (FAB), hand length (HAL), thigh length (THL), tibia length (TL), tarsal length (TAL), foot length (FL, distance from proximal margin to inner metatarsal tubercle to tip of toe IV), third finger disk diameter (TFD) and fourth toe disk diameter (FTD). Color in life based on field notes and digital photos. Localities, coordinates and elevations were determined with a 60Cx Garmin GPS.

Comparisons were directly made with examined specimens (see Appendix 1) and/or the literature (when the latter was the case, the reference is given in parentheses). The type series of the new species resides in the Museo de Herpetología Universidad de Antioquia (MHUA) in Medellín, Colombia. Other institutional abbreviations used throughout this paper are ICN (Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia), CSJ (Museo Colegio San José, Instituto Tecnológico Metropolitano, Medellín, Colombia).

Laboratory procedures

To provide a molecular phylogenetic position of the new species within *Pristimantis*, we amplified and sequenced two mitochondrial gene fragments from *P. dorsopictus* and *P. boulengeri*, traditionally assigned to the *P. lacrimosus* species group. Total genomic DNA was extracted from ethanol preserved tissues using the DNeasy kit (Qiagen, Inc.). We amplified the mitochondrial region including partial sequence of the ribosomal gene 16S using the primers 16SC–16SD (Santos et al., 2003) and a fragment of the cytochrome c oxidase subunit 1 (COI) gene, using the primers dgLCO and dgHCO (Pinto et al., 2012). Amplification protocols followed Santos et al. (2003) and Pinto et al. (2012). PCR products were purified and sequenced at the Macrogen facilities in Korea (Macrogen, Inc.). Chromatographs were aligned and manually edited using Geneious 8.1.4 (created by Biomatters and available from http://www.geneious.com). Genbank accession numbers for the sequences are provided in Appendix 2.

Phylogenetic analyses

We assembled a large *Pristimantis* dataset including 10 genomic regions, four from the mitochondrial genome (12S, 16S, COI, cyt-b) and six nuclear markers (CXCR4, NCX1, POMC, Rag-1, SLC8A3 and Tyr) . Taxon sampling included 179 terminals representing 135 nominal species within *Pristimantis* and 14 species used as outgroups (Appendix 2). Each genomic region was aligned separately using default parameters in Muscle (Edgar, 2004). We inferred the best partition scheme and evolution model for each partition using PartitionFinder under the BIC criterion (Lanfear et al., 2012). We inferred a phylogenetic tree using the Bayesian method implemented in Beast 1.8.1 (Drummond et al., 2012). We used a rate prior using a

lognormal distribution and a Yule prior for the tree model. Two independent tree searches from random starting trees were initiated and ran for 40 million generations. Trees were sampled every 1000 generations and convergence and stationarity were verified by examining ESS values of the posterior using Tracer 1.5 (Rambaut and Drummond, 2007). We discarded as burn-in the first four million generations from each run and combined the two runs to summarize the posterior distribution of nodes on the maximum clade credibility tree.

Genetic divergence

To have an estimate of genetic divergence among species we included intraspecific sampling within the clade the new species belongs. We compared with the new taxon four of the species assigned to the *P. lacrimosus* species group: *P. angustilineatus*, *P. boulengeri*, *P. brevifrons* and *P. dorsopictus*. We calculated uncorrected genetic distances, for both the COI and 16S fragments, among species with 1000 bootstrap replicates using the program MEGA 6.0 (Tamura et al., 2013).

RESULTS

Phylogenetic relationships

The entire dataset included 8978 aligned sites: 1067 sites of 12S, 1759 of 16S, 690 of COI, 675 of CXCR4, 705 of cyt-b, 1275 of NCX1, 504 of POMC, 640 of Rag-1, 1131 of SLC8A3, and 532 of Tyr. Alignments are available upon request. The best partition scheme and evolution model for each partition are shown in Appendix 3. The inferred phylogenetic tree recovers, in general, well-supported nodes within *Pristimantis* (Appendix 4) and it is in agreement with previous studies (Pinto et al., 2012; Padial et al., 2014; Rivera-Prieto et al., 2014). Two non-reciprocally monophyletic clades represent the current *P. lacrimosus* species group (Fig. 1). One clade (Clade "A") includes species distributed in the Northern Andes in Colombia. The second clade (Clade "B") includes species distributed on the Andes from Ecuador and Perú, in Brazil, probably in western Colombia and Central America. Both clades received Bayesian support of 1.0 and 0.9, respectively. The new species is most closely related to *P. brevifrons* from southwestern Colombia although with low node support. Genetic distances among species within the Clade "A" range between 2.3 and 6.7 % for 16S and 4.1 and 11.9 % for COI (Table 1). The genetic distance between the new taxon and the sister species *P. brevifrons* is 4.3 % for 16S and 10.6 % for COI.

Morphological description

Pristimantis urani sp. nov.
(Figs 2,5)

Holotype. MHUA-A 7471, adult female. Colombia, Departamento de Antioquia, Municipio de Urrao, Corregimiento La Encarnación, Vereda El Maravillo, (6°30'36" N, 76°08'40" W; 2295 m a.s.l.), collected on March 14, 2012 by José Fang.

Paratypes. MHUA-A 7467–68 adult males; MHUA-A 7469–70, MHUA-A 7472 adult females; all collected with holotype.

Diagnosis

We assigned the new species to the genus *Pristimantis* on the basis of our phylogenetic results (Fig. 1). The new species is characterized by a combination of (1) skin texture of the dorsum smooth, venter weakly areolate; dorsolateral folds and discoidal fold absent; (2) tympanic membrane and tympanic annulus evident, supratympanic fold not differentiated; horizontal diameter of tympanum 33-38% of eye diameter; (3) snout broadly rounded in dorsal view (truncated by protruding nostrils), truncate in profile; (4) tubercles on upper eyelids and cranial crests absent; (5) dentigerous process of the vomer absent; (6) males with vocal slits and median

Table 1. Uncorrected genetic distances among *Pristimantis* species within the Clade "A" (Fig. 1). Upper right matrix shows genetic distances for the 16S fragment. Lower-left matrix shows the distances for the COI fragment. PA (Paraguas locality); PE (Peñas Blancas locality).

	P. angustilineatus	*P. boulengeri*	*P. brevifrons* PA	*P. brevifrons* PE	*P. dorsopictus*	***P. urani*** sp. nov.
P. angustilineatus	--	0.067	0.045	0.038	0.057	0.039
P. boulengeri	0.090	--	0.054	0.057	0.046	0.057
P. brevifrons PA	0.110	0.089	--	0.023	0.049	0.044
P. brevifrons PE	0.112	0.094	0.041	--	0.055	0.041
P. dorsopictus	0.103	0.101	0.119	0.117	--	0.062
P. urani sp. nov.	0.097	0.101	0.104	0.108	0.109	--

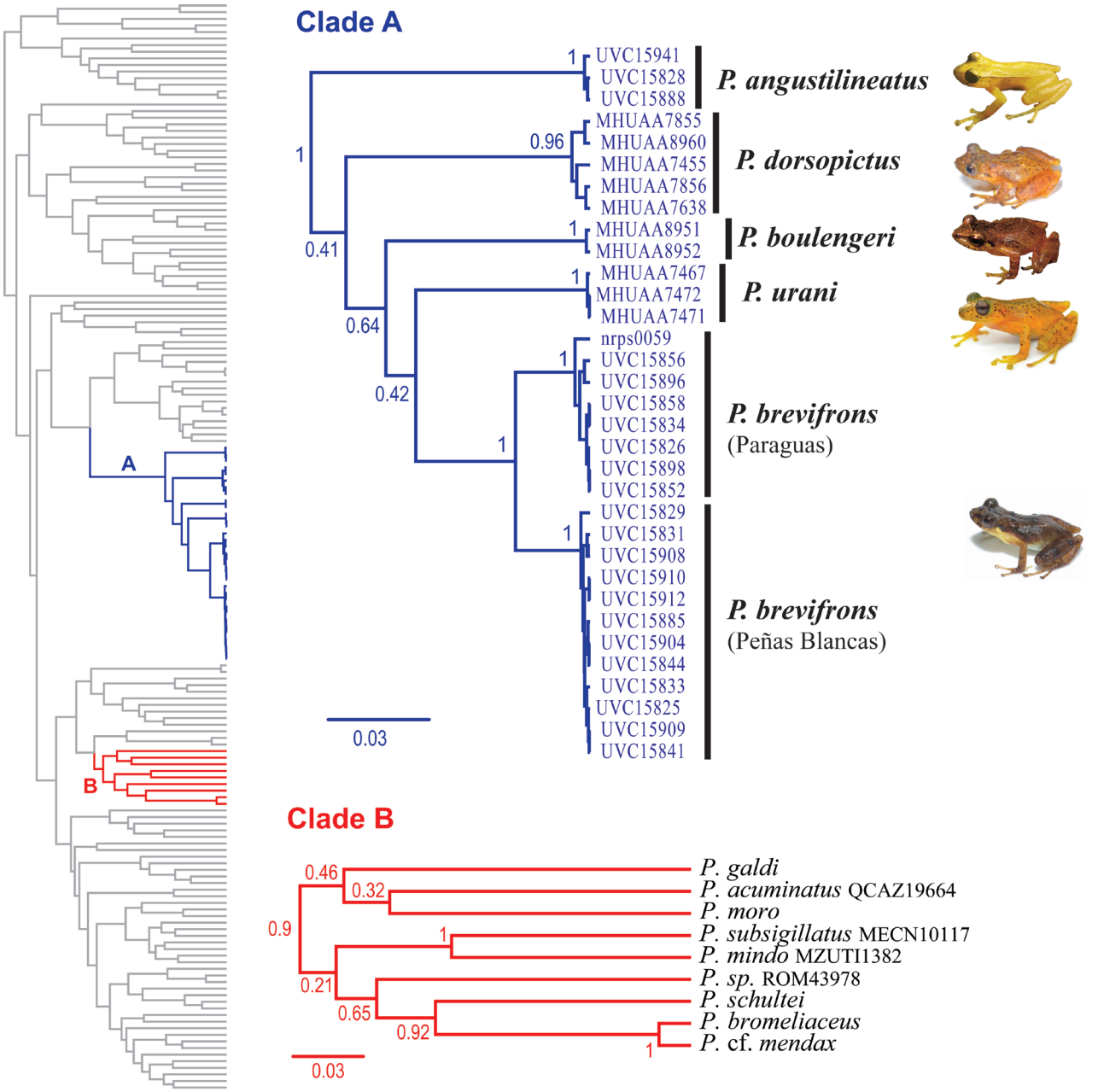

Fig. 1. Maximum clade credibility tree depicting the phylogenetic relationships within the *Pristimantis lacrimosus* species group. Clades on the right are highlighted in the *Pristimantis* phylogeny on the left. Numbers on nodes represent posterior probabilities.

subgular vocal sac; white, nonspinous nuptial pads present; (7) first finger shorter than the second; fingers III–IV bearing expanded and rounded discs about twice as wide as digits; (8) fingers with narrow lateral fringes; (9) antebrachial and ulnar tubercles absent, but a low ulnar fold present; (10) tubercles on heel and outer edge of tarsus absent; inner tarsal fold absent; (11) inner metatarsal tubercle oval, two-to-three times as long as round outer metatarsal tubercle; supernumerary plantar tubercles small and low, at the base of toes III and IV; (12) toes with narrow lateral fringes; webbing absent; fifth toe longer than third; (13) in life, dorsum light yellow to green-yellow with dark brown marks and blotches (Fig. 2); venter creamy white; (14) adults small, SVL in males 18.7–19.1 mm (18.9 ± 0.28, n = 2), in females 21.0–23.4 mm (22.5 ± 1.02, n = 4).

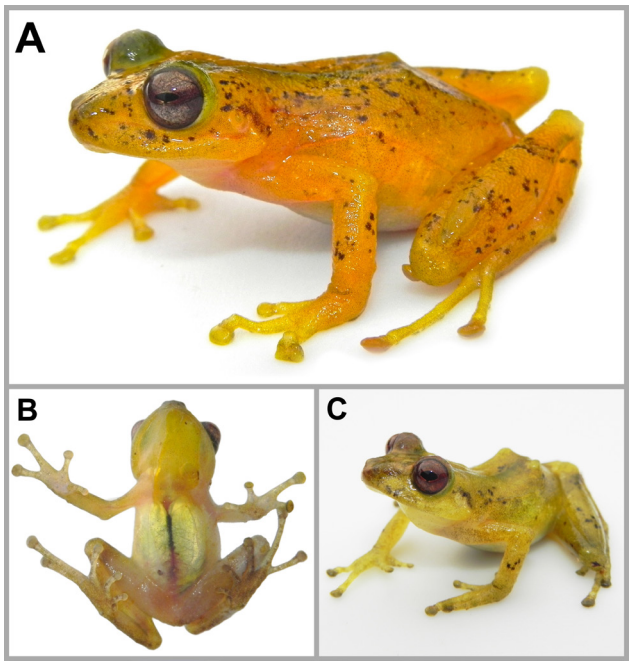

Fig. 2. *Pristimantis urani* sp. nov. in life: (A) MHUA-A 7471, SVL 24.2 mm, holotype, adult female; (B) MHUA-A 7467, SVL 19.1 mm, paratype, adult male; (C) MHUA-A 7472, SVL 23.4 mm, paratype, adult female. Photos by F. Duarte-Cubides.

Fig. 3. *Pristimantis* species of Clade "A". (A) *Pristimantis angustilineatus* TG 1484 (Vereda Las Amarillas, Municipio del Cairo, Valle del Cauca, Colombia), adult female; (B) *Pristimantis boulengeri* MHUA-A 8952 (Parque Regional Natural Ucumarí, Pereira, Risaralda, Colombia), SVL 22.0 mm, adult male; (C) *Pristimantis brevifrons* (Finca San Pedro, Municipio de Dagua, Valle del Cauca, Colombia; not collected), adult male; (D). *Pristimantis dorsopictus* MHUA-A 7855, (Corregimiento de Santa Elena, Medellín, Antioquia, Colombia), SVL 24.0 mm, adult male. TG: Taran Grant field number. Photos by Taran Grant (A), J.J. Ospina-Sarria (C), M. Rivera-Correa (B, D).

Comparison with related species

Pristimantis urani sp. nov. differs from related species (Fig. 3) by lacking a rostral papilla (present in *P. angustilineatus*, *P. boulengeri*, *P. brevifrons* and *P. dorsopictus*), tubercles on upper eyelids absent (present in *P. boulengeri*, *P. brevifrons* and *P. dorsopictus*). The snout in *P. urani* is broadly rounded in dorsal view (acuminate in *P. boulengeri*, subacuminate in *P. angustilineatus*, *P. brevifrons* and *P. dorsopictus*). *Pristimantis urani* lacks tubercles on heel and outer edge of tarsus (present in *P. brevifrons* and *P. dorsopictus*). Furthermore, *P. angustilineatus* have dorsolateral stripe yellow bordered below by brown to nearly black (absent in *P. urani*). See Table 2, for a summary of diagnostic characters of other similar species to *P. urani* sp. nov.

Description of the holotype

Adult female (Fig. 4), head as wide as long; snout broadly rounded in dorsal view and truncate in lateral view, relatively short (snout–eye distance 13% SVL), without small papilla at tip; canthus rostralis indistinct; loreal region slightly concave; nostrils protuberant, directed anterolaterally, internostrils area slightly concave; interorbital area flat, as broad as upper eyelid; cranial crests absent; upper eyelid with small and low tubercles; tympanic membrane and tympanic annulus distinct, round; supratympanic fold not differentiated (Fig. 2); tympanum diameter 33% of eye diameter; postrictal tubercles low. Choanae small, nearly rounded, not concealed by palatal shelf of maxillary; dentigerous process of the vomer absent; tongue longer that wide, posterior one-half free from floor of mouth. Texture of skin of dorsum and flanks smooth, dorsolateral folds absent; venter areolate; thoracic fold and discoidal fold absent; cloacal sheath absent.

Forearm slender; radio–ulna length 28.2% SVL; ulnar tubercles and low ulnar fold present; hand length longer than radio–ulna length (hand length 33.8% SVL); fingers with narrow lateral fringes; relative lengths of fingers I < II < IV < III; palmar tubercle bifid, thenar tubercle oval; subarticular tubercles round, low; supernumerary palmar tubercles present at the base of all fingers, low, inconspicuous; disc cover of finger I slightly expanded, those of fingers II–IV extensively expanded; outer discs of fingers as wide as those of toes; all disc covers with elliptical ventral pads defined by circummarginal grooves. Hind limbs relatively slender; tibia length 56.4% SVL; foot length 100% of tibia length; tarsal fold and tarsal tubercles absent; heel (tibiotarsal articulation) with low tubercles; toes with narrow lateral fringes; subarticular tubercles round, low; inner metatarsal tubercle oval, about 2x

Table 2. Character states in some species currently placed in the polyphyletic *Pristimantis lacrimosus* group and for which we do not have molecular evidence to infer their phylogenetic position. Some of them are phenotypically similar to *P. urani* sp. nov. and may be closely related to it. Source: 1. Guayasamin et al., 2006; 2. Lynch, 1980; 3. Lehr et al., 2013; 4. Lynch and Duellman, 1997; 5. Batallas-R. and Brito-M., 2014; 6. Köhler et al., 1998; 7. Moravec et al., 2010; 8. Lehr et al., 2006; 9. Lynch and Duellman, 1980; 10. Shreve, 1935; 11. Morales, 2007; 12. Lehr et al., 2007; 13. Lynch and Ruiz-Carranza, 1985; 14. McCracken et al., 2007; 15. Heyer and Hardy, 1991; * this work.

Species	Rostral Papilla	**Eyelid tubercle**	**Heel tubercle**	**Snout shape**	**Source**
P. aureolineatus	Present	Absent	Absent	Acuminate	1
P. eremitus	Present	Present	Present	Subacuminate	2
P. deyi	Present	Present	Present	Acuminate	3
P. lacrimosus	Present	Absent	Absent	Broadly rounded	4
P. latericius	Present	Present	Absent	Acuminate	5
P. olivaceus	Present	Present	Absent	Subacuminate	6
P. padiali	Absent	Absent	Present	Acuminate	7
P. pardalinus	Present	Present	Present	Acuminate	8
P. petersi	Present	Present	Present	Rounded	9
P. pseudoacuminatus	Present	Absent	Absent	Acuminate	10
P. royi	Absent	Present	Absent	Broadly rounded	11
P. urani **sp. nov.**	Absent	Absent	Absent	Broadly rounded	*
P. tantanti	Present	Absent	Absent	Acuminate	12
P. tayrona	Present	Present	Present	Acuminate	13
P. waoranii	Absent	Absent	Absent	Subacuminate	14
P. zimmermanae	Present	Present	Absent	Acuminate	15

as long as wide; subconical outer tubercle; supernumerary plantar tubercles inconspicuous; disc covers slightly expanded; toes with defined pads; disc pads nearly elliptical; relative lengths of toes I < II < III < V < IV; tip of toe V reaching distal border of distal subarticular tubercle of toe IV; tip of toe III reaching proximal border of medial subarticular tubercle of toe IV.

Coloration of the holotype

In life, the holotype of *Pristimantis urani* sp. nov. is yellow on the dorsum with many dark brown blotches; limbs, flanks and thighs cream with scattered brown blotches; axillae, undersides and posterior surfaces of thighs immaculate with light yellow coloration, venter and throat white; palmar and plantar side light yellow; ventral side of limbs and thighs light yellow without spots or marks; iris cooper with fine brown reticulation and with a maroon horizontal streak (Fig. 2). In preservative, the holotype has dorsum and flanks cream with numerous darker brown blotches; arm and legs cream with dark brown spots and blotches, cream belly without marks (Fig. 4).

Fig. 4. Holotype of *Pristimantis urani* sp. nov. in preservative. MHUA-A 7471, SVL 24.2 mm, adult female.

Measurements of the holotype (in millimeters)

SVL: 23.4; HL: 9.2; HW: 9.4; ED: 3.3; END: 2.7; NSD: 1.2; IND: 2.5; AMD: 4.5; TD: 1.1; FAL: 6.6; FAB: 1.8; HAL: 7.9; THL: 13.0; TL: 13.2; TAL: 6.3; FL: 13.3; TFD: 1.6; FTD: 1.5.

Variation

Males are smaller than females (see Table 3). In life, dorsum light yellow to green-yellow. The dorsal pigmentation in *P. urani* is variable, presenting conspicuous blotches, spots and marks, mainly in the holotype (MHUA-A 7471); marks more diffuse in the two paratype males (MHUA-A 7467–7468). A supra-tympanic dark brown stripe present in all individuals except in (MHUA-

Table 3. Morphological variation (in mm) of the type series of *Pristimantis urani* sp. nov.. See text for abbreviations. Meas = Measurement; min = minimum value; max = maximum value; x = arithmetic mean value; SD = standard deviation.

Meas	Females (n = 4)				Males (n = 2)			
	min	max	x	SD	min	max	x	SD
SVL	21.0	23.4	22.5	1.02	18.7	19.1	18.9	0.28
HL	7.9	9.2	8.8	0.61	7.0	7.5	7.3	0.35
HW	7.9	9.5	9.0	0.74	6.9	7.1	7.0	0.14
ED	3.0	3.3	3.1	0.15	2.6	2.7	2.7	0.07
END	2.5	2.8	2.7	0.13	1.9	2.3	2.1	0.28
NSD	1.2	1.2	1.2	0	1.2	1.3	1.3	0.07
IND	2.0	2.5	2.3	0.21	1.9	1.9	1.9	0
AMD	4.2	5.0	4.6	0.34	3.6	1.2	3.7	0.14
TD	1.0	1.2	1.1	0.10	1.0	5.0	1.1	0.14
FAL	5.0	6.6	5.7	0.67	4.8	1.6	4.9	0
FAB	1.5	1.8	1.6	0.14	1.4	1.6	1.5	0.14
HAL	5.8	7.9	7.3	0.99	5.9	5.9	5.9	0
THL	11.5	13.0	12.3	0.62	10.1	10.5	10.3	0.28
TL	11.5	13.2	12.5	0.72	10.7	5.3	10.7	0
TAL	5.6	6.4	6.1	0.36	4.7	9.6	5.0	0.42
FL	10.2	13.3	12.1	1.36	9.8	1.2	9.7	0.14
TFD	1.2	1.6	1.4	0.17	1.1	1.2	1.2	0.07
FTD	1.2	1.5	1.3	0.14	1.0	1.1	1.1	0.07

A 7470), instead of the above is weakly stained. Vocal sac in males is yellow.

Geographic distribution and natural history

Pristimantis urani sp. nov. is known only from type locality at elevations ca. 2300 m a.s.l., on the north-western flank of the Cordillera Occidental, Antioquia department of Colombia (Fig. 5). Limited natural history data were obtained during the collection of the series type, except that specimens were found near a creek inside a cloud montane forest, hidden in the axils of Araceae plants. The individuals were collected inactive in the morning (between 11:00–12:20 h). Female MHUA-A 7470 had developed follicles. The only other *Pristimantis* found to be syntopic with *P. urani* was *P. erythropleura*. Vocal behaviour or any aspect of the reproductive ecology of the species is currently unknown.

Etymology

The specific name is a patronym for Rigoberto (Rigo) Uran, a Colombian cyclist born in Urrao, Antioquia, type locality of the new species. Rigo Uran represents, despite adversity, the struggle to become a great athlete. The new taxon is native to the northern Andes, mountains widely conquered by the Colombian cyclists.

DISCUSSION

Our phylogenetic inference suggests that the *Pristimantis lacrimosus* species group consists of two non-sister clades and, therefore, does not represent a natural group. Previous phylogenetic studies found the species group to be monophyletic (Padial et al., 2014) but did not include species from the *lacrimosus* group distributed in the northern Andes of Colombia. We recovered Clade "A" as endemic to Colombia while Clade "B" is composed by species distributed in Central America, Ecuador and Peru. In this sense, we prefer not to name any of the clades recovered in our phylogenetic hypothesis as *P. lacrimosus* species group until the phylogenetic position of the species *P. lacrimosus* is inferred. According to Padial et al. (2014) acuminate snout, smooth dorsal skin, round and ovate finger and toe discs are suggested as putative synapomorphies, character states founded in our two unrelated lineages. Therefore, we recommend that these characters must be carefully considered when allocating taxa to species groups. Once we include the remaining species assigned to this taxonomic group we will better understand the evolution and usefulness of several phenotypic characters, widely used in the definition of the group.

Genetic divergence among species in Clade "A" is high and intraspecific sampling within *P. brevifrons* indicates that phylogeographic structure occurs in these montane settings (García-R. et al., 2012). The northern Andes of Colombia are divided in three mountain ranges and species from Clade "A" are distributed in the Cordillera Occidental (*P. angustilineatus*, *P. brevifrons* and *P. urani* sp. nov.) and Cordillera Central (*P. boulengeri* and *P. dorsopictus*). A more thorough sampling along the entire distribution of these species may uncover new phylogeographic patterns and even cryptic species diversity. Also, adding the putative related species from the Cordillera Oriental (i.e., *P. prolixodiscus* and *P. uisae*) will improve our understanding of the evolution of these phenetic similar species.

Pristimantis angustilineatus was not assigned to a species group according to Padial et al. (2014). Since its original description (Lynch, 1998), the species was considered a member of the diverse and known polyphyletic *P. unistrigatus* group (Padial et al., 2014). Lynch (2003) suggested a close relationship of this species with *P. baiotis*, *P. boulengeri*, *P. brevifrons*, *P. dorsopictus*, *P. eremitus* and *P. uisae*, species associated to *P. lacrimosus* species group. Our study, the first to include *P. angustilineatus* in

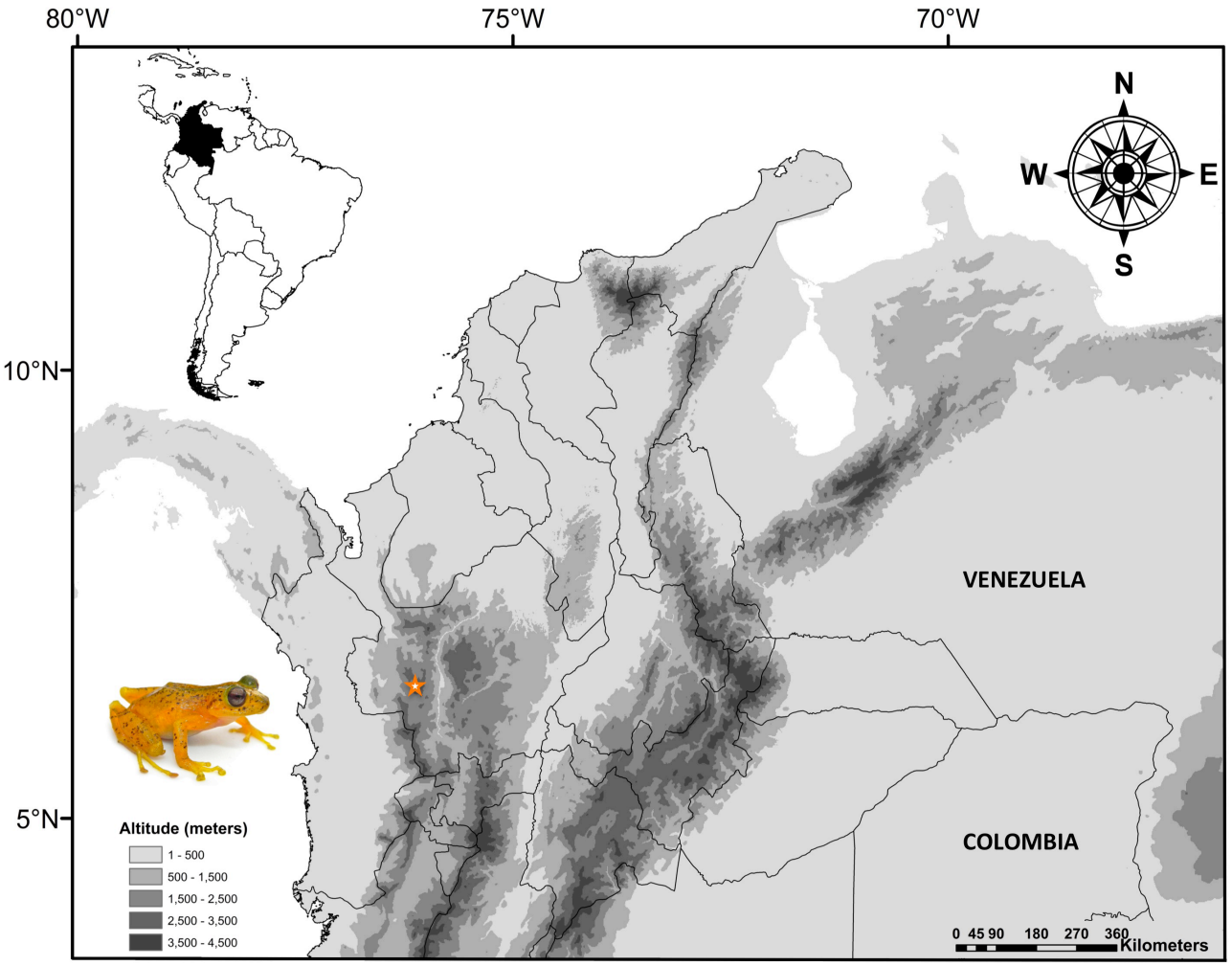

Fig. 5. Map showing the type locality of *Pristimantis urani* sp. nov. Vereda El Maravillo, Corregimiento La Encarnación, Municipio de Urrao, Antioquia, Colombia.

a wide-scale *Pristimantis* phylogeny, partially support the hypothesis suggested by Lynch (2003).

Taxonomic arrangements in the highly diverse genus *Pristimantis* are still highly dependent on species groups, but the majority of species are not assigned to named groups. Here, we show phylogenetic evidence that even named groups are not monophyletic and therefore inferences in phenotypic trait evolution, ecology and biogeography are waiting for more complete and robust phylogenetic hypotheses that in turn will unravel the amazing diversification in the most specious anuran clade.

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APPENDIX

Appendix 1. Specimens examined.

*Pristimantis angustilineatus***:** El Boquerón, vereda Las Amarillas, Municipio de El Cairo, Departamento del Valle del Cauca, Colombia (ICN 39598, holotype); Vereda Las Amarillas, Municipio de El Cairo, Departamento del Valle del Cauca, Colombia (ICN 29286, 29281, 39598, 39601, 39611).
*Pristimantis boulengeri***:** Refugio Ecológico La Pastora (4,707971, -75,492928), Parque Regional Natural Ucumarí, Pereira, Risaralda, Colombia (MHUA-A 8951, 8952).
*Pristimantis brevifrons***:** Vereda Las Amarillas, Municipio El Cairo, Valle del Cauca (ICN 29269-29270).
*Pristimantis dorsopictus***:** Páramo de Sonsón, Sonsón, Antioquia (CSJ 1535, 1537, 1548-53, paratypes); Corregimiento de Santa Elena (6,211347, -75,489166), Medellín, Antioquia, Colombia (MHUA-A 7855-7856); San Felix (6,331388, -75,636111), Bello, Antioquia, Colombia (MHUA-A 8960).

Appendix 2. GenBank accession numbers for loci and terminals used in this study. Names in bold are new sequences added for this study.

Taxon	12S	16S	COI	Cyt-b	CXCR4	NCX1	POMC	Rag-1	SLC8A3	Tyr
Bufo melanostictus	AB331714	FJ882791	---	AF249082	DQ306508	AY948805	DQ158317	EU712821	AY948851	---
Craugastor longirostris	EF493395	EF493395	---	DQ350242	---	---	---	EF493454	---	EF493482
Craugastor podiciferus	EF493360	EF493360	---	---	GQ345182	GQ345230	GQ345258	EF493450	GQ345328	EF493481
Eleutherodactylus caribe	EF493385	EF493385	---	---	---	---	---	---	---	EF493472
Eleutherodactylus marnockii	DQ283102	DQ283101	---	---	EF107463	EF107238	---	EF107300	EF107388	EF493476
Oreobates cruralis	EU186666	EU186666	---	EU368881	---	---	---	EU186743	---	EU186764
Oreobates saxatilis	EU186726	EU186708	---	---	---	---	---	EU186742	---	EU186763
Phyllomedusa hypochondrialis	FJ882741	AY948748	---	AY843969	GQ366014	AY948826	---	AY948929	AY948882	AY844153
Pristimantis acerus	EF493678	EF493678	---	---	---	---	---	---	---	---
Pristimantis achatinus	EF493827	EF493660	JN991349	---	---	---	---	---	---	---
Pristimantis actites	EF493696	EF493696	---	---	---	---	---	EF493432	---	EF493494
Pristimantis acuminatus	---	EU130579	---	---	---	---	---	---	---	---
Pristimantis affinis	JN991487	JN991424	---	---	---	---	---	---	---	JN991554
Pristimantis altae	JN991496	---	JN991361	---	---	---	---	JQ025174	---	JN991560
Pristimantis altamazonicus	EF493670	EF493670	---	---	---	---	---	EF493441	---	EU186778
P. angustilineatus UVC15828	---	JN371034	JN371123	---	---	---	---	---	---	---
P. angustilineatus UVC15888	---	JN104677	JN371124	---	---	---	---	---	---	---
P. angustilineatus UVC15941	---	JN371035	JN371125	---	---	---	---	---	---	---
Pristimantis aniptopalmatus	EF493390	EF493390	---	---	---	---	---	---	---	---
Pristimantis appendiculatus	EF493524	EF493524	---	---	---	---	---	---	---	---
Pristimantis ardalonychus	EU186664	EU186664	---	---	---	---	---	---	---	---
Pristimantis bipunctatus	EF493702	EF493702	---	---	---	---	---	EF493430	---	EF493492
Pristimantis bogotensis	JN991497	JN991432	JN991362	---	---	---	---	---	---	---
***P. boulengeri* MHUAA8951**	---	**KU724435**	**KU724444**	---	---	---	---	---	---	---
***P. boulengeri* MHUAA8952**	---	**KU724436**	**KU724445**	---	---	---	---	---	---	---
P. brevifrons nrps0059	JN991498	JN991433	---	---	---	---	---	---	---	---
P. brevifrons UVC15825	---	JN104678	JN371051	---	---	---	---	---	---	---
P. brevifrons UVC15826	---	JN370960	JN371043	---	---	---	---	---	---	---
P. brevifrons UVC15829	---	JN370966	JN371049	---	---	---	---	---	---	---
P. brevifrons UVC15831	---	JN370964	JN371047	---	---	---	---	---	---	---
P. brevifrons UVC15833	---	JN370967	JN371050	---	---	---	---	---	---	---
P. brevifrons UVC15834	---	JN370962	JN371045	---	---	---	---	---	---	---
P. brevifrons UVC15841	---	JN370969	JN371053	---	---	---	---	---	---	---
P. brevifrons UVC15844	---	JN370971		---	---	---	---	---	---	---
P. brevifrons UVC15852	---	JN370961	JN371044	---	---	---	---	---	---	---

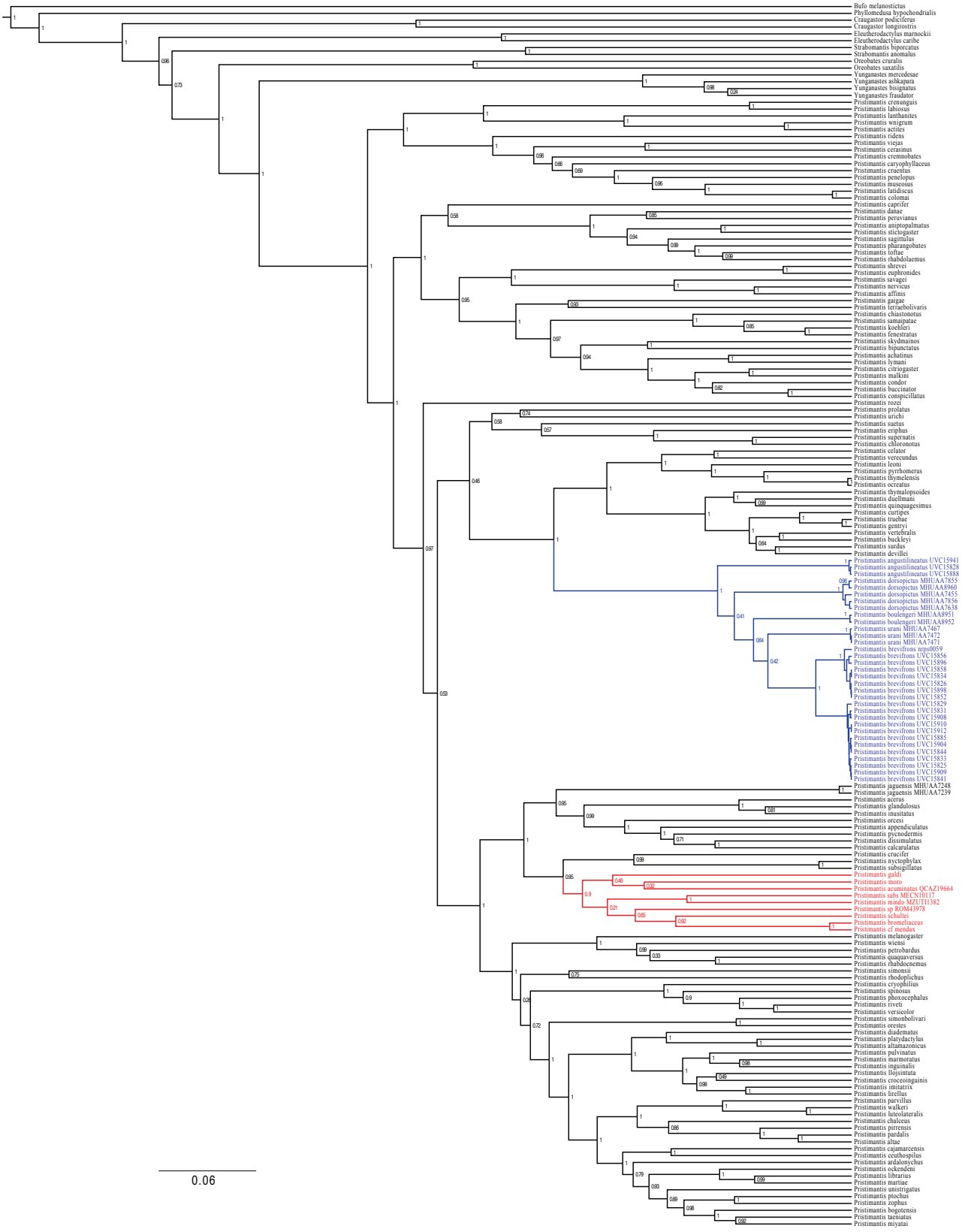
Taxon	12S	16S	COI	Cyt-b	CXCR4	NCX1	POMC	Rag-1	SLC8A3	Tyr
P. brevifrons UVC15856	---	JN370957	JN371041	---	---	---	---	---	---	---
P. brevifrons UVC15858	---	JN370956	JN371040	---	---	---	---	---	---	---
P. brevifrons UVC15885	---	JN370972		---	---	---	---	---	---	---
P. brevifrons UVC15896	---	JN370958		---	---	---	---	---	---	---
P. brevifrons UVC15898	---	JN370959	JN371042	---	---	---	---	---	---	---
P. brevifrons UVC15904	---	JN370973	JN371055	---	---	---	---	---	---	---
P. brevifrons UVC15908	---	JN370968	JN371052	---	---	---	---	---	---	---
P. brevifrons UVC15909	---	JN370963	JN371046	---	---	---	---	---	---	---
P. brevifrons UVC15910	---	JN370965	JN371048	---	---	---	---	---	---	---
P. brevifrons UVC15912	---	JN370970	JN371054	---	---	---	---	---	---	---
Pristimantis bromeliaceus	EF493351	EF493351	---	---	---	---	---	---	---	---
Pristimantis buccinator	---	EU712631	---	---	---	---	---	---	---	---
Pristimantis buckleyi	EF493350	EF493350	---	---	---	---	---	---	---	---
Pristimantis cajamarcensis	EF493823	EF493663	---	---	---	---	---	---	---	---
Pristimantis calcarulatus	EF493523	EF493523	---	---	---	---	---	---	---	---
Pristimantis caprifer	EF493391	EF493391	---	---	---	---	---	---	---	---
Pristimantis caryophyllaceus	EU186686	EU186686	JN991363	---	---	---	---	---	---	---
Pristimantis celator	EF493685	EF493685	---	---	---	---	---	---	---	---
Pristimantis cerasinus	---	JN991437	JN991366	---	---	---	---	JQ025177	---	JN991564
Pristimantis ceuthospilus	EF493520	EF493520	---	---	---	---	---	---	---	---
Pristimantis cf. *mendax*	EU186659	EU186659	---	---	---	---	---	---	---	---
Pristimantis chalceus	EF493675	EF493675	---	---	---	---	---	---	---	---
Pristimantis chiastonotus	---	EU201061	---	---	---	---	---	---	---	---
Pristimantis chloronotus	AY326007	AY326007	---	---	---	---	---	---	---	---
Pristimantis citriogaster	EF493700	EF493700	---	---	---	---	---	---	---	---
Pristimantis colomai	EF493354	EF493354	---	---	---	---	---	EF493440	---	EF493502
Pristimantis condor	EF493701	EF493701	---	---	---	---	---	EF493443	---	EF493504
Pristimantis conspicillatus	EF493529	EF493529	---	---	---	---	---	EF493437	---	EF493499
Pristimantis cremnobates	EF493528	EF493528	---	---	---	---	---	EF493424	---	EF493486
Pristimantis crenunguis	EF493693	EF493693	---	---	---	---	---	---	---	---
Pristimantis croceoinguinis	EF493669	EF493665	---	---	---	---	---	---	---	---
Pristimantis crucifer	EU186736	EU186718	---	---	---	---	---	---	---	---
Pristimantis cruentus	FJ882746	EF493697	JN991369	---	---	AY948836	---	AY948935	AY948898	---
Pristimantis cryophilius	EF493672	EF493672	---	---	---	---	---	---	---	---
Pristimantis curtipes	EF493513	EF493513	---	---	---	---	AY819092	DQ679272	---	EF493497
Pristimantis danae	---	EU192272	---	EU368882	---	---	---	---	---	---
Pristimantis devillei	EF493688	EF493688	---	---	---	---	---	---	---	---
Pristimantis diadematus	EU186668	EU186668	---	---	---	---	---	---	---	---
Pristimantis dissimulatus	EF493522	EF493522	---	---	---	---	---	---	---	---
***P. dorsopictus* MHUAA7455**	---	**KU724437**	**KU724446**	---	---	---	---	---	---	---
P. dorsopictus MHUAA7638	KP082864	KP082874	---	---	---	---	---	---	---	KP082879
***P. dorsopictus* MHUAA7855**	---	**KU724438**	**KU724447**	---	---	---	---	---	---	---
***P. dorsopictus* MHUAA7856**	---	**KU724439**	**KU724448**	---	---	---	---	---	---	---
***P. dorsopictus* MHUAA8960**	---	**KU724440**	---	---	---	---	---	---	---	---
Pristimantis duellmani	AY326003	AY326003	---	---	---	---	---	EF493438	---	EF493500
Pristimantis eriphus	EU186671	EU186671	---	---	---	---	---	---	---	---
Pristimantis euphronides	EF493527	EF493527	---	---	---	---	---	EF493427	---	EF493489
Pristimantis fenestratus	FJ438809	EF493703	---	EU368884	---	---	---	---	---	---
Pristimantis gaigae	JN991512	JN991448	---	---	---	---	---	JQ025184	---	JN991570
Pristimantis galdi	EU186670	EU186670	---	---	---	---	---	EU186746	---	EU186767
Pristimantis gentryi	EF493511	EF493511	---	---	---	---	---	---	---	---

Taxon	12S	16S	COI	Cyt-b	CXCR4	NCX1	POMC	Rag-1	SLC8A3	Tyr
Pristimantis glandulosus	EF493676	EF493676	---	---	---	---	---	---	---	---
Pristimantis imitatrix	EF493824	EF493667	---	---	---	---	---	---	---	---
Pristimantis inguinalis	EU186676	EU186676	---	---	---	---	---	---	---	---
Pristimantis inusitatus	EF493677	EF493677	---	---	---	---	---	---	---	---
Pristimantis jaguensis	KP082860	KP082865	---	---	---	---	---	---	---	KP082878
Pristimantis jaguensis	KP082861	KP082869	---	---	---	---	---	---	---	---
Pristimantis koehleri	FJ438810	FJ438799	---	---	---	---	---	---	---	---
Pristimantis labiosus	EF493694	EF493694	---	---	---	---	---	---	---	---
Pristimantis lanthanites	EF493695	EF493695	---	---	---	---	---	---	---	---
Pristimantis latidiscus	EF493698	EF493698	---	---	---	---	---	---	---	---
Pristimantis leoni	EF493684	EF493684	---	---	---	---	---	EF493433	---	EF493495
Pristimantis librarius	JN991515	JN991451	JN991379	---	---	---	---	JQ025188	---	JN991571
Pristimantis lirellus	EF493521	EF493521	---	---	---	---	---	---	---	---
Pristimantis llojsintuta	---	EU712642	---	---	---	---	---	---	---	---
Pristimantis luteolateralis	EF493517	EF493517	---	---	---	---	---	---	---	---
Pristimantis lymani	EF493392	EF493392	---	---	---	---	---	---	---	---
Pristimantis malkini	EU186663	EU186663	---	---	---	---	---	---	---	---
Pristimantis marmoratus	EU186692	EU201063	---	---	---	---	---	---	---	---
Pristimantis martiae	JN991516	---	JN991380	---	---	---	---	JQ025190	---	---
Pristimantis melanogaster	EF493826	EF493664	---	---	---	---	---	---	---	---
Pristimantis mindo	---	KF801584	---	---	---	---	---	---	---	---
Pristimantis miyatai	JN991518	JN991452	JN991382	---	---	---	---	---	---	JN991573
Pristimantis moro	JN991519	JN991453	JN991383	---	---	---	---	JQ025192	---	JN991574
Pristimantis museosus	JN991521	JN991455	JN991385	---	---	---	---	JQ025193	---	JN991576
Pristimantis nervicus	JN991522	JN991456	JN991386	---	---	---	---	JQ025194	---	JN991577
Pristimantis nyctophylax	EF493526	EF493526	---	---	---	---	---	EF493425	---	EF493487
Pristimantis ockendeni	EF493519	EF493519	JN991387	EU130680	---	---	---	EF493434	---	EF493496
Pristimantis ocreatus	EF493682	EF493682	---	---	---	---	---	---	---	---
Pristimantis orcesi	EF493679	EF493679	---	---	---	---	---	---	---	---
Pristimantis orestes	EF493388	EF493388	---	---	---	---	---	---	---	---
Pristimantis pardalis	JN991526	---	JN991390	---	---	---	---	JQ025197	---	JN991579
Pristimantis parvillus	EF493352	EF493352	---	---	---	---	---	---	---	---
Pristimantis penelopus	JN991524	JN991459	JN991389	---	---	---	---	---	---	JN991578
Pristimantis peruvianus	EF493707	EF493707	JN991392	---	---	---	---	EF493436	---	EF493498
Pristimantis petrobardus	EF493825	EF493367	---	---	---	---	---	---	---	---
Pristimantis pharangobates	AY843586	AY843586	---	---	---	---	---	---	---	AY844035
Pristimantis phoxocephalus	EF493349	EF493349	---	---	---	---	---	---	---	---
Pristimantis pirrensis	JN991528	JN991462	JN991393	---	---	---	---	JQ025199	---	JN991580
Pristimantis platydactylus	FJ438811	EU192255	JN991394	EU368888	---	---	---	---	---	---
Pristimantis prolatus	EU186701	EU186701	---	---	---	---	---	---	---	---
Pristimantis ptochus	JN991530	---	JN991395	---	---	---	---	---	---	JN991581
Pristimantis pulvinatus	EU186741	EU186723	---	---	---	---	---	---	---	---
Pristimantis pycnodermis	EF493680	EF493680	---	---	---	---	---	---	---	---
Pristimantis pyrrhomerus	EF493683	EF493683	---	---	---	---	---	---	---	---
Pristimantis quaquaversus	JN991532	JN991463	JN991396	EU130578	---	---	---	JQ025201	---	JN991583
Pristimantis quinquagesimus	EF493690	EF493690	---	---	---	---	---	---	---	---
Pristimantis rhabdocnemus	EU186724	EU186706	---	---	---	---	---	---	---	---
Pristimantis rhabdolaemus	EF493706	EF493706	---	---	---	---	---	---	---	---
Pristimantis rhodoplichus	EF493674	EF493674	---	---	---	---	---	---	---	---
Pristimantis ridens	EF493355	EF493355	JN991398	---	---	---	---	---	---	---
Pristimantis riveti	EF493348	EF493348	---	---	---	---	---	---	---	---

Taxon	12S	16S	COI	Cyt-b	CXCR4	NCX1	POMC	Rag-1	SLC8A3	Tyr
Pristimantis rozei	EF493691	EF493691	---	---	---	---	---	EF493429	---	EF493491
Pristimantis sagittulus	EF493705	EF493705	---	---	---	---	---	EF493439	---	EF493501
Pristimantis samaipatae	FJ438814	FJ438803	---	EU368890	---	---	---	---	---	---
Pristimantis savagei	JN991536	JN991467	JN991401	---	---	---	---	JQ025205	---	JN991587
Pristimantis schultei	EF493681	EF493681	---	---	---	---	---	---	---	---
Pristimantis shrevei	EF493692	EF493692	---	---	---	---	---	---	---	---
Pristimantis simonbolivari	EF493671	EF493671	---	---	---	---	---	---	---	---
Pristimantis simonsii	EU186665	EU186665	---	---	---	---	AY819155	---	---	---
Pristimantis skydmainos	EF493393	EF493393	---	---	---	---	---	---	---	---
Pristimantis sp. ROM43978	EU186678	EU186678	---	---	---	---	---	---	---	---
Pristimantis spinosus	EF493673	EF493673	---	---	---	---	---	---	---	---
Pristimantis stictogaster	EF493704	EF493704	---	---	---	---	---	EF493445	---	EF493506
P. subsigillatus MECN10117	---	KF801580	---	---	---	---	---	---	---	---
Pristimantis subsigillatus	EF493525	EF493525	---	---	---	---	---	---	---	---
Pristimantis suetus	JN991537	JN991469	---	---	---	---	---	---	---	---
Pristimantis supernatis	AY326005	AY326005	---	---	---	---	---	---	---	---
Pristimantis surdus	EF493687	EF493687	---	---	---	---	---	---	---	---
Pristimantis taeniatus	JN991538	JN991470	JN991407	---	---	---	---	JQ025208	---	JN991588
Pristimantis terraebolivaris	EU186650	EU186650	---	---	---	---	---	---	---	---
Pristimantis thymalopsoides	EF493514	EF493514	---	---	---	---	---	---	---	---
Pristimantis thymelensis	AY326009	AY326009	---	---	---	---	---	EF493442	---	EF493503
Pristimantis toftae	EF493353	EF493353	---	---	---	---	---	---	---	---
Pristimantis truebae	EF493512	EF493512	---	---	---	---	---	---	---	---
Pristimantis unistrigatus	EF493387	EF493387	---	---	---	---	---	EF493444	---	EF493505
***P. urani* MHUAA7467**	---	**KU724441**	**KU724449**	---	---	---	---	---	---	---
***P. urani* MHUAA7471**	---	**KU724442**	---	---	---	---	---	---	---	---
***P. urani* MHUAA7472**	---	**KU724443**	**KU724450**	---	---	---	---	---	---	---
Pristimantis urichi	EF493699	EF493699	---	---	---	---	---	EF493426	---	EF493488
Pristimantis verecundus	EF493686	EF493686	---	---	---	---	---	---	---	---
Pristimantis versicolor	EF493389	EF493389	---	---	---	---	---	EF493431	---	EF493493
Pristimantis vertebralis	EF493689	EF493689	---	---	---	---	---	---	---	---
Pristimantis viejas	JN991546	JN991476	JN991409	---	---	---	---	JQ025212	---	JN991595
Pristimantis w-nigrum	AY326004	AY326004	---	---	---	---	DQ158260	DQ158344	---	---
Pristimantis walkeri	EF493518	EF493518	---	---	---	---	---	EF493428	---	EF493490
Pristimantis wiensi	EF493377	EF493668	---	---	---	---	---	---	---	---
Pristimantis zophus	JN991549	JN991479	JN991413	---	---	---	---	JQ025213	---	JN991598
Strabomantis anomalus	EF493534	EF493534	---	---	---	---	---	EF493447	---	---
Strabomantis biporcatus	EU186691	EU186691	---	---	GQ345188	GQ345236	GQ345265	EU186754	GQ345334	EU186775
Yunganastes ashkapara	FJ438807	FJ438796	---	---	---	---	---	JF809919	---	JF809898
Yunganastes bisignatus	FJ438808	EU192235	---	---	---	---	---	JF809918	---	JF809897
Yunganastes fraudator	---	FJ539065	---	---	---	---	---	JF809916	---	JF809895
Yunganastes mercedesae	FJ539071	FJ539066	---	---	---	---	---	JF809920	---	JF809899

Appendix 3. Partition scheme and evolution model for each partition obtained with PartitionFinder 1.1.1.

Partition	Model
12S	GTR+G+I
16S	GTR+G+I
COI pos1	TrNef+G+I
COI pos2	HKY+I
COI pos3	TrN+G
CytB	GTR+G+I
Nuclear	GTR+G+I



Appendix 3. Bayesian tree of *Pristimantis* depicting the phylogenetic position of *Pristimantis urani* sp. nov. Numbers on nodes represent posterior probabilities.