

# A new small golden frog of the genus *Pristimantis* (Anura: Craugastoridae) from an Andean cloud forest of Colombia

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**Abstract.** A new species of *Pristimantis* is described from an Andean cloud forest at 2650 m in the Cordillera Oriental of Colombia. *Pristimantis dorado* sp. nov. is similar to and could be closely related to *P. acutirostris*, but can be readily distinguished from this latter species by the absence of a tympanic annulus, vocal slits, vocal sac and reticulations on concealed surfaces, and by having a metallic gold iris with a brown horizontal streak. The phylogenetic position of the new species is recovered and we provide its advertisement call, which this species manages to emit despite lacking a vocal sac and vocal slits. This discovery reminds us that despite the extensive research on the alpha-taxonomy of *Pristimantis* in Colombia, fieldwork in high montane forests continues to yield previously unknown species.

**Keywords:** advertisement call, Amphibia, Brachycephaloidea, Eastern Cordillera, morphology, South America, Terrarana.

## Introduction

The genus *Pristimantis* is a principal component of anuran diversity in the Neotropics. Currently 465 species are recognized, 205 of which are found in Colombia, making it the country with the highest diversity of *Pristimantis* (AmphibiaWeb, 2015; Frost, 2015). Much of our knowledge of these species comes from the extensive taxonomic work exerted over the last four decades by Dr. John D. Lynch (e.g. Lynch, 1971; Lynch, 1980; Lynch and Rueda-Almonacid, 1998). Despite these efforts, the Andes of Colombia still continue to yield a plethora of new species, especially from montane localities. Thus, the anuran diversity of Colombia is still underestimated and many aspects of the biology of known species, such as

morphological variation, calls and reproduction are still poorly understood. During field expedition to cloud forest habitat in the buffer area of the Chingaza National Park in the Eastern Cordillera of Colombia, we discovered a small, distinctive species of *Pristimantis* calling from the bushes along a roadside. The aim of the present paper is to describe this new species and document its advertisement call.

## Materials and methods

### Morphology

Specimens were sacrificed in a solution of Lidocaine 2% and fixed in 10% formaldehyde solution. Adult specimens were transferred to and kept in 70% ethanol within five days of fixation. Prior to fixation, tissue samples from some specimens were collected and preserved in 96% ethanol. Terminology used to describe the morphological characters of the frogs follows Lynch and Duellman (1997) and Duellman and Lehr (2009). Abbreviations are SVL (snout-vent length), HL (head length), HW (head width), ED (eye diameter), END (eye-nostril distance), NSD (nostril to tip of snout distance), IND (inter-nostril distance), AMD (distance between the anterior margins of eyes), FAL (forearm length), FAB (forearm breadth); HAL (hand length), TFD (third finger disc diameter), THL (thigh length), TL (tibia length), TAL (tarsal length), FL (foot length) and FTD (fourth toe disc diameter). All measurements were taken using dial callipers accurate to the nearest 0.1 mm. Observations on the colour of the frogs in life were based on field notes and colour slides of specimens. Sex was determined by examination of secondary sexual characters (i.e., nuptial pads). Illustrations were made using a Zeiss stereomicroscope with a drawing tube attached.

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### Vocalization

Call recordings were made at the type locality on 26 February 2009 at 22:08 h (ANDES-A 1028, temperature not recorded), with an Astone mp3 player model Niva using the incorporated omnidirectional microphone. All recordings were made at a distance of approximately 0.6 m from the signaller. Calls were analysed using the software RAVEN version 1.3 (Charif et al., 2004) with a FFT of 512 points, at a sampling rate of 44.1 kHz and 16-bit precision. The following call variables were obtained as defined in Crocote and Ryan (1995): call duration, interval between calls, call type (tonal or pulsed), and peak (dominant) frequency. Morphological information on additional species was taken from preserved specimens (Appendix), photos in life, and from the literature. Generic and familial allocations follow Padial, Grant and Frost (2014). Institutional acronyms are MHUA (Museo de Herpetología Universidad de Antioquia, Medellín, Colombia); ICN (Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia); ANDES (Museo de Historia Natural ANDES, Bogotá, Colombia).

### Molecular analysis

In order to provide a molecular genetic evaluation of the distinctiveness and phylogenetic position of the new species, we sequenced DNA from two mitochondrial gene fragments, a roughly 540 base pair (bp) fragment of the 16S ribosomal RNA gene (16S) and 654 bp of the Folmer fragment of cytochrome oxidase I (COI-5'), commonly referred to as the animal Barcode of Life (Hebert et al., 2003), from two paratypes of the new species (see below) and from one specimen each of *P. lutitus* from Colombia and *P. cf. fenestratus* from Peru (see online supplementary table S1). From liver tissue samples of these four specimens we extracted genomic DNA following the standard instructions in a DNeasy Blood and Tissue Kit (QIAGEN). We PCR-amplified the COI marker using the primers dgHCO2198 (5'-TAA ACT TCA GGG TGA CCA AAR AAY CA-3') and dgLCO1490 (5'-GGT CAA CAA ATC ATA AAG AYA TYG G-3'; Meyer et al., 2005) and included 0.25  $\mu\text{g}/\mu\text{l}$  of bovine serum albumin in the reaction. The 16S fragment was amplified using 16SB-H (aka, 16Sbr-H) (5'-CCG GTC TGA ACT CAG ATC ACG T-3') and 16SA-L (aka, 16Sar-L) (5'-CGC CTG TTT ATC AAA AAC AT-3') (Kessing et al., 2004). All PCR contained 2.0 mM  $\text{Mg}^{2+}$  and utilized an annealing temperature of 49°C, with other reaction conditions standard (Kessing et al., 2004). PCR products were cleaned using Exonuclease I and Shrimp Alkaline Phosphatase enzymes (Werle et al., 1994) and Sanger-sequenced on an ABI 3500 automated sequencer (Life Technologies). Chromatograms were assembled and cleaned using Sequencher 5.0 (Gene Codes Corporation). Resulting DNA sequences were deposited in GenBank and chromatograms plus DNA sequences, collection data and photographs were posted at the Barcode of Life Data Systems (Ratnasingham and Hebert, 2007; table S1).

### Phylogenetic inference

To compare genetic data from the new species with published molecular data (supplementary table S1), we conducted BLAST searches (Altschul et al., 1990) in GenBank for sequences similar to the resulting COI and 16S gene fragments. We found more published 16S data than COI data for *Pristimantis*. Thus, we focused our phylogenetic inference exclusively on 16S and report COI genetic distances below. The first 100 BLAST hits to the 16S fragment obtained from the new species were visualized preliminarily using neighbour-joining (NJ) trees (Saitou and Nei, 1987). Redundant and genetically very distant sequences were removed, leaving us with 13 published DNA sequences similar to the new species, including the morphologically similar *P. sagittulus* from central Peru. To this combined data set of 13 published and four new DNA sequences, we added as an out-group a sample from the sister genus *Yunganastes* (Padial, Grant and Frost, 2014). For these 14 published 16S sequences only six also had COI sequence available (Pinto-Sánchez et al., 2012).

Alignment of COI was trivial as no length variation was inferred, whereas for 16S we aligned the sequences using the 'quick-set ML' default parameter options in SATÉ-II version 2.2.7, which iteratively optimizes both the tree and the alignment (Liu et al., 2012). We then fixed the resulting 16S alignment and conducted phylogenetic inference based on maximum parsimony (MP; Fitch, 1971) and based on maximum likelihood (ML; Felsenstein, 1981). Because inferred gaps clustered in the difficult-to-align loop regions of the 16S rRNA molecular, we conservatively removed all gapped sites prior to phylogenetic inference, resulting in a 16S data matrix of 474 total characters of which 40 were parsimony-informative and 127 were parsimony-informative.

Heuristic tree searching for MP inference was conducted using PAUP\* (Swofford, 2000) version 4.0a136 for Unix based on 5000 replicate searches each from random starting trees (not just random addition sequence) with MaxTrees set to 100 000 and the rearrangement limit on each tree set also to 100 000 to indirectly constrain the search time. To evaluate the potential completeness of the resulting search, the same search conditions were run five more times and no new trees were found after the first search, despite the use of random starting trees.

Prior to ML inference we used jModeltest2 version 2.1.6 (Darriba et al., 2012) to select the best-fit model of molecular evolution among seven possible substitution patterns each with or without a gamma rate heterogeneity parameter (Yang, 1993). Akaike, Bayesian and Decision Theory criteria agreed on recommending a general time-reversible model with rate heterogeneity, GTR +  $\Gamma$  (Tavaré, 1986). ML tree searches were performed using GARLI version 2.0.1019 for Unix (Zwickl, 2006) that uses a genetic algorithm to more efficiently search tree space and optimize parameter values. Here we employed default search parameter values and present the best tree obtained across ten independent ML searches.

Statistical support for clades was assessed by non-parametric bootstrapping (Felsenstein, 1985) under the MP and ML criteria. For MP bootstrapping in PAUP\*, we

used 2000 replicate samples (with replacement) of the data matrix, with 100 tree searches performed on each re-sampled character matrix starting from random trees and with other search parameters as above. For ML bootstrapping in GARLI we used 1000 re-sampling replicates with two ML searches per re-sampled character matrix but with the search parameter ‘number of generations without topology improvement required for termination’ lowered from the default of 20 000 to 5000. Results of ML bootstrap were summarized using version 3.3.1 of SumTrees (Phylogenetic Tree Split Support Summarization) for Unix (Sukumaran and Holder, 2010).

As a heuristic measure of divergence between the new species and other available samples, we calculated the raw, uncorrected genetic distance, or *p*-distance, between each of the eleven samples in the 16S phylogeny that also had data available on the COI gene fragment. While we do not support the concept of a widely applicable threshold of genetic distance separating within- versus between-species divergence levels, we find that mtDNA data may highlight lineages of interest that may warrant further integrative taxonomic investigations (Padial et al., 2010). We chose the *p*-distance measure because recent studies have shown that uncorrected distances perform as well as other distance measures in delimiting species (Collins et al., 2012; Srivathsan and Meier, 2012), and by definition this measure will provide a minimum estimate of divergence at our two mtDNA markers.

## Results

*Pristimantis dorado* sp. nov. (figs 1-3)

*Pristimantis* aff. *acutirostris*. Rivera-Correa (2012:37; fig. 2, bottom left)

*Holotype*. MHUA-A 7313, adult male. Colombia: Meta: Municipio San Juanito: Vereda San Luis El Plan: Alto Buenavista, km 9 road San Juanito – Chingaza (4.487642, –73.682584; 2650 m), collected on 16 April 2010 by Mauricio Rivera-Correa, Faride Lamadrid-Feris, Marco Rada and Santiago Castro-viejo-Fisher.

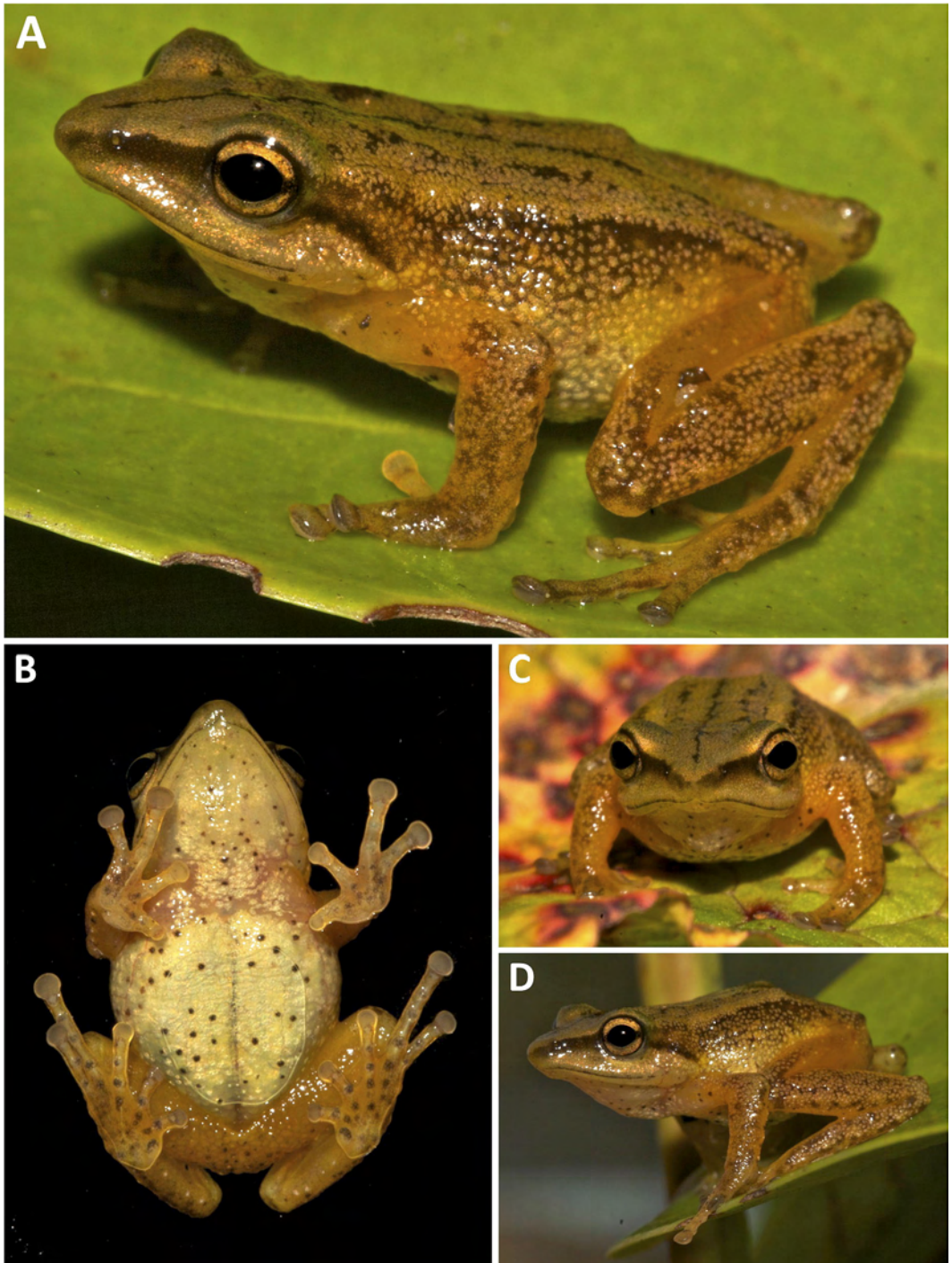
*Paratypes*. Six specimens. MHUA-A 7310, 7312, two adult males; MHUA-A 7311, subadult female, collected with holotype. MHUA-A 7308, ANDES-A 1028-29, three adult males. Colombia: Meta: Municipio San Juanito: Vereda San Luis El Plan: Alto Buenavista, km 9 road San Juanito – Chingaza (4.48947, –73.68650; 2650 m), collected on 26 February

2009 by Faride Lamadrid-Feris, Diego Gonzalez, Olga Nieto and Ivan Sánchez.

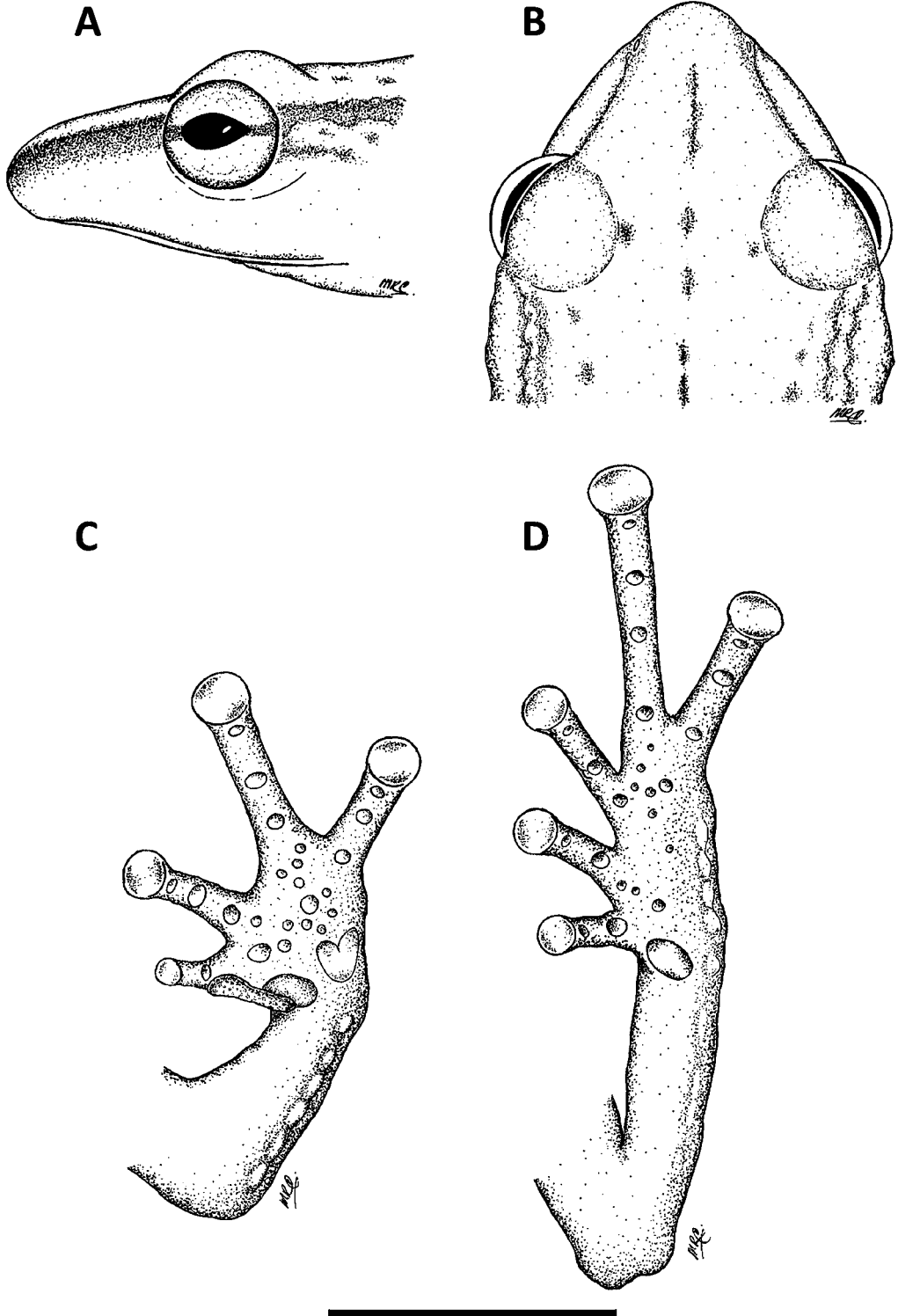
*Referred specimens*. MHUA-A 7314-15 (two juvenile), collected with the holotype. MHUA-A 7309 (adult female) collected with the paratypes.

*Generic allocation*. We designate this new species to the genus *Pristimantis* based on its morphological similarity to other species of *Pristimantis*, and corroborate this inference using mitochondrial DNA (mtDNA) sequence data (see below).

*Diagnosis*. This new species of *Pristimantis* is characterized and defined by the following combination of characters: (1) skin on dorsum is shagreen, ventral skin areolate; dorsolateral fold present, supratympanic fold barely evident; (2) tympanic membrane present but inconspicuous and tympanic annulus absent; (3) snout acuminate in dorsal view and rounded in profile, *canthus rostralis* distinct, loreal region slightly concave to flat; nostrils slightly protuberant, directed laterally; area between nostrils convex; (4) upper eyelid rugose and lacking an enlarged supraocular tubercle; cranial crests absent; (5) choanae small, rounded and partly concealed by palatal shelf of maxilla arch; denticulous process of vomers present; (6) vocal slits and vocal sac absent; males with extensive nuptial pads on thumbs almost reaching the proximal region of the disc of Finger I, without thorny structures; (7) Finger I shorter than Finger II; disks on Fingers III and IV expanded; (8) fingers and toes lacking lateral fringes; (9) small ulnar tubercles present; (10) inner and outer edges of tarsus with very small tubercles; heel bearing single small and subconical tubercle; (11) inner metatarsal tubercle ovoid and elevated; outer metatarsal tubercle small, subconical; a small and elongate inner tarsal; supernumerary plantar tubercles present; (12) rounded and projecting subarticular tubercles on toes, webbing absent, Toe III shorter than Toe V, toe disks smaller than finger disks; (13) dorsum golden with brown stripes and blotches; lip cream



**Figure 1.** *Pristimantis dorado* sp. nov. in life (MHUA-A 7311, paratype, female, SVL 19.2 mm). (A, D) lateral view; (B) ventral view; (C) frontal view. Photographs: S. Castroviejo-Fisher. This figure is published in colour in the online version.



**Figure 2.** *Pristimantis dorado* sp. nov. (MHUA-A 7313, holotype). (A) Head in lateral view; (B) head in dorsal view; (C) left hand in ventral view; (D) left foot in ventral view. Scale bar = 5 mm. Note the nuptial pad on Finger I. Drawings: MRC.



**Figure 3.** Dorsal and ventral view of the holotype of *Pristimantis dorado* sp. nov. (MHUA-A 7313, SVL 17.9 mm). This figure is published in colour in the online version.

with a brown line at lip margin; concealed surfaces of thigh immaculate light orange; gular translucent yellow, venter cream and dark brown spots present; iris metallic golden with a brown horizontal streak (14) SVL in adult males 14.2-17.9 mm ( $n = 6$ ) and one female 25.6 mm.

*Pristimantis acutirostris* (Lynch, 1984) is superficially similar to *P. dorado*, but differ in the states of the following characters: vocal slits, vocal sac and tympanic annulus present (absent in *P. dorado*); concealed surfaces yellow with brown reticulation (light orange without reticulations in *P. dorado*); iris pale blue with reddish horizontal streak (metallic golden with a brown horizontal streak in *P. dorado*). According to our phylogenetic evidence, the species with closer genetic affinity is *P. lutitus* (Lynch, 1984), but this latter species has tympanic annulus prominent, conical supraocular tubercle (absent in *P. dorado*), dorsum brown with darker markings (dorsum golden with brown stripes and blotches in *P. dorado*) and, limbs with bars broad (absent in *P. dorado*). *Pristimantis dorado* is also superficially similar to *P. sagittulus* (Lehr, Aguilar and Duellman, 2004), but differs in the states of the following characters: nuptial pads in males absent (nuptial pads present in *P. dorado*); fingers and toes with lateral fringes (lateral fringes absent in *P. dorado*); tympanic annulus present (absent in *P. dorado*);

and posterior surfaces of thighs brown with red spots and broad red longitudinal stripe (light orange without spots and stripe in *P. dorado*). *Pristimantis dorado* differs from *P. merostictus* (Lynch, 1984) because the males of latter has vocal slits and tympanic annulus present (absent in *P. dorado*), posterior surfaces of thighs black with small yellow spots (light orange with dark brown spots in *P. dorado*). In addition, *P. atratus* (Lynch, 1979) is similar in dorsal pattern to *P. dorado*, but the former has more abundant ridges, hidden areas with black spots, less acuminate snout, dentigerous process of vomers conspicuous and a conic heel tubercle more prominent.

**Description of the holotype.** A small frog, 17.9 mm SVL; head wider than body, head width 35% of SVL; head barely wider than long; head length 34% of SVL. *Canthus rostralis* sharp, essentially straight, loreal region slightly concave to flat; nostrils slightly protuberant, directed laterally; area between nostrils convex; eyes directed laterally; eye-to-nostril distance equal to eye diameter; lips not flared; upper eyelid rugose but lacking an enlarged supraocular tubercle; eyes extend beyond jaw in dorsal view; snout acuminate in dorsal view and rounded in profile; tympanic membrane present although depressed and inconspicuous, tympanic annulus absent; supratympanic fold

barely evident; postrictal tubercle indistinct, but post-tympanic fold evident; interorbital distance greater than width of upper eyelid; choanae small, rounded and partly concealed by palatal shelf of maxilla arch; dentigerous processes of vomers present but inconspicuous; tongue longer than wide, posterior one-half free from floor of mouth; vocal sac and vocal slits absent.

Skin of dorsum is finely shagreen; dorsolateral folds low; skin of throat smooth; ventral and lateral skin areolate; discoidal folds absent; small ulnar tubercles present, without forming a distinct fold; thenar tubercle oval, smaller than divided palmar tubercles; supernumerary palmar tubercles present; subarticular tubercles prominent, rounded, including the most distal tubercle; lateral fringes absent; Finger I shorter than Finger II; tips of digits round to subtruncated; inner digits of hand bearing much narrower discs than outer digits; all fingers bearing pads on digital tips; tip of Finger IV reaches beyond distal subarticular tubercle on Finger III; tip of Finger II barely reaches distal subarticular tubercle of Finger III; nuptial pads on thumbs almost reaching the proximal region of the disc of Finger I, without thorny structures.

Hind limbs moderate; tibia long, 47% of SVL; foot long, 44% of SVL; toe length formula I < II < III < V < IV; tip of Toe V reaches distal subarticular tubercle on Toe IV; tip of Toe III does not reach the distal subarticular tubercle on Toe IV; discs of toes slightly smaller to those on outer fingers, round to subtruncated; webbing absent; subarticular tubercles on toes distinct, projecting and ovoid; few supernumerary plantar tubercles at bases of toes present; inner metatarsal tubercle ovoid and elevated, almost half the length of Toe I; lateral fringes absent; outer metatarsal tubercle small, subconical; tarsus without fold, but small subconical tubercles along inner and outer border; a small subconical heel calcar; cloacal sheath and tubercles present, cloacal opening directed posteroventrally at level of thighs.

*Coloration in life.* Dorsum with many fine dark brown blotches forming middorsal, par-

avertebral and dorsolateral stripes (interrupted partially); canthal stripe dark brown, reaches the tip of the snout; post-ocular stripe dark brown; lip pale cream, unmarked except for thin brown line at lip margin; limbs, flanks and thighs cream with scattered brown blotches; axillae, undersides and posterior surfaces of thighs immaculate with light orange colouration, venter and throat white with darker brown spots; palmar and plantar side light orange; ventral side of limbs and thighs light orange with dark brown spots; iris metallic golden with a brown horizontal streak.

*Colouration in preservative.* Dorsum and flanks dirty brown to grey with numerous darker brown blotched, partially forming a middorsal and dorsolateral stripes; canthal and postocular stripes darker brown; arms and legs cream with dark brown spots and blotches, cream belly with dark brown spots.

*Measurements of holotype (in mm).* SVL 17.9; HL 6.1; HW 6.3; ED 2.0; END 2.1; NSD 1.2; IND 1.9; AMD 4.1; FAL 3.5; FAB 1.3; HAL 4.9; THL 7.2; TL 8.5; TAL 4.5; FL 7.8; TFD 1.0; FFD 1.0.

*Variation.* Morphometric variation in adult males is described in table 1. The only known adult female is noticeably larger than males [SVL 25.6 vs. 14.2-19.2 mm (mean = 17.0), respectively]. The forearm of males is swollen and nuptial pads are evident covering dorsal surface of Finger I. In the female, the dentigerous processes of the vomers are conspicuous, oblique, each bearing three teeth and separated by a distance approximately the size of the choanae. Female is more pigmented ventrally relative to males and its dorsum is darker than that of the males. Dorsal colour is reddish-gold in the female and golden in males.

*Distribution and natural history.* The new species is known only from the type locality, the buffer zone of Chingaza National Park and Farallones de Gachalá, Municipio de Medina, Departamento de Cundinamarca, Colombia

**Table 1.** Morphological variation (in mm) of the type series of *Pristimantis dorado* sp. nov. See text for abbreviations. Min = minimum value; Max = maximum value; Mean = arithmetic mean value; S.D. = standard deviation.

Measurements	Males (n = 6)			
	Min	Max	Mean	S.D.
SVL	14.2	19.2	17.0	1.5
HL	5.4	7.0	6.1	0.5
HW	5.7	7.1	6.3	0.5
ED	1.9	2.2	2.0	0.1
END	1.7	2.3	2.0	0.2
NSD	1.2	1.8	1.4	0.2
IND	1.0	2.2	1.8	0.4
AMD	3.4	4.5	4.1	0.4
FAL	3.0	4.6	3.6	0.6
FAB	1.2	1.5	1.3	0.1
HAL	4.4	5.5	5.0	0.4
THL	7.2	9.5	8.0	0.8
TL	8.0	9.9	8.7	0.6
TAL	4.3	5.1	4.6	0.3
FL	5.2	8.7	7.3	1.1
TFD	0.8	1.1	1.0	0.1
FFD	0.7	1.0	0.9	0.1

(fig. 4). Specimens were found in an Andean cloud forest between 19:00 h and 22:30 h during a light drizzle. *Pristimantis dorado* was found along the roadside in association with plants such as *Miconia summa*, *Weinmannia* spp. and *Hedyosmum bomplandianum* and near palms of the species *Ceroxylon quindiuense*. *Pristimantis dorado* is vocally active at night; all adult males captured were calling from vegetation at a height of 50 to 150 cm. We observed two or more males vocalizing from the same plant at different heights above the ground. By placing males together to be photographed, we observed that the frogs produced a typical advertisement call but increased the call rate and decreased the intercall interval, which may represent an agonistic behaviour. However, no orientation behaviour or physical contact was observed. *Pristimantis dorado* was found in sympatry with *P. frater*.

**Vocalization.** The advertisement call is characterized by a series of short, indistinctly pulsed clicks with notes not modulated in amplitude (fig. 5). The peak frequency ranged between 2930.0 and 3320.5 Hz; the mean value of the

maximum energy among calls was  $60.4 \text{ dB} \pm 3.51$ ,  $n = 22$  calls (fig. 5b). The mean note length was 0.011 s with an internote interval ranging between 0.0010 and 0.041 s (mean =  $0.026 \pm 0.03$  s;  $n = 20$  calls). The mean interval between calls was 1.223 s, and call duration ranged from 0.001 to 0.049 s.

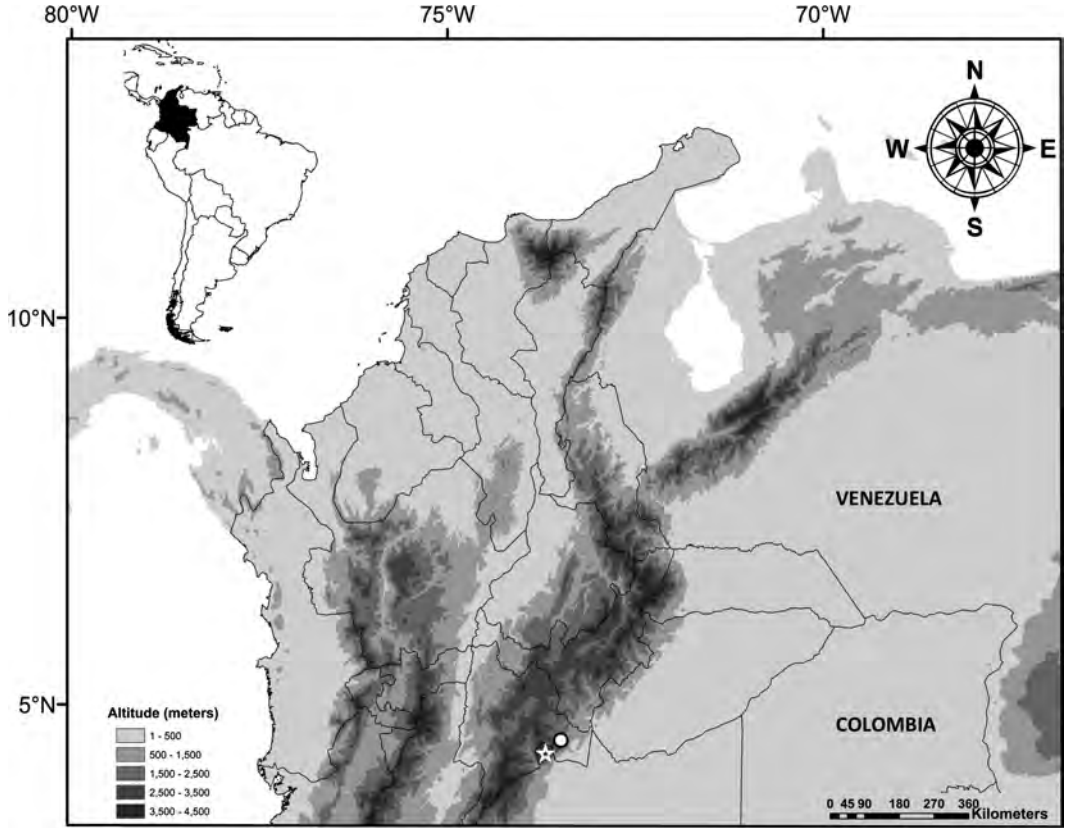
**Molecular phylogenetics and similarity.** MP tree searching found 28 shortest trees of 488 steps length. MP trees varied widely in topology among each other and in comparison to the ML tree (fig. 6). Bootstrap analyses under MP and ML criteria, however, supported roughly the same nodes and with quite similar bootstrap scores (fig. 6). The ML tree and all 28 MP trees placed *P. dorado* as sister to *P. lutitus* (reported here for the first time) + *P. cf. anolirex*. Because these latter two samples are so genetically similar, and the latter voucher has not been recovered, we hypothesize that both of these samples may, in fact, be *P. lutitus*. Little agreement existed among MP trees in the placement of the *P. dorado* + *P. lutitus* clade, nor was there any support in either MP or ML bootstrap analyses. The morphologically similar *P. sagittulus* is placed with high statistical support in a clade with another Peruvian and a Bolivian sample (fig. 6), and this clade was not sister to the *P. dorado* + *P. lutitus* clade in any of the 28 MP trees.

Based on the COI data set, the species most genetically similar to *P. dorado* was *P. lutitus*, which showed a  $p$ -distance of 17.4%, while at the 16S markers, the most similar species to *P. dorado* were *P. affinis* and *P. gaigei*, with genetic distances of 11.1% and 11.8%, respectively (table 2). These distances are far beyond any thresholds used previously to identify cryptic species (Crawford et al., 2013), adding further support for the distinctiveness of *P. dorado*, based on available DNA sequence data.

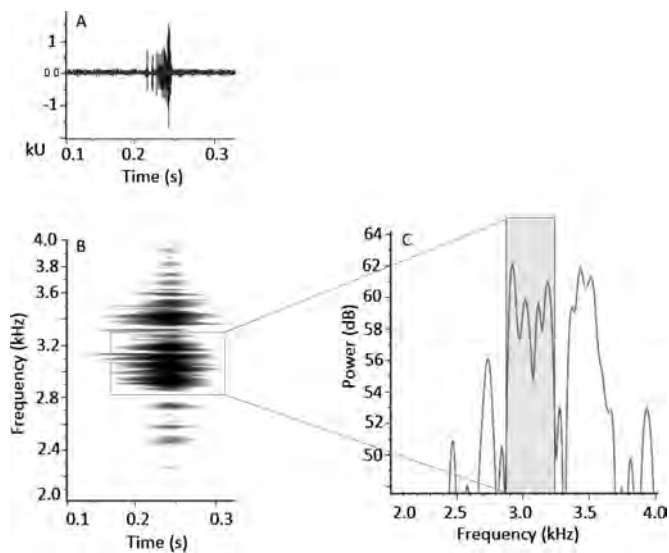
### Etymology

The specific name is an adjective in Spanish meaning “golden”, in allusion to the gold colour of the dorsum in this species. This name is also a

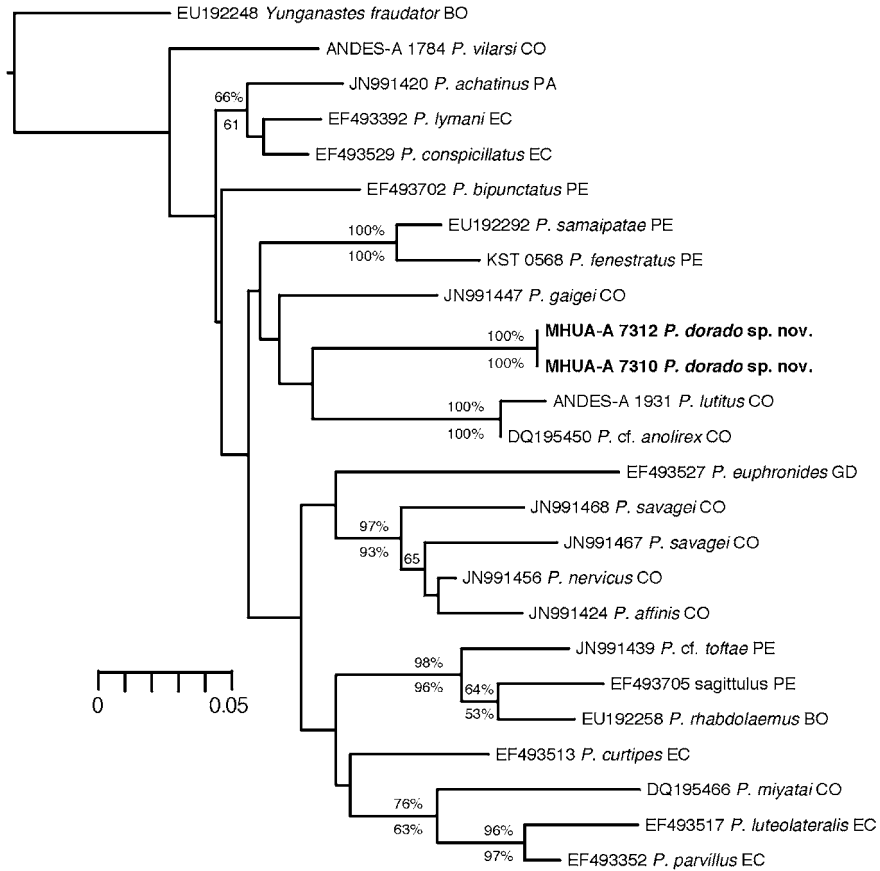




**Figure 4.** Map showing the known localities of *Pristimantis dorado* sp. nov.: Municipio de San Juanito, Departamento de Meta, type locality (white star), and Farallones de Gachalá, Municipio de Medina, Departamento de Cundinamarca (white circle).



**Figure 5.** Advertisement call of *Pristimantis dorado* sp. nov. (A) Oscillogram showing the amplitude and call duration; (B) spectrogram showing the frequency range of calls; (C) power spectrum showing the peak frequency of the call.



**Figure 6.** Maximum likelihood (ML) phylogenetic inference based on 474 aligned base pairs of the 16S gene, including 127 parsimony-informative sites, for a Ln-likelihood value of  $-2866.67$ . Numbers on branches represent support values for likelihood bootstrap percentages above the branch and maximum parsimony bootstrap percentages below the branch. Bootstrap values below 50% are not shown. Scale bar shows ML branch length estimated under a General Time Reversible model of substitution with four gamma-distributed rate categories described by an  $\alpha = 0.21305$  shape parameter.

tribute to “El Dorado”, a legendary but mythical city of gold sought by Spaniard *conquistadores* throughout South America.

## Discussion

In the Neotropics, the terraranan frogs of the genus *Pristimantis* are extraordinary diverse, particularly in the northern region of the Andean Mountains of South America (Frost, 2015). The heterogeneous Andean topography accompanied by the life histories of *Pristimantis* (such as direct development, high endemism and the ability to colonize a large variety of habitat types including high altitudes) could be partly

responsible for this notorious biodiversity. *Pristimantis* is characterized by remarkable morphological variability (Hedges, Duellman and Heinicke, 2008). For example, body sizes range from tiny species such as *P. andinognomus* with an adult male SVL of 10.0-14.5 mm (Lehr and Coloma, 2008) to relatively large species such as *P. labiosus*, with males reaching up to 50.8 mm (Lynch et al., 1994). *Pristimantis dorado* belongs to the group of small species (sensu Lynch and Duellman, 1997). In our type series, for example, one male (MHUA-A 7308) with SVL = 14.2 mm has well-developed nuptial pads, a secondary sexual character found exclusively in reproductively active individuals

**Table 2.** Matrix of pairwise distances between all samples for which mitochondrial DNA (mtDNA) sequences were available for both gene fragments, cytochrome oxidase I (COI, above the diagonal) and the 16S ribosomal RNA (16S, below the diagonal). Genetic distances are uncorrected (*p*-distances) to provide a minimal estimate of divergence among species. Further specimen data are provided in table S1. New species in bold.

	<i>P. achatinus</i> JN991349	<i>P. gaigei</i> JN991376	<i>P. lutinus</i> ANDES-A1931	<i>P. vilarsi</i> ANDES-A1784	<i>P. fenestratus</i> KST 0568	<i>P. dorado</i> MHUA-A7310	<i>P. dorado</i> MHUA-A7312	<i>P. affinis</i> JN991353	<i>P. nervicus</i> JN991386	<i>P. savagei</i> JN991401	<i>P. savagei</i> JN991402
<i>P. achatinus</i> JN991349	*	0.1781	0.2206	0.2173	0.2129	0.1781	0.1781	0.1957	0.2029	0.2337	0.2177
<i>P. gaigei</i> JN991376	0.0879	*	0.1863	0.2059	0.2145	0.1846	0.1846	0.2101	0.2065	0.1957	0.1974
<i>P. lutinus</i> ANDES-A1931	0.1289	0.1214	*	0.2080	0.2212	0.1743	0.1743	0.2138	0.2047	0.2120	0.2066
<i>P. vilarsi</i> ANDES-A1784	0.0859	0.0776	0.1429	*	0.2148	0.1820	0.1820	0.2192	0.2319	0.2210	0.2177
<i>P. fenestratus</i> KST 0568	0.1033	0.1031	0.1606	0.1285	*	0.2085	0.2085	0.2047	0.2120	0.2011	0.2454
<b><i>P. dorado</i> MHUA-A7310</b>	0.1398	0.1182	0.1448	0.1483	0.1580	*	0.0000	0.1975	0.1957	0.2101	0.1900
<b><i>P. dorado</i> MHUA-A7312</b>	0.1405	0.1188	0.1454	0.1490	0.1587	0.0000	*	0.1975	0.1957	0.2101	0.1900
<i>P. affinis</i> JN991353	0.1224	0.1022	0.1238	0.1102	0.1216	0.1111	0.1116	*	0.1239	0.1884	0.1679
<i>P. nervicus</i> JN991386	0.1127	0.1066	0.1167	0.1043	0.1070	0.1280	0.1286	0.0408	*	0.2004	0.1790
<i>P. savagei</i> JN991401	0.1163	0.1184	0.1235	0.1242	0.1070	0.1368	0.1374	0.0671	0.0509	*	0.2085
<i>P. savagei</i> JN991402	0.1145	0.1022	0.1310	0.1041	0.1031	0.1442	0.1449	0.0672	0.0571	0.0732	*

(Lynch, 1971; Luna et al., 2012), indicating that this small animal was a reproductively mature adult.

Nuptial excrescences are secondary sexual traits consisting of modified epidermal and dermal tissues typically located on the first finger (Noble, 1931), and occasionally on the remaining fingers (Lynch, 1971; Cisneros-Heredia and McDiarmid, 2007). Their function is to facilitate the male's grip on the female during amplexus (Lataste, 1876; Boulenger, 1897; Noble, 1931; Liu, 1936). Well-developed nuptial pads may be associated with breeding in water, with less developed structures being found in terrestrial breeders (Duellman and Trueb, 1986), presumably because females would be more difficult to clasp in water (Wells, 2007), although this has not been experimentally tested. In *Pristimantis*, a genus without aquatic breeding, nuptial pads are taxonomically widely distributed (Lynch and Duellman, 1997; Duellman and Lehr, 2009). Despite the lack of direct observations of the reproductive behaviour of *P. dorado* sp. nov., we assume that, as with the rest of the genus, these species are terrestrial breeders. Clearly, the presence of nuptial pads is not strictly related to breeding in water. The functional implications of variation in nuptial pad morphology are presently unknown for *Pristimantis*. More research is needed to understand the diversity and evolution of these characters.

A distinctive feature of *P. dorado* is the ability of males to call in the absence of both a vocal sac and vocal slits, morphological structures widely associated with the production of calls (Wells, 2007; Duellman and Lehr, 2009). Among Terrarana, several species of the *Craugastor gollmeri* species group lack these structures yet they may emit calls (Ibañez et al., 2012; Salazar-Zuñiga and García-Rodríguez, 2014), conditions not previously reported for *Pristimantis* (J.D. Lynch, pers. comm.). We suspect that in more species of *Pristimantis*, and perhaps of other genera, males without these structures may also be able to vocalize, but that this has been overlooked.

The discovery of a morphologically distinctive species not far from a major metropolis such as Bogotá emphasizes the fact that, despite recent advances in the alpha taxonomy of *Pristimantis* (or *Eleutherodactylus* sensu lato), our knowledge of the true diversity of this genus remains quite incomplete. The large genetic distances among species observed here based on the conservative *p*-distance measure of divergence among the COI and 16S genes (table 2) support the idea that many species of *Pristimantis* are missing from our tree. The unsatisfactory resolution especially of the basal relationships in our tree highlights the fact that much larger molecular datasets are needed in order to confidently designate new species to well-defined species groups within the genus or infer the biogeographic history of these lineages.

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**Appendix. Specimens examined**

*Pristimantis acutirostris*: COLOMBIA: Santander, Charalá, Virolín, 1780, ICN 12374 (holotype); Santander, Gámbita, ICN 11063; Santander, Charalá, ICN 11281.

*Pristimantis atratus*: ECUADOR: Morona Santiago, Rancho Suro, USNM 199675 (holotype).

*Pristimantis lutitus*: COLOMBIA: Santander, Charalá, Rio Luisito, ICN 5192 (holotype).

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## A new small golden frog of the genus *Pristimantis* (Anura: Craugastoridae) from an Andean cloud forest of Colombia

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### Supplementary material

**Supplementary Table S1.** Sample information for DNA sequences used in molecular phylogenetic analyses, including species of the genus *Pristimantis* (Anura: Craugastoridae) in alphabetical order (plus two outgroup samples in last two rows), field collector number, museum voucher number, locality information, GenBank accession numbers for mitochondrial 16S ribosomal RNA subunit (16S) and cytochrome oxidase subunit I (COI) gene fragments, and the publication where the DNA data were originally published, where applicable. Field collection acronyms include: AJC (Andrew J. Crawford), JDL (John D. Lynch), KST (Karen Siu-Ting), LAC (Luis Coloma), MNCN DNA (Museo Nacional de Ciencias Naturales, colección de material genético), MRC (Mauricio Rivera-Correa) nrps (Nelsy Rocío Pinto-Sánchez), SC (*serie de campo*), USNM (National Museum of Natural History, not typically used as field number). Museum acronyms include: ANDES-A (Museo de Historia Natural ANDES [Amphibian Collection], Bogotá, Colombia), BWMC (Bobby Witcher Memorial Collection, Avila University, Kansas City, USA), KU (Biodiversity Institute and Natural History Museum, Kansas University, Lawrence, USA), MHUA-A (Museo de Herpetología de la Universidad de Antioquia [Amphibian Collection], Medellín, Colombia), MNCN (Museo Nacional de Ciencias Naturales, Madrid, Spain), MNKA (Museo de Historia Natural Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia [Amphibian Collection]), MUSM (Universidad Nacional Mayor de San Marcos, Lima Peru), MVUP (Museo de Vertebrados de la Universidad de Panamá, Panama City, Panama), QCAZ (El Museo de Zoología de la Pontificia Universidad Católica del Ecuador, Quito). Countries abbreviated by their ISO 3166 two-letter codes.

Species	Collector number	Voucher number	Approximate locality	16S	COI	Reference
<i>P. achatinus</i>	AJC 0573	MVUP 1859	Caná, Darién, PA	JN991420	JN991349	Pinto-Sánchez et al. (2012)
<i>P. affinis</i>	nrps 0031	ANDES-A 0026	Parque Nacional Chingaza, Cundinamarca, CO	JN991424	JN991353	Pinto-Sánchez et al. (2012)
<i>P. anolirex</i>	JDL 26115		Santander, CO	DQ195450	N/A	Mahecha et al. (unpub.)
<i>P. bipunctatus</i>	USNM 171021	KU 291638	Oxapampa, Pasco, PE	EF493702	N/A	Heinicke, Duellman and Hedges (2007)
<i>P. conspicillatus</i>	SC 13732	QCAZ-A 28448	Monte Tour, Sucumbios, EC	EF493529	N/A	Heinicke, Duellman and Hedges (2007)
<i>P. curtipes</i>	LAC 584	KU 217871	Bosque de Pasochoa, Pichincha, EC	EF493513	N/A	Heinicke, Duellman and Hedges (2007)
<i>P. dorado</i>	MRC 636	MHUA-A 7310	Vereda San Luis El Plan, San Juanito, Meta, CO	KU496877	KU496873	this study
<i>P. dorado</i>	MRC 638	MHUA-A 7312	Vereda San Luis El Plan, San Juanito, Meta, CO	KU496875	KU496871	this study
<i>P. euphronides</i>	266624 (no letters)	BWMC 6918	Grand Etang, GD	EF493527	N/A	Heinicke, Duellman and Hedges (2007)
<i>P. fenestratus</i>	KST 0568	BSAPE373-12	Inkaterra Lodge, Madre de Dios, PE	KU496876	KU496872	This study
<i>P. gaipei</i>	AJC 1339	MHUA-A 4812	Maceo, Antioquia, CO	JN991447	JN991376	Pinto-Sánchez et al. (2012)
<i>P. luteolateralis</i>	JDL 9069	KU 177807	Tandapi, Pichincha, EC	EF493517	N/A	Heinicke, Duellman and Hedges (2007)
<i>P. lutitus</i>	AJC 3493	ANDES-A 1931 (BSECO362-11)	El Rasgón, Santander, CO	KU496878	KU496874	this study
<i>P. lymani</i>	LAC 1823	KU 218019	Loja-Zamora Road, Loja, EC	EF493392	N/A	Heinicke, Duellman and Hedges (2007)
<i>P. miyatai</i>	JDL 26118		Santander, CO	DQ195466	N/A	Mahecha et al. (unpub.)
<i>P. nervicus</i>	nrps 0048	ANDES-A 42	Parque Nacional Chingaza, Cundinamarca, CO	JN991456	JN991386	Pinto-Sánchez et al. (2012)
<i>P. parvillus</i>	JDL 9109	KU 177821	Tandapi, Pichincha, EC	EF493352	N/A	Heinicke, Duellman and Hedges (2007)
<i>P. rhabdolaemus</i>	MNCN DNA3940	MNKA 6628	Serrano de la Siberia, Santa Cruz, BO	EU192258	N/A	Padial et al. (2007)
<i>P. sagittulus</i>	USNM 171098	KU 291635	Oxapampa, Pasco, PE	EF493705	N/A	Heinicke, Duellman and Hedges (2007)
<i>P. samaipatae</i>	MNCN DNA3899	MNCN 42987	Angostura-Samaipata road, Santa Cruz, BO	EU192292	N/A	Padial and De la Riva (2009)
<i>P. savagei</i>	nrps 0085	ANDES-A 79	Restrepo, Meta, CO	JN991467	JN991401	Pinto-Sánchez et al. (2012)
<i>P. savagei</i>	nrps 0087	ANDES-A 81	Restrepo, Meta, CO	JN991468	JN991402	Pinto-Sánchez et al. (2012)
<i>P. cf. toftae</i>	KST 0208	MUSM 26791	Panguana, Huánuco, PE	JN991439	N/A	Pinto-Sánchez et al. (2012)
<i>P. vilarsi</i>	AJC 3944	ANDES-A 1784	San Juan de Arama, Meta, CO	KP149278	KP149083	Guarnizo et al. 2015
<i>Yunganastes fraudator</i>	MNCN DNA5984	MNCN 43107	Rd. to Villa Tunari, Cochabamba, BO	EU192248	N/A	Padial et al. (2007)