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# A new, critically endangered species of *Pristimantis* (Amphibia: Anura: Strabomantidae) from a mining area in the Cordillera Occidental of northern Peru (Región Cajamarca)

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**Abstract.** We describe a new species of *Pristimantis* from high Andean grasslands (jalca) at 3600 m above sea level in northern Peru (Región Cajamarca) based on morphological and molecular characters. The new species is known from four males and five females, which were found sheltering in the rosettes of *Puya fastuosa* (Bromeliaceae). The phylogenetic analysis of a fragment of the mitochondrial 16S rRNA gene suggests that the new species is a sister taxon of *Pristimantis simonsii*. The new species differs from its congeners by having a black dorsum speckled with white flecks and a dark brown groin with white spots. Furthermore, adult males have a snout–vent length of 23.6–27.2 mm (n = 4), and adult females of 25.6–32.8 mm (n = 5). Intensive mining activities apparently have extirpated the new species at its type locality and it is therefore considered critically endangered. We discuss the impact of mining on biodiversity and biological surveys in Peru.

Key words. Extirpation, gold mining, Hualgayoc, jalca, morphology, phylogeny, systematics, *Pristimantis astralos* new species, Peru.

**Resumen.** Describimos una nueva especie de *Pristimantis* de pastizales altoandinos (jalca) a 3600 m s.n.m. en el norte de Perú (Región Cajamarca) en base a caracteres morfológicos y moleculares. La nueva especie se conoce de cuatro machos y cinco hembras que encontramos escondidos en rosetas de *Puya fastuosa* (Bromeliaceae). El análisis filogenético de un fragmento del gen mitocondrial 16S rRNA sugiere que la nueva especie es el taxón hermano de *Pristimantis simonsii*. La nueva especie se distingue de sus congéneres por tener un dorso negro con manchas blancas rociadas y una ingle de color marrón oscuro con manchas blancas. Además, los machos adultos tienen una longitud hocico-cloaca de 23.6–27.2 mm (n = 4), y las hembras adultas de 25.6–32.8 mm (n = 5). Debido a las intensas actividades mineras, la nueva especie ha sido extirpada en su localidad tipo. Discutimos el impacto de la minería sobre la biodiversidad y los estudios biológicos en Perú.

## Introduction

Peru's complex oreographic structure provides a wide variety of microclimates and ecological zones along its altitudinal gradients, harbouring exceptionally rich biodiversity characterized by high endemism in both plants and animals, placing Peru amongst the top "megadiverse" countries in the world (McNeely et al. 1990, Mittermeier et al. 1997, Myers et al. 2000). Conservation International designated the Tropical Andes as one of the 25 global biodiversity "hotspots" because of their high species richness and endemism (Myers et al. 2000). The National Service of Natural Protected Areas (SERNANP) in Peru is aware of its responsibility and preserves 17.5% of its national territory with 76 national, 23 regional, and 136 private natural areas (SERNANP 2010). Among them are fifteen national parks, which are the areas with the highest protection status. However, fauna and flora outside of protected areas suffer from diverse anthropogenic influences such as agriculture, deforestation, pollution, and mining. Peru has some of the world's richest mineral deposits (Bury 2005), one of which is the Hualgayoc district (Regíon Cajamarca), ca. 80 km north of the city Cajamarca. The Spanish discovered the silver deposits of the Hualgayoc district in 1771 (O'Phelan Godoy 2004), and they have been mined since colonial times (Berquist Soule 2014). Today, other minerals such as zinc, lead, copper, and gold are extracted through conventional open-pit mining as well (Canchaya 1990). Mines are required to provide reports of biological surveys conducted by consultants (e.g., biologists) to facilitate evaluation of the environmental impacts of proposed mining operations by the Ministerio de Energia y Minas (MINEM) and Servicio Nacional Forestal y de Fauna Silvestre (SERFOR) (Gold Fields 2018, 2019).

A review of the collection of *Pristimantis* specimens at the Museo de Historia Natural Universidad Nacional Mayor de San Marcos in 2014 made the senior author discover unidentifiable specimens (Figs 1, 2) that had been collected during a biological survey in the Hualgayoc district. Herein, we describe these as a new species based on molecular and morphological data, discuss the threat status of the new species, and the impact of mining on the conservation of the new species.

## Materials and methods

### Morphology and voucher specimens

The format for the description follows Lynch & Duellman (1997), except that the term "dentigerous processes of vomers" is used instead of "vomerine odontophores" (Duellman et al. 2006), and diagnostic characters follow those of Duellman & Lehr (2009). Taxonomic classification follows Hedges et al. (2008) and Heinicke et al. (2009), except that we follow Pyron & Wiens (2011) for family placement, and Padial et al. (2014) for names of species groups in *Pristimantis*. Information on species for comparative diagnoses was obtained from Duellman & Lehr (2009) and from original species descriptions. Specimens were fixed in 10% formol and stored in 70% ethanol. Specimens were sexed externally by the presence or absence of vocal slits and internally by the condition of the gonads. Measurements, which were taken with digital callipers under a microscope by SL and rounded to the nearest 0.1 mm, are: snout–vent length (SVL), tibia length (TL, distance from the knee to the distal end of the tibia), foot length (FL, distance from the proximal margin of inner metatarsal tubercle to tip of Toe IV), head length (HL, from angle of jaw to tip of snout), head width (HW, at level of angle of jaw), horizontal eye diameter (ED), horizontal tympanum diameter (TY), interorbital distance (IOD), upper eyelid width (EW), internarial distance (IND), eye–nostril distance (E-N, straight line distance between anterior corner of orbit and posterior margin of external nares), and egg diameter. Fingers and toes are numbered preaxially to postaxially from I–IV and I–V, respectively. We compared the lengths of toes III and V by adpressing both toes against Toe IV; lengths of fingers I and II were compared by adpressing the fingers against each other. All drawings were made by SL using a stereomicroscope with a drawing tube attachment. Photographs of preserved specimens where taken by SL. Photographs of life specimens taken by Carlos Diaz were used for descriptions of skin texture and coloration in life.

Specimens examined are listed in Appendix I; collection acronyms are: AMNH = American Museum of Natural History, New York, USA; MCZ = Museum of Comparative Zoology, Cambridge, USA; MUSM = Museo de Historia Natural Universidad Nacional Mayor de San Marcos, Lima, Peru; NMP6V = National Museum Prague, Prague, Czech Republic. Threat status was assessed using the IUCN criteria (2016). The book "Composition of Scientific Words" by Brown (1956) was used to form the name for the new species.

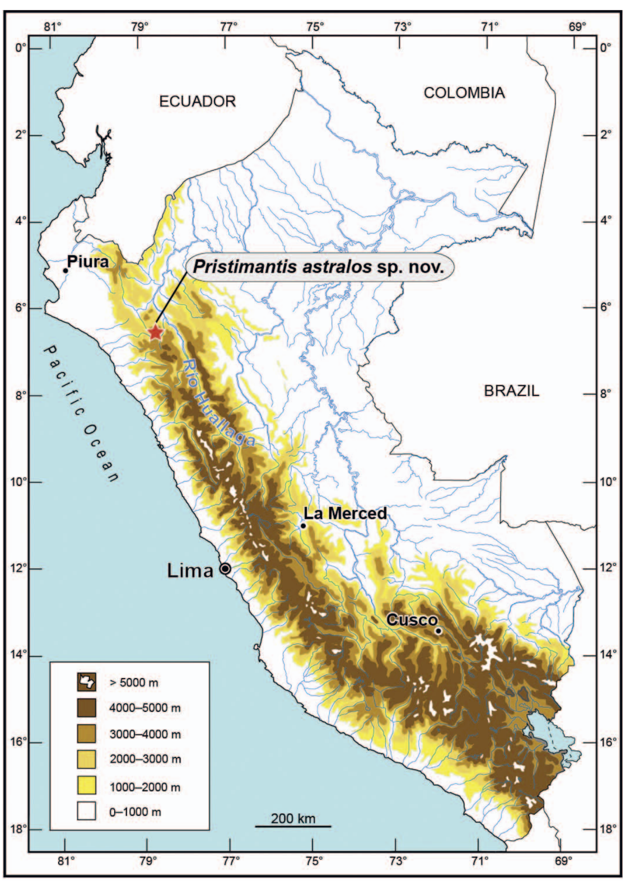


Figure 1. Map of Peru indicating the type locality (red star) of *Pristimantis astralos* sp. n. Map by E. Lehr.

### Molecular genetics

We used phylogenetic analyses to confirm the generic placement of the new species within *Pristimantis*. We used a fragment of the mitochondrial 16S rRNA fragment, because this gene is the most frequently sequenced gene for species of *Pristimantis* (Fouquet et al. 2007, Hedges et al. 2008, Vences et al. 2005). For our focal specimens, we used liver tissues from MUSM 32753 and 32755, and extracted DNA with a commercial extraction kit (IBI Scientific, Peosta, USA). We also obtained DNA sequences from morphologically similar, or putatively related, species (on the basis of similarity from BLAST search) of *Pristimantis* from GenBank (Appendix 2). We followed Hedges et al. (2008) for DNA amplification and sequencing, with the 16Sar (forward) and 16Sbr (reverse) primers. The thermocycling conditions during the polymerase chain reaction (PCR) using a Proflex thermal cycler (Applied Biosystems) were: one cycle at 96°C/3 min; 35 cycles at 95°C/30 s, 55°C/45 s, 72°C/1.5 min; and one cycle at 72°C/7 min. We purified PCR products with Exosap-IT (ThermoFisher), and shipped purified samples to MCLAB (South San Francisco, CA, USA) for sequencing.

We used Geneious, version 11.1.5 (Biomatters, http://www.geneious.com/) to align sequences with the MAFFT v7.017 alignment program (Katoh & Standley 2013), after manually removing hyper-variable regions, and trimmed sequences to a length of 503 bp. Our analysis included 30 terminals. We used MEGA v. 7 (Kumar et al. 2016) to determine the best evolutionary model. We employed a Maximum Likelihood (ML) approach to infer a molecular phylogeny using RAxML v. 8.2.11 (Stamatakis 2014) based on the GTR+G+I model. We assessed node support using 10,000 bootstrap replicates. We also estimated pairwise, uncorrected genetic distances (p-distances) for 16S rRNA between the new species and other species of *Pristimantis*.

## Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: urn:lsid:zoobank.org:pub:5D11192D-89D0-49F8-B9E7-C27C9E49BD7E. The electronic edition of this work was published in a journal with an ISSN, has been archived, and is available from the following digital repositories: www.salamandra-journal.com and www.zenodo.org.

## Results

According to our maximum likelihood phylogeny (Fig. 3), the new species is most closely related to *Pristimantis simonsii*. These two sister species are part of a polytomy that includes *P. rhodoplichus*, as well as a group of species consisting of *P. simonbolivari* and species related to *P. croceoinguinis* and *P. platydactylus*. However, there is little support for several of these nodes (Fig. 3), as could be expected given the known issues with the alignment of 16S fragments, reduced fragment length, and small sample size. Future analyses should expand the dataset to include other mitochondrial genes. Uncorrected genetic distances for 16S and BLAST searches confirm that the most similar species is *P. simonsii* (p-distance 8.2–8.6%,


Figure 2. Satellite imagery from GoogleEarth showing the Cerro Corona-Gold Fields Cima Mine (Regíon Cajamarca, Hualgayoc district) and the type locality (yellow star) of *Pristimantis astralos* sp. n. This satellite image was taken prior to the destruction of the type locality at the end of 2014. Figure by E. Lehr.

94.7–94.8% pairwise identity from BLAST search). Furthermore, genetic distances and BLAST searches returned only three additional specimens with values ~10%. These specimens are a paratype of *P. rhodoplichus*, KU219788 (p-distance 9.6%, 91.2% pairwise identity), a paratype of *P. simonbolivari*, KU218254 (p-distance 10.4%, 91.0% pairwise identity), and a member of the *P. platydactylus* Group (p-distance 10.4%, 90.6% pairwise identity; GenBank accession code EU712678). All other species of *Pristimantis* available on GenBank had uncorrected p-distances >10% compared to the focal specimens.

## ***Pristimantis astralos*** **sp. n.**
(Figs 4–8)

urn:lsid:zoobank.org:act:DF587647-FB37-47BA-84C7-30339181925F

Holotype: MUSM 32752, adult male (Figs 3–5), from the Cerro Corona mining concession (6°45'38.11" S, 78°38'53.71" W), 3600 m a.s.l., Comunidad Tingo, Distrito Hualgayoc, Provincia Bambamarca, Regíon Cajamarca, Peru, collected on 3 January 2014 by C. Diaz.

Paratypes: five adult females (MUSM 32753, 32755, 32758, 32759, 32760, Fig. 8), four adult males (MUSM 32752, 32754, 32756, 32757, Fig. 7), all collected at the type locality along with the holotype by C. Diaz.

Generic placement: We assign this species to *Pristimantis* based on our molecular data (Fig. 3) and its overall morphological similarity to other members of this genus.

Diagnosis: (1) Skin on dorsum tuberculate, skin on venter coarsely areolate; discoidal and thoracic folds present, dorsolateral folds present; (2) tympanic membrane and tympanic annulus present, distinct, visible externally; (3) snout broadly rounded to truncate in dorsal view, round in lateral view; (4) upper eyelid lacking conical tubercles; EW smaller than IOD; cranial crest absent; (5) dentigerous processes of vomers oblique; (6) males with vocal slits, subgular vocal sac, and with nuptial pads; (7) Finger I shorter than Finger II; discs of digits broadly expanded, round, bearing circumferential grooves; (8) fingers with lateral fringes; (9) ulnar and tarsal tubercles present; (10) heel lacking conical tubercles; inner tarsal fold present; (11) inner metatarsal tubercle ovoid, 2–3 times larger than outer one, outer metatarsal tubercle small, ovoid; numerous supernumer-

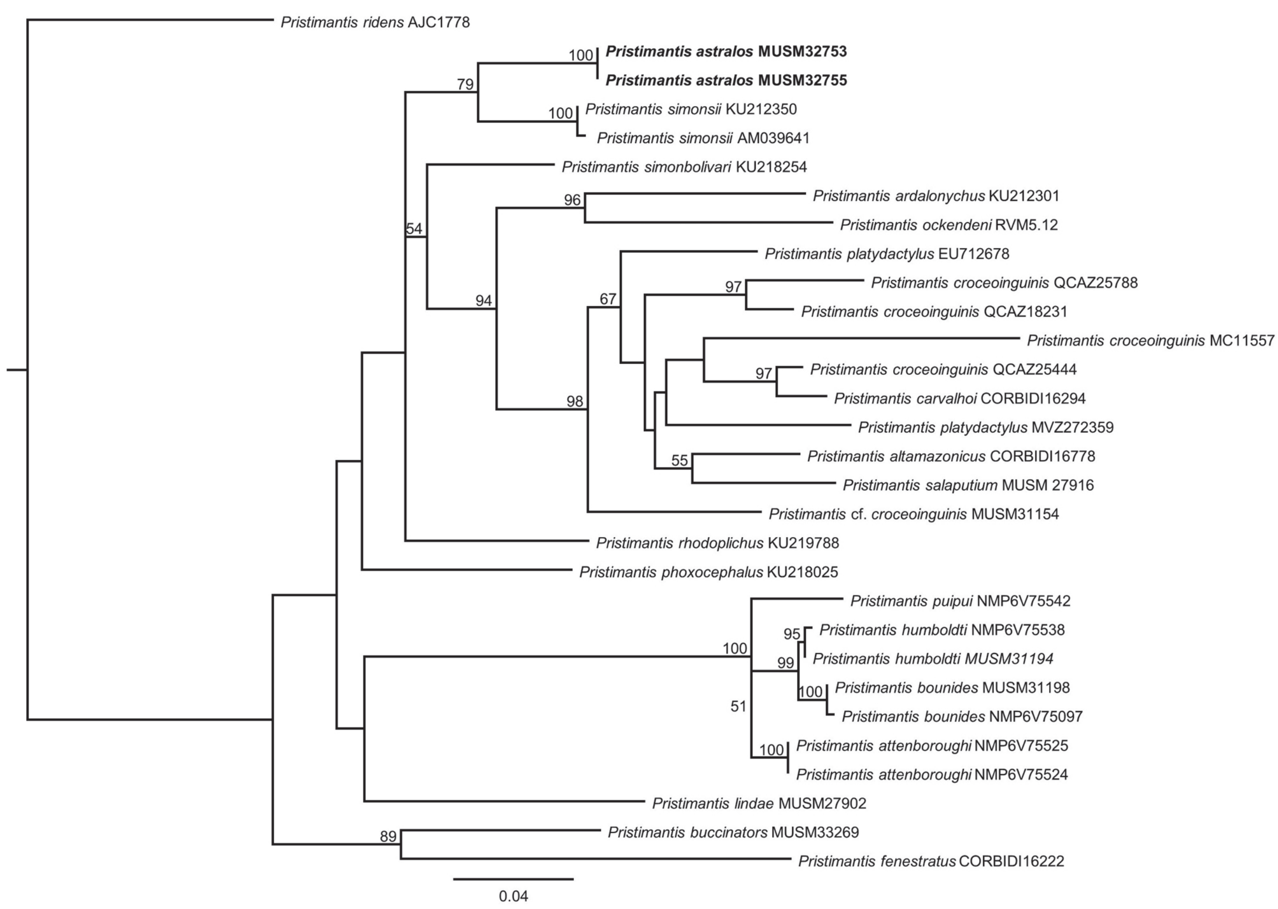


Figure 3. Maximum Likelihood (ML) phylogeny (best tree) based on a sequence of 503 bp of 16S ribosomal RNA inferred by using the GTR + G + I model of nucleotide substitution in RAxML. ML bootstrap values > 50% are indicated at each node.

ary tubercles; (12) toes with lateral fringes; basal toe webbing present; Toe V longer than Toe III; toe discs about as large as those on fingers, bearing circumferential grooves; (13) in life, dorsum ranges from black to dark brown with or without white flecks; anterior and posterior face of thighs black to dark brown with white spots; flanks black to dark tan, with or without white or cream flecks; groin black to dark brown with or without white spots; venter dark to pale grey with black dots; iris dark copper-colored with fine black vermiculations; (14) SVL in adult females 25.6–32.8 mm (n = 5), in adult males 23.6–27.2 mm (n = 4).

Comparisons: *Pristimantis astralos* is distinguished from its congeners in Peru (139 species, AmphibiaWeb 2020) by the following combination of characters: skin on dorsum tuberculate, tympanum and tympanic annulus distinct, weakly defined dorsolateral folds, uniform black to dark brown dorsal ground coloration with or without irregular white flecks, groin black to dark brown with white spots, and anterior and posterior faces of thighs dark brown with white spots. *Pristimantis astralos* can be distinguished from other Andean species of Peruvian *Pristimantis* (*P. attenboroughi, P. ardalonychus, P. atrabracus, P. coronatus,* and *P. vilcabambae*) that have a dark dorsum as follows (characters of *P. astralos* in parenthesis):

*Pristimantis attenboroughi* Lehr & May, 2017 from altitudes of 3400–3936 m a.s.l. in central Peru (Regíon Junín) shares with *P. astralos* the presence of dorsolateral folds, and the usually dark grey dorsal coloration. However, *P. attenboroughi* lacks a visible tympanic membrane (present), a visible tympanic annulus (present), circumferential grooves (present), vocal slits (present), and nuptial pads (present). *Pristimantis ardalonychus* (Duellman & Pramuk, 1999) from altitudes of 680–2550 m a.s.l. in northern Peru (Regíon San Martín) shares with *P. astralos* a dark dorsal coloration, a tympanic membrane, tympanic annulus, a reticulated pattern on venter and lateral fringes. However, *P. ardalonychus* has the dorsum smooth with scattered low tubercles (tuberculate), and smaller females with SVL 19.7 and 21.9 mm (23.6–27.2 mm, n = 4; Duellman & Pramuk 1999). *Pristimantis atrabracus* (Duellman & Pramuk, 1999) from altitudes of 2963–3330 m a.s.l. in northern Peru (Regíon Amazonas) and *P. astralos* both have a tympanic membrane, tympanic annulus, vocal slits, lateral fringes on fingers and toes, and a reticulated pattern on their venters. However, *P. atrabracus* has the skin on dorsum shagreen (tuberculate), dentigerous processes of vomers absent (present), and no nuptial pads (present). *Pristimantis coronatus* Lehr & Duellman, 2007 from 2850 m a.s.l. in northern Peru (Regíon Piura) shares with *P. astralos* a blackish brown dorsum with white flecks. However, *P. coronatus* has the groin marked with orange-red blotches (white), lacks a tympanic membrane (present), lacks a tympanic annulus (present), and lacks dorsolateral folds (present). *Pristimantis vilcabambae* Lehr, 2007 from 2050 m a.s.l in southern Peru (Regíon Cusco) shares with *P. astralos* a dark dorsal coloration and white spots on the groin. However, *P. vilcabambae* lacks dorsolateral folds (present), a tympanic membrane (present), tympanic annulus (present), vocal slits (present), and nuptial pads (present).

*Pristimantis chimu* Lehr, 2007 from 3000–3100 m a.s.l. of the Pacific versants of northern Peru (Regíon Cajamarca) occurs syntopically with *P. astralos*. *Pristimantis chimu* shares with *P. astralos* a tympanic membrane and annulus, rounded snout, dentigerous processes of vomers, and lateral fringes on toes and fingers. However, *P. chimu* lacks nuptial pads (present), vocal slits (present), and has a white groin with brown blotches (black with white dots). Furthermore, *P. chimu* has smaller males (SVL 19.4–20.5 mm [n = 2] vs. SVL 23.6–27.2 mm [n = 4] in *P. astralos*) and smaller females (SVL 22.6–25.7 mm [n = 16] vs. SVL 25.6–32.8 mm [n = 5] in *P. astralos*; Lehr 2007).

*Pristimantis pinguis* (Duellman & Pramuk, 1999) from altitudes of 3000–3916 m a.s.l. (Duellman & Pramuk 1999, Duellman & Lehr 2009) occurs syntopically with *P. astralos*. *Pristimantis pinguis* has smaller females (SVL 24.6–29.8 mm, n = 10 vs. 25.6–32.8 mm, n = 5, in *P. astralos*), lacks dorsolateral folds (present), and has the venter dull yellow with greyish brown reticulations (dark to pale grey with black dots).

*Pristimantis mariaelenae* Venegas & Duellman, 2012 from 3569 m a.s.l. in the humid puna (Regíon Lambayeque) in northeastern Peru shares with *P. astralos* a visible tympanic membrane and tympanic annulus, rounded snout, dentigerous processes of vomers, vocal slits, nuptial pads, and white blotches in the groin area. However, *P. mariaelenae* lacks lateral fringes on fingers and toes (present), and its dorsal coloration is reddish brown (black to dark brown). Furthermore, *P. mariaelenae* has smaller males (SVL 16.0–19.4 mm [n = 2] vs. SVL 23.6–27.2 mm [n = 4] in *P. astralos*) and smaller females (SVL 23.7–27.8 mm [n = 3] vs SVL 25.6–32.8 mm [n = 5] in *P. astralos*; Venegas & Duellman 2012). *Pristimantis stipa* Venegas & Duellman, 2012 from 3569 m a.s.l. in the humid puna (Regíon Lambayeque) in northeastern Peru shares with *P. astralos* a similar SVL, dorsolateral folds, and a visible tympanic membrane and tympanic annulus. However, *P. stipa* has digits without circumferential grooves (present), and ulnar tubercles that are coalesced into a low fold (absent; Venegas & Duellman 2012).

Genetically, *P. astralos* is most closely related (8.2–8.6% 16S genetic distance, Fig. 3) to *P. simonsii* (Boulenger, 1900). *Pristimantis simonsii* inhabits the humid puna at altitudes of 3050–3760 m a.s.l. in northern Peru (Regíon Cajamarca) (Duellman & Lehr 2009) and occurs syntopically with *P. astralos*. Both species are of similar size (male SVL 19.3–25.9 mm in *P. simonsii* vs. 23.6–27.2 mm [n = 4] in *P. astralos*; female SVL 26.2–33.3 mm vs. 25.6–32.8 mm [n = 5] in *P. astralos*; Duellman & Lehr 2009), share the presence of dorsolateral folds, and nuptial pads. However, *P. simonsii* lacks a tympanic membrane and a tympanic annulus (both present), has males that lack vocal slits (present), lacks fingers with lateral fringes (present), lacks circumferential groves (present), lacks dentigerous processes of vomers (present), and has in life the dorsum dull brown to pinkish tan with dark brown spots (black to dark brown with irregular scattered white flecks).

Description of the holotype: Head narrower than the body, slightly longer than wide; head width 34.9% of SVL; head length 38.6% of SVL; cranial crest absent; snout bluntly rounded in dorsal and lateral views (Figs 5A, B), eye–nostril distance 76.7% of eye diameter; nostrils protuberant, directed dorsolaterally; canthus rostralis straight in dorsal view, rounded in profile; loreal region slightly concave; lips rounded; upper eyelid without enlarged tubercles; upper eyelid width 70.0% of IOD; supratympanic fold distinct, narrow, extending diagonally from posterior margin of upper eyelid towards insertion of arm, covering upper margin of the tympanum (Fig. 5A); tympanic membrane and tympanic annulus distinct, externally visible, tympanum 46.7% of ED; two conical postrictal tubercles present bilaterally (Fig. 5A). Choanae small, teardrop-shaped; dentigerous processes of vomers oblique and small; tongue width is half of the tongue length, notched posteriorly, posterior third free; vocal slits slightly curved, located in posterior half of mouth floor between tongue and margin of jaw; subgular vocal sac distinct (Fig. 5C).

Skin on dorsum tuberculate, dorsolateral fold weakly defined and in weak contrast to the tuberculate skin texture (Fig. 5A); skin on flanks tuberculate with tubercles coalescing into short ridges; skin on throat and chest shagreen, belly coarsely areolate; discoidal and thoracic weakly defined (Fig. 5C); cloacal sheath short.

Outer surface of ulnar with minute tubercles; outer palmar tubercle bifid (Fig. 6A); distinct supernumerary tubercles, ovoid, approximately half the size of subarticular tubercles; subarticular tubercles well defined, most prominent on fingers, round in ventral view, conical in lateral view; fingers with narrow lateral fringes; Finger I shorter than Finger II (Fig. 6A); discs on digits of fingers broadly expanded, rounded, bearing circumferential grooves.

Hind limbs short, slender, tibia length 36.8% of SVL; foot length 35.3% of SVL; upper surface of hind limbs tuberculate with few scattered tubercles; inner surface of thighs smooth, posterior and ventral surfaces of the thighs areolate; heels without conical tubercles; outer surface of tarsus with minute tubercles; inner tarsal fold short (Fig. 6B); inner metatarsal tubercle ovoid, two times the size of ovoid outer metatarsal tubercle; subarticular tubercles well defined, round in ventral view, conical in lateral view; plantar supernumerary tubercles distinct, about one-fourth the size of subarticular tubercles; toes with narrow lateral fringes, fringe of Toe V undulated; basal webbing present, most prominent between toes IV and V; discs expanded, slightly truncated, approximately the same size as discs on fingers, bearing circumferential grooves; relative length of toes: 1<2<3<5<4 (Fig. 6B).

Measurements (in mm) of the holotype: SVL 27.2; TL 10.0; FL 9.6; HL 10.5; HW 9.5; ED 3.0; TY 1.4; IOD 3.0; EW 2.1; IND 2.0; E-N 2.3.

Coloration in preservative (Fig. 4): The dorsal ground coloration is dark charcoal with irregular creamy white

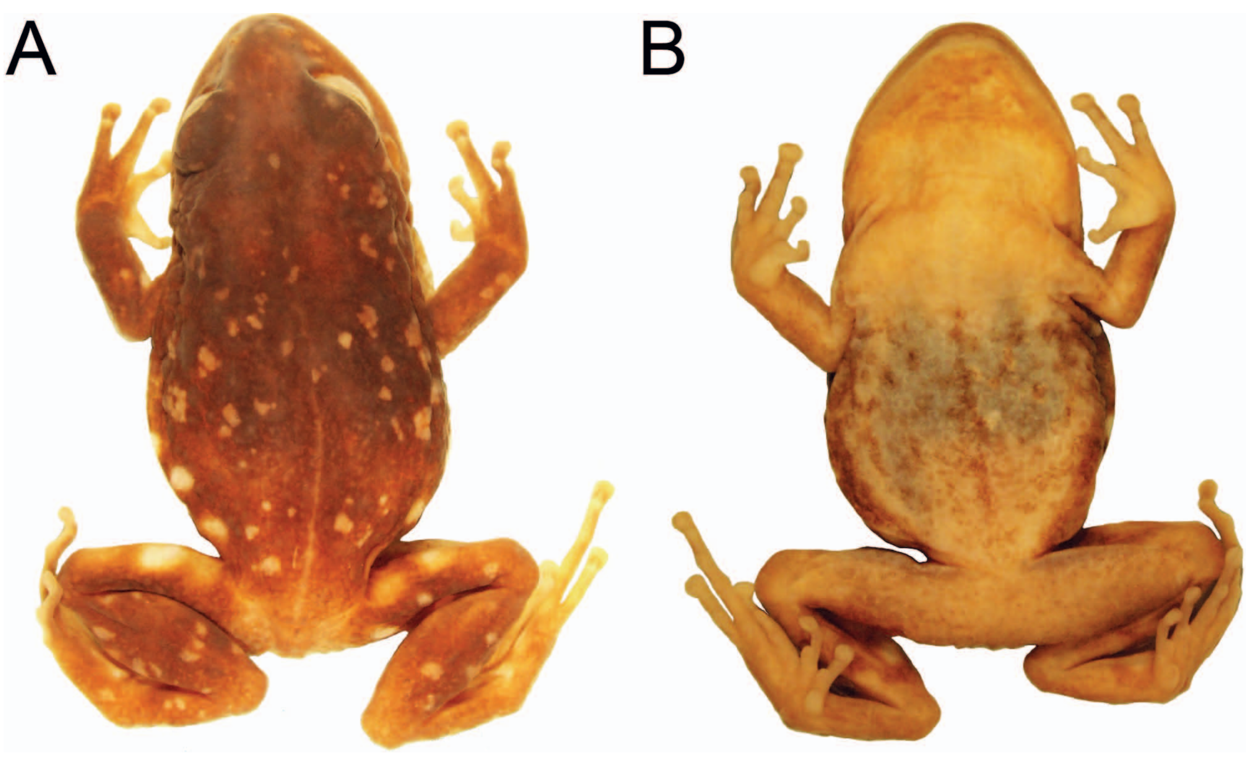


Figure 4. Holotype of *Pristimantis astralos* sp. n. (MUSM 32752) in alcohol in dorsal (A) and ventral (B) views. SVL = 27.2 mm. Photos by S. Lyu.

flecks; flanks charcoal with relatively larger, irregular, cream, rounded dorsolateral white flecks; groin and anterior faces of thighs brown with a cream tint; chest and belly cream and dark brown, with reticulations forming an irregular dark brown stripe along the midline of venter; ventral faces of thighs cream, throat pale cream and mottled with pale brown; palmar and plantar faces, and fingers and toes cream; iris pale bluish grey.

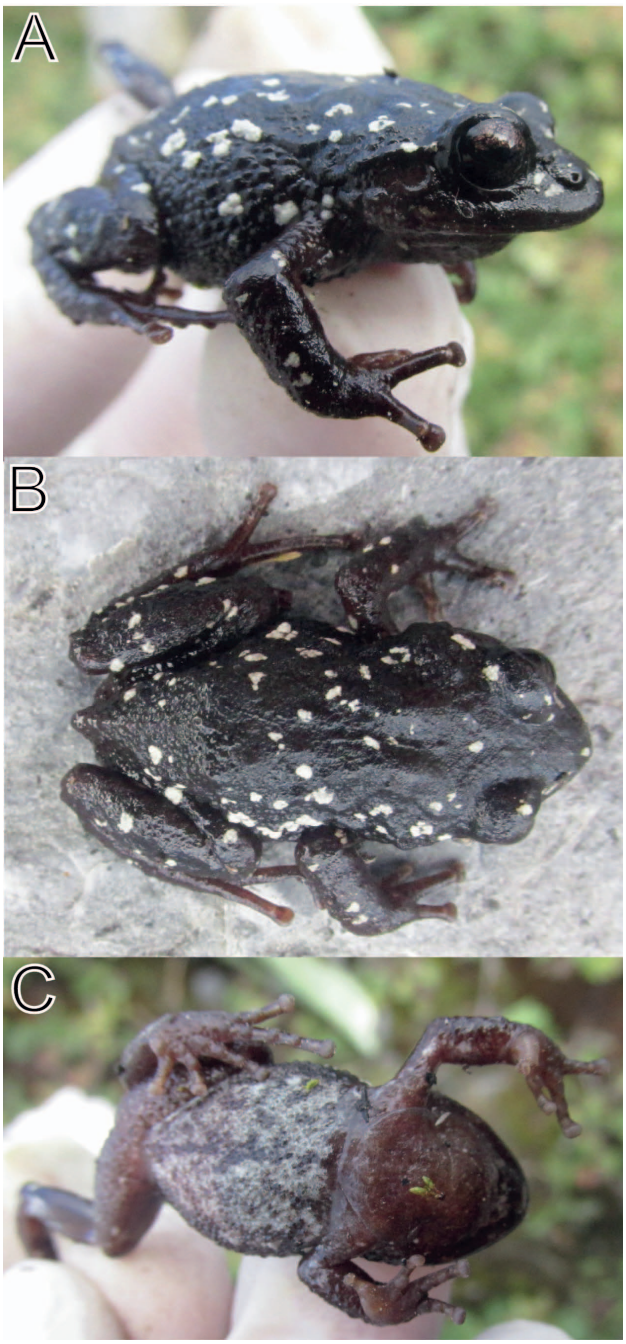


Figure 5. Holotype of *Pristimantis astralos* sp. n. (MUSM 32752) in life, in lateral (A), dorsal, (B), and ventral (C) views. Photos by C. Diaz.

Coloration in life (Fig. 5): The dorsal ground coloration is black with white flecks and spots; flanks black, with relatively large, irregular, rounded white spots; groin and anterior faces of thighs dark charcoal with white spots; chest and belly pale grey and dark brown, with an irregularly reticulated dark brown stripe along the midline of venter; posterior faces of thighs black with white spots; throat greyish purple with a pale grey tint; palmar and plantar faces, and fingers and toes brown; iris dark copper with fine black vermiculations.

Variation: All paratypes (Figs 7, 8) are similar to the holotype regarding morphology and proportions. Besides differences in size, morphological variation is noticeable in the dorsolateral folds, which may be prominent (MUSM 32755, 32760, Figs 8G, H) or weakly defined (MUSM 32753, 32754, Figs 7A, B; MUSM 32756, Figs 7D, E; MUSM 32757, 32758, Figs 8A, B; MUSM 32759, Figs 8D, E). Skin texture on the flanks is also variable, with tubercles either fused into prominent, continuous ridges (MUSM 32760, Fig. 8G) or weakly defined ridges (MUSM 32753, 32754, Figs 7A, B; MUSM 32755, 32756, Figs 7D, E; MUSM 32757, 32758, Figs 8A, B; MUSM 32759, Figs 8D, E). Most specimens have bilaterally two distinct conical postrictal tubercles, whereas some specimens have the postrictal tubercles fused into short ridges (MUSM 32758, Fig. 8B), being elongated (MUSM 32756, Fig. 7E), or weakly defined (MUSM 32755). The dorsal coloration in life ranges from black (MUSM 32754, Fig. 7B; MUSM 32755, 32756, Fig. 7D; MUSM 32758,

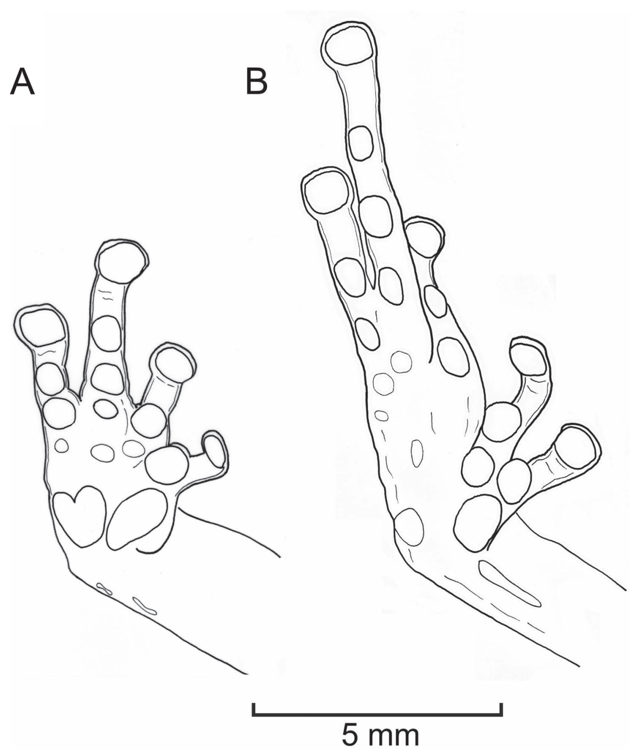


Figure 6. Ventral views of hand (A) and foot (B) of the holotype of *Pristimantis astralos* sp. n. (MUSM 8796). Drawings by S. Lyu.

Table 1. Morphometrics (in mm) of the individual type specimens of *Pristimantis astralos* sp. n.

| Characters | MUSM 32755 | MUSM 32759 | MUSM 32753 | MUSM 32760 | MUSM 32758 | MUSM 32752 | MUSM 32757 | MUSM 32754 | MUSM 2756 |
|---|---|---|---|---|---|---|---|---|---|
| Sex | F | F | F | F | F | M | M | M | M |
| SVL | 32.8 | 31.5 | 31.0 | 27.8 | 25.6 | 27.2 | 27.0 | 24.9 | 23.6 |
| TL | 12.1 | 11.5 | 12.3 | 12.0 | 11.0 | 10.0 | 10.0 | 9.3 | 8.5 |
| FL | 12.2 | 12.7 | 11.9 | 12.2 | 11.5 | 9.6 | 11.0 | 9.6 | 9.4 |
| HL | 11.6 | 10.6 | 12.0 | 9.1 | 8.8 | 10.5 | 9.9 | 8.8 | 8.8 |
| HW | 12.6 | 11.2 | 12.0 | 9.8 | 8.2 | 9.5 | 10.3 | 9.4 | 8.9 |
| ED | 3.1 | 3.2 | 3.2 | 3.2 | 3.2 | 3.0 | 3.2 | 3.1 | 2.9 |
| TY | 1.8 | 1.8 | 1.6 | 1.8 | 1.6 | 1.4 | 1.6 | 1.4 | 1.3 |
| IOD | 3.3 | 3.5 | 3.9 | 3.5 | 3.2 | 3.0 | 3.2 | 2.9 | 3.0 |
| EW | 2.7 | 2.5 | 2.1 | 2.3 | 2.2 | 2.1 | 2.3 | 2.0 | 2.3 |
| IND | 2.5 | 2.2 | 2.3 | 3.7 | 2.0 | 2.0 | 2.3 | 1.9 | 2.5 |
| E–N | 3.2 | 2.7 | 3.2 | 3.7 | 2.4 | 2.3 | 2.1 | 2.3 | 2.0 |

Table 2. Morphometrics (in mm) and proportions of the type series of *Pristimantis astralos* sp. n.; ranges followed by means and one standard deviation in parentheses.

| Characters | Males (n = 4) | Females (n = 5) |
|---|---|---|
| SVL | 23.6–27.2 (25.7 ± 1.5) | 25.6–32.6 (29.7 ± 2.6) |
| TL | 8.5–10.0 (9.5 ± 0.6) | 11.0–12.3 (11.8 ± 0.5) |
| FL | 9.4–11.0 (9.9 ± 0.6) | 11.5–12.7 (12.1 ± 0.4) |
| HL | 8.8–10.5 (9.5 ± 0.7) | 8.8–12.0 (10.4 ± 1.3) |
| HW | 8.9–10.3 (9.5 ± 0.5) | 8.2–12.6 (10.8 ± 1.6) |
| ED | 2.9–3.2 (3.1 ± 0.1) | 3.1–3.2 (3.2 ± 0.0) |
| TY | 1.3–1.6 (1.4 ± 0.1) | 1.6–1.8 (1.7 ± 0.1) |
| IOD | 2.9–3.2 (3.0 ± 0.1) | 3.2–3.9 (3.5 ± 0.2) |
| EW | 2.0–2.3 (2.2 ± 0.1) | 2.1–2.7 (2.4 ± 0.2) |
| IND | 1.9–2.5 (2.2 ± 0.2) | 2.0–3.7 (2.5 ± 0.6) |
| E–N | 2.0–2.3 (2.2 ± 0.1) | 2.4–3.7 (3.0 ± 0.4) |
| TL/SVL | 0.36–0.37 | 0.37–0.43 |
| FL/SVL | 0.35–0.41 | 0.37–0.45 |
| HL/SVL | 0.35–0.39 | 0.33–0.39 |
| HW/SVL | 0.35–0.38 | 0.32–0.39 |
| HW/HL | 0.9–1.0 | 0.9–1.1 |
| E–N/ED | 0.66–0.77 | 0.75–1.16 |
| EW/IOD | 0.69–0.77 | 0.54–0.82 |
| TY/ED | 0.45–0.50 | 0.50–0.58 |

Fig. 8A; MUSM 32759, Fig. 8D) to dark brown (MUSM 32753, 32757, Fig. 7D; MUSM 32760, Fig. 8G). Some specimens have white, irregularly shaped flecks scattered on the dorsum (MUSM 32752, 32758, Fig. 8A). While the groin is dark grey with creamy white patches in all specimens, the number of patches ranges from one (MUSM 32757, 32760, Fig. 8H) to four (MUSM 32753). The venter may be dark grey (MUSM 32755, 32756, Fig. 7F), pale grey (MUSM 32753, 32754, Fig. 7C) or creamy white (MUSM 32757, 32758, Fig. 8C; MUSM 32759, Fig. 8F; MUSM 32760, Fig. 8I). Some specimens have the reticulated dark brown ventral blotches fused into a thin midline (MUSM 32754, Fig. 7C; MUSM 32760, Fig. 8I), or dark brown dots (MUSM 32753, 32755, 32756, Fig. 7F; MUSM 32758, Fig. 8C; MUSM 32759, Fig. 8F). Only one female has a reddish brown interorbital bar (MUSM 32760, Fig. 8G).

Etymology: The species epithet *astralos* is a Greek adjective meaning "spotted with stars" or "speckled". The name refers to the dorsal white spots on black background of the species that is reminiscent of stars in the night sky.

Distribution, natural history, and threat status: Hualgayoc is situated in the Cajamarca Regíon of northern Peru, between 2400 and 3500 m a.s.l., about 80 km north of the city Cajamarca, and east of the continental divide of the Cordillera Occidental (Canchaya 1990, Fig. 1). Specimens were obtained on 3 January 2014 in the concession of the Mina Cerro Corona-Gold Fields La Cima, Comunidad Tingo, in the high Andean grasslands (jalca) consisting of Peruvian feather grass and *Puya fastuosa* Mez, 1906 (Bromeliaceae). Specimens of *P. astralos* were found sheltering in *P. fastuosa* plants. Up to 10 specimens (juveniles, males, females) were found in the same plant, and *P. astralos* apparently prefers hiding at the bases of larger plants under dead leaves where humidity levels are higher (C. Diaz, pers. comm.). Syntopic frogs at the time of the find included *Pristimantis chimu, P. pinguis, P. simonsii,* and a syntopic lizard was *Stenocercus stigmosus* Cadle, 1998, all of which use *Puya fastuosa* plants as refuges (C. Diaz, pers. comm.).

*Pristimantis astralos* is so far only known from its type locality at 3600 m a.s.l. in the Cordillera Occidental. Mining operations, and especially large-scale dumping of mining debris at the type locality (pers. comm. C. Diaz) by the end of 2014, have destroyed the type locality and caused the extirpation of the population of *P. astralos* at the type locality (Figs 1, 2). We are unaware of similar habitats with *Puya fastuosa* in the proximity of the type locality where *P. astralos* could be still in existence. Consequently, we suggest the threat status for *P. astralos* to be 'critically endangered' (CR). The current conservation status of *Puya fastuosa* is 'endangered' (EN, Treviño Zevallos 2019).

## Discussion

Peru is the leading gold producer in South America, and with 155 metric tons per year the sixth largest in the world

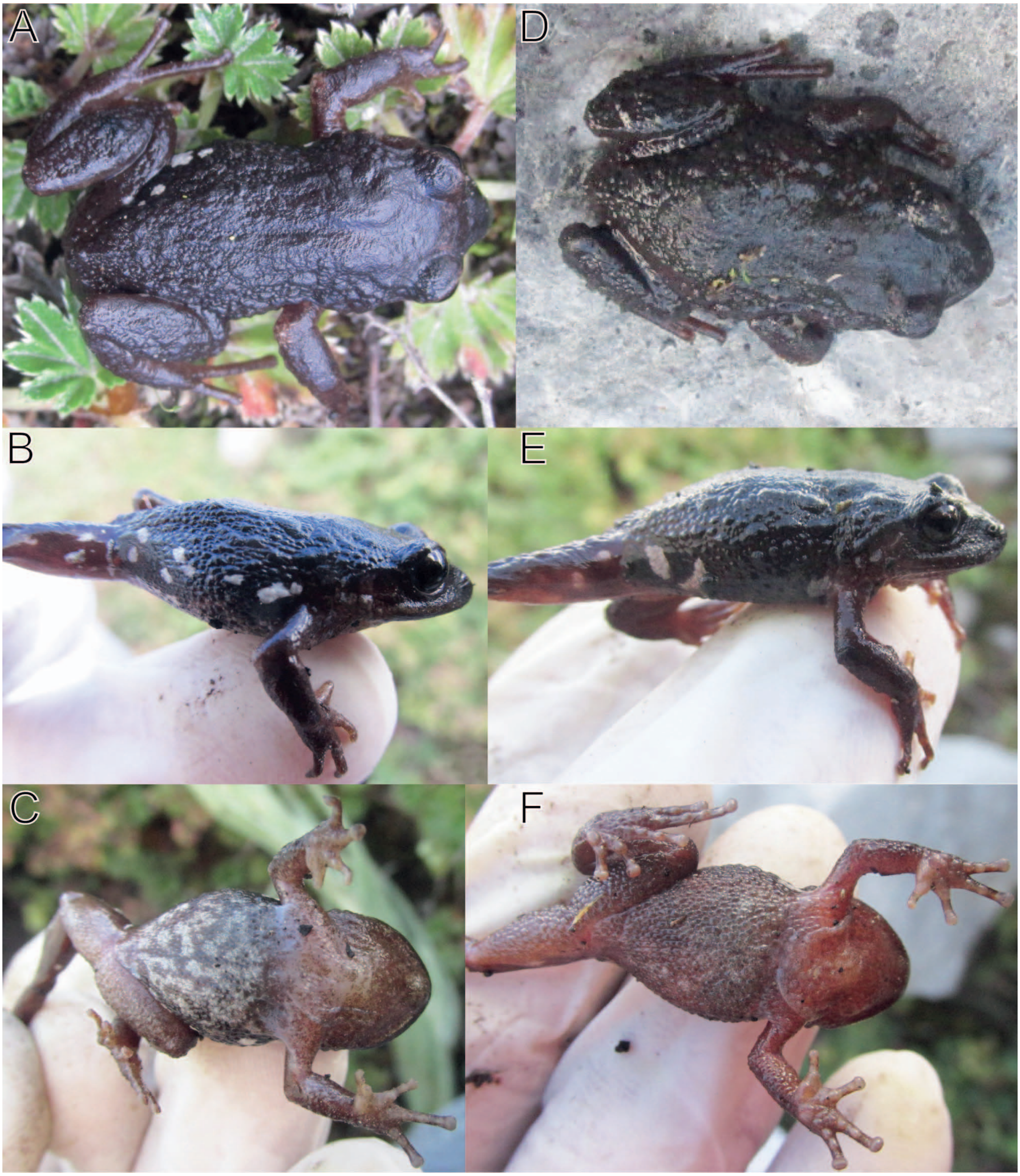


Figure 7. Coloration variation of male *Pristimantis astralos* sp. n.: Left column MUSM 32754, SVL 24.9 mm; right column MUSM 32756, SVL 23.6 mm in dorsal (A, D), lateral (B, E), and ventral (C, F) views. Photos by C. Diaz.

in 2020 (World Atlas 2020). Metals have been mined for thousands of years in Peru and used by various pre-Columbian cultures for artworks and tools long before the Spanish colonization. Alexander von Humboldt visited the Andean silver mines of Hualgayoc in 1802 and commented critically on the destructive techniques employed and the inhumane treatment of the workers (O'Phelan Godoy 2004). The widespread habitat destruction has continued until today (Fig. 2). The Cerro Corona – Gold Fields La Cima Mine in Peru produces copper and gold by conventional open-pit mining methods. With a gold content of ca. 1 g per ton, and the expectation to mine 2.1 MOZ (= 59.5 metric tons) of gold over a 15-year period (Gold Fields 2019), the accumulated debris is immense.

In areas with intensive mining, habitat destruction and pollution can be drastic and threaten endemic species. According to Peruvian law, companies that have an expected impact on nature are obliged to hire consultants (e.g., biologists, engineers) to conduct environmental impact assessments. These assessments include lists of species found before a company starts their activities. The final technical reports are then sent for review to the Ministry of the Environment.

The Tropical Andes are recognized as a biodiversity hotspot (Meyers et al. 2000) for animals and plants, many of which have small-scale distributions that render them highly sensitive to environmental impacts. Mining companies are aware that the presence of potentially new or endangered species can interfere with their activities. As a result, new species may not be highlighted in survey reports by environmental consulting firms under pressure from mining operators. Instead, potential new species may be identified only to genus level (e.g., as *Pristimantis* sp.), or tentatively assigned to the most similar species (e.g., as *Pristimantis* cf. *simonsii*, as was done for the new species described herein). In order to better understand the threat caused by mining activities in the Peruvian Andes, Aguilar et al. (2012) surveyed amphibians in mining concessions, and noted a decline in species richness in two concessions where two previously recorded species (*Atelopus peruensis* Gray & Cannatella, 1985; *Nannophryne cophotis* (Boulenger, 1900)) are now absent. For the latter two

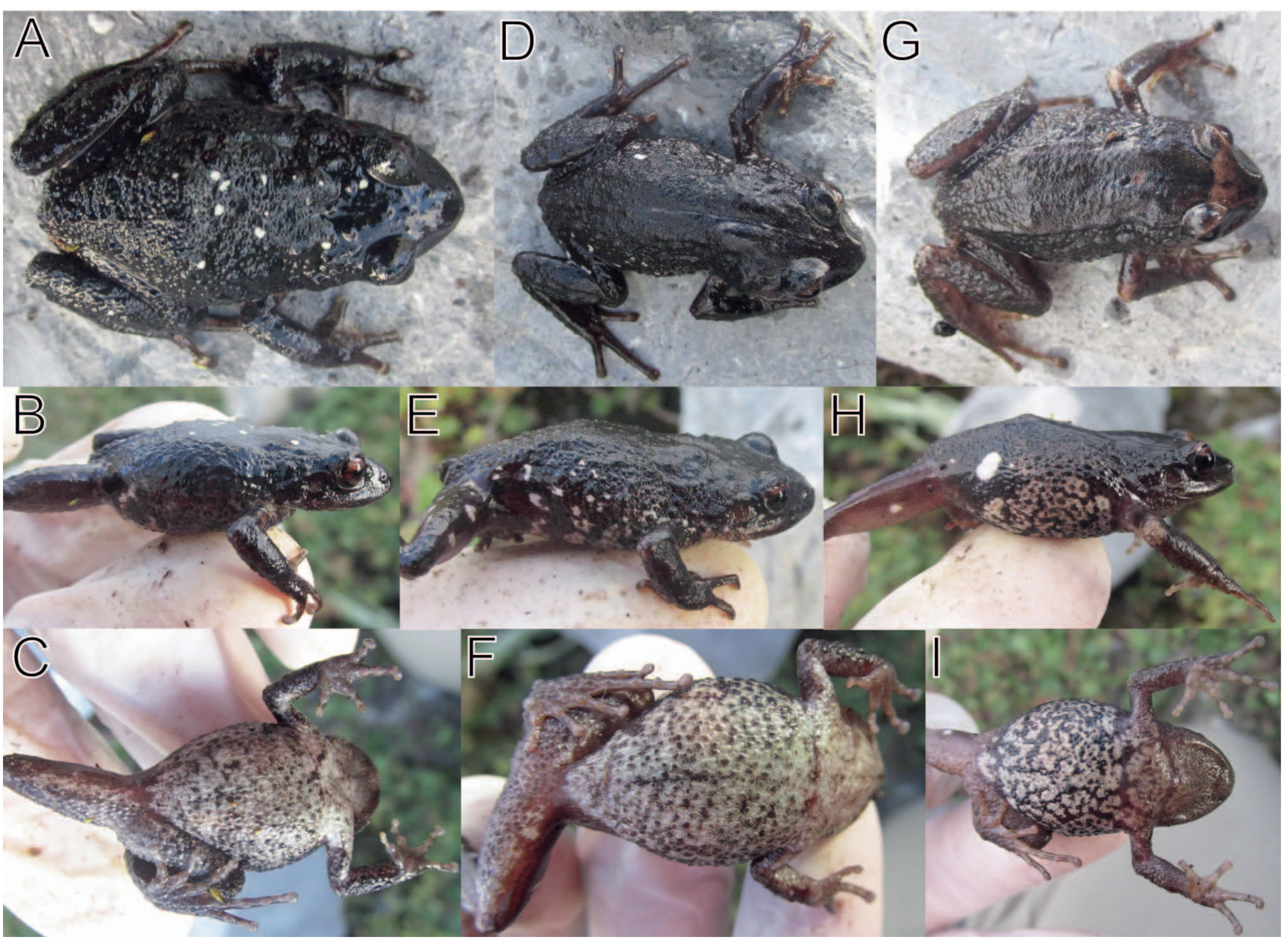


Figure 8. Coloration variation of female *Pristimantis astralos* sp. n.: Left column MUSM 32758, SVL = 25.6 mm; middle column MUSM 32759, SVL = 31.5 mm; right column MUSM 32760, SVL = 27.8 mm in dorsal (A, D, G), lateral (B, E, H), and ventral views (C, F, I). Photos by C. Diaz.

critically endangered species (IUCN SSC Amphibian Specialist Group 2018a, b), additional factors apart from habitat destruction/pollution such as diseases (e.g., *Batrachochytrium dendrobatidis*, also see Catenazzi 2015) may have contributed to their local disappearance.

Bax et al. (2019) analysed the land-use conflicts between vertebrate biodiversity conservation and extractive industries in the forested eastern Peruvian Andes from 500 to 3000 m a.s.l., and found that 16% of endemic-rich areas overlapped with mining concessions. Furthermore, Bax et al. (2019) reported that 5% of all endemic species have geographical distributions that overlap by more than 90% with concession areas, and that less than 2% of all endemic mammal, bird, amphibian and reptile species are protected within reserves (Bax & Francesconi 2019). We assume that conservation gaps of similar severity exist for endemic species in the western Andes of Peru. *Pristimantis astralos* exemplifies how quickly habitat destruction in a mining concession can extirpate species. In their 2018 report, Gold Fields mentioned that *Lupinus peruvianus* (flora), amphibians, and reptiles had been relocated in 2017 (Gold Fields 2018: 83), but the success of these relocations remains unevaluated and unknown. The naming of new species is the first important step towards conservation, and we hope that MINEM and SERFOR will initiate surveys at Hualgayoc and its surroundings to assess the current population status of *P. astralos*.

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## Appendix 1

Comparative material examined.

*Pristimantis aaptus*: Colombia: Amazonas: Puerto Nariño: MCZ A-93635. *Pristimantis attenboroughi*: Peru: Junín: Pui Pui Protected Forest: Hatunpata, 3710 m: NMP6V 75076, Antuyo Bajo, 3400 m a.s.l.: NMP6V 75534. *Pristimantis chimu*: Peru: Cajamarca: 2–3 km NW El Pargo (Llama-Huambos Rd.), 3000–3100 m a.s.l.: MCZ 136072–75. *Pristimantis mariaelenae*: Peru: Lambayeque: Cañaris, 3406–3494 m a.s.l.: MUSM 26478. *Pristimantis puipui*: Peru: Junín: Pui Pui Protected Forest, Laguna Sinchon, 3890 m a.s.l.: MUSM 31982 (holotype), MUSM 31981, 31983, NMP6V 75056–57, 75541–42, all paratypes. *Pristimantis seorsus*: Peru: Cusco: Cordillera Vilcabamba, 3350 m: AMNH A153052–53. *Pristimantis simonsii*: Peru: Cajamarca: 23.5 km NE Encanada, 3510 m a.s.l.: MUSM 1163–1179. *Pristimantis stipa*: Peru: Lambayeque: Cañaris, 3406–3494 m a.s.l.: MUSM 26481–82. *Pristimantis vilcabambae*: Peru: Cusco: Cordillera Vilcabamba, 2050 m: AMNH A153058–59.

## Appendix 2

GenBank accession codes for the taxa used in the molecular genetic analysis in this study.

| Species | Voucher | 16S rRNA |
|---|---|---|
| *Pristimantis altamazonicus* | CORBIDI 16778 | MG820143 |
| *Pristimantis aradalonychus* | KU 212301 | EU186664 |
| *Pristimantis astralos* sp. n. | MUSM 32753 | MT968733 |
| *Pristimantis astralos* sp. n. | MUSM 32755 | MT968732 |
| *Pristimantis attenboroughi* | NMP6V 75524 | KY594754 |
| *Pristimantis attenboroughi* | NMP6V 75525 | KY594755 |
| *Pristimantis bounides* | NMP6V 75097 | KY962797 |
| *Pristimantis bounides* | MUSM 31198 | KY962794 |
| *Pristimantis buccinator* | MUSM 33269 | KY652650 |
| *Pristimantis* cf. *carvalhoi* | CORBIDI 16294 | KY652651 |
| *Pristimantis croceoinguinis* | MC 11557 | DQ195455 |
| *Pristimantis croceoinguinis* | QCAZ 18231 | MH516171 |
| *Pristimantis croceoinguinis* | QCAZ 25444 | MH516173 |
| *Pristimantis croceoinguinis* | QCAZ 25788 | MH516176 |
| *Pristimantis* cf. *croceoinguinis* | MUSM 31154 | KY594759 |
| *Pristimantis fenestratus* | CORBIDI 16222 | MT968731 |
| *Pristimantis humboldti* | MUSM 31194 | KY962798 |
| *Pristimantis humboldti* | NMP6V 75538 | KY962799 |
| *Pristimantis lindae* | MUSM 27902 | KY652653 |
| *Pristimantis ockendeni* | RvM 5.12 | KY652654 |
| *Pristimantis phoxocephalus* | KU 218025 | EF493349 |
| *Pristimantis platydactylus* | MVZ 272359 | KY652656 |
| *Pristimantis platydactylus* | MNCN-DNA 4138 | EU712671 |
| *Pristimantis puipui* | NMP6V 75542 | KY962800 |
| *Pristimantis rhodoplichus* | KU 219788 | EF493674 |
| *Pristimantis ridens* | AJC 1778 | KR863320 |
| *Pristimantis simonbolivari* | KU 218254 | EF493671 |
| *Pristimantis simonsii* | N/A | AM039641 |
| *Pristimantis simonsii* | KU 212350 | EU186665 |
| *Pristimantis salaputium* | MUSM 27916 | KY652658 |