



Contributing to the taxonomic inventory of green-colored rain frogs: A new species of the *Pristimantis lacrimosus* group (Anura: Strabomantidae) from the southern Cordillera Azul, central Peru

Ernesto Castillo-Urbina^{1,2}, Miguel Vences³, César Aguilar-Puntriano¹, Frank Glaw⁴, Jörn Köhler⁵

¹ Universidad Nacional Mayor de San Marcos, Museo de Historia Natural, Departamento de Herpetología, Av. Arenales 1256, Lima 11, Peru

² Asociación GRUPO RANA, Calle 5 Manzana G Lote 14, Urbanización Praderas del Naranjal, Lima, Peru

³ Zoological Institute, Technische Universität Braunschweig, Mendelssohnstr. 4, 38106 Braunschweig, Germany

⁴ Zoologische Staatssammlung München (ZSM-SNSB), Münchhausenstr. 21, 81247 München, Germany

⁵ Hessisches Landesmuseum Darmstadt, Friedensplatz 1, 64283 Darmstadt, Germany

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Corresponding author: Jörn Köhler (joern.koehler@hlmd.de)

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Abstract

We studied the taxonomic status of a population of *Pristimantis* from the southern Cordillera Azul, Departamento Huánuco, central Peru. A phylogenetic analysis based on the mitochondrial 16S rRNA gene revealed that it represents a lineage within the *Pristimantis lacrimosus* species group, being the closest relative of a species of uncertain taxonomic status from a lowland rainforest in central Peru (Panguana), and *P. pulchridormientes* from the Tingo Maria National Park. However, the focal lineage is divergent from all nominal species in the *P. lacrimosus* group for which respective data are available by >7.9% uncorrected pairwise distance in the 16S rRNA gene fragment. An integrative taxonomic approach, including morphological and bioacoustic analyses, provided multiple lines of evidence for the focal specimens belonging to an unnamed evolutionary lineage at the species level that we describe and name herein. The systematics of Peruvian populations associated with the *P. lacrimosus* group are discussed, particularly highlighting problematic taxa with uncertain taxonomic status and unknown relationships. We point to scientific challenges and actions needed to achieve a better taxonomic resolution of this species-rich clade of frogs.

Keywords

Amphibia, bioacoustics, integrative taxonomy, molecular genetics, morphology, systematics

Introduction

Within the species-rich Neotropical anuran genus *Pristimantis*, 17 species groups are currently recognized (Köhler et al. 2022; Ortega et al. 2022; Székely et al.

2023). Among these is the *Pristimantis lacrimosus* species group, which historically has been simply an assemblage within the purely phenetic *P. unistrigatus* group

(Lynch and Duellman 1980) that has been demonstrated to be paraphyletic (see Hedges et al. 2008; Padial et al. 2014). Hedges et al. (2008) formally erected a *P. lacrimosus* species group and recognized 18 species within this group. In a comprehensive phylogenetic study, Padial et al. (2014) redefined the group according to monophyly criteria, and recognized 25 species as group members. Subsequently, Rivera-Correa and Daza (2016), by adding numerous Colombian samples, demonstrated that the group is composed of two distinct clades, one of which was later referred to as the *P. boulengeri* species group (González-Durán et al. 2017). Finally, Ron et al. (2020) presented a comprehensive phylogeny of the group and related taxa, clarified the identity of the name-giving species *P. lacrimosus*, and removed and added species to the group, resulting in 36 recognized species. Due to subsequent species descriptions, exclusions, and additions to the group (Rivera-Correa and Daza 2020; Carrión-Olmedo and Ron 2021; Ttito and Catenazzi 2021), currently 40 *Pristimantis* species are placed in the *P. lacrimosus* group.

As demonstrated by the most recent progresses in understanding the phylogenetic relationships and taxonomic identity of various populations, the systematics of the *P. lacrimosus* group are far from being clarified. There are several documented cases of misidentifications and confusion among species, as the result of either the presence of intra-specific morphological variation, and/or inter-specific morphological crypsis (e.g., Paéz and Ron 2019; Ron et al. 2020; Carrión-Olmedo and Ron 2021). Evidently, with progressing research efforts, this group of frogs awaits the discovery of numerous new species as well as the clarification of so far unsolved taxonomic problems. This might be particularly true for Peru, where on the one hand new species of this group are regularly discovered (e.g., Morales 2007; Chávez and Catenazzi 2016; Shepack et al. 2016; Ttito and Catenazzi 2021), but on the other hand several taxonomic problems remain with respect to the identity of many populations (e.g., Morales and Icochea 2000). Duellman and Lehr (2009) listed eight species of the group to occur in Peru. Their species account was based on the assumption that the nominal species *P. bromeliaceus*, *P. lacrimosus*, and *P. mendax* occupy wide ranges along the eastern Andean slopes and the adjacent lowlands (see also Lehr et al. 2006). However, Ron et al. (2020) demonstrated that *P. bromeliaceus* and *P. lacrimosus* are actually restricted to Ecuador and northernmost Peru, rendering the majority of Peruvian samples previously identified as belonging to these two species actually unidentified and potentially belonging to unnamed species. Another uncertainty is connected with the name *P. mendax*, which to our knowledge has not been re-collected at its type locality and has not been genetically studied, leaving its identity partly obscure (see also Morales and Icochea 2000). As a consequence, many Peruvian populations from montane rainforests at the eastern Andean slopes, superficially similar to *P. mendax*, have been called *P. mendax*, *P. aff. mendax* or *P. cf. mendax* in provisional ways, as the data available prevented a proper evaluation of their

taxonomic identity (e.g., Tasker and Twomey 2015; Angulo et al. 2016; Whitworth et al. 2016; Lehr and von May 2017; Lehr et al. 2017a, b; Moravec et al. 2020). The fact that species that were believed to have a wide distribution in Peru, actually occupy much more restricted ranges (*P. lacrimosus* and *P. bromeliaceus*; Ron et al. 2020), plus further uncertainties (identity of *P. mendax*), leaves a considerable lack of information concerning the taxonomic identity of numerous populations that occur in central and southern Peru. Furthermore, some *Pristimantis* species from Peru share morphological characters with the *P. lacrimosus* group (e.g., *P. rhodostichus*; see Chávez and Catenazzi 2016; Shepack et al. 2016), but they are unstudied genetically and thus their relationships remain unknown.

Summarizing the current knowledge, we can expect 16 species of the *Pristimantis lacrimosus* group to occur in Peru: *P. achupalla*, *P. aureolineatus*, *P. acuminatus*, *P. deyi*, *P. galdi*, *P. lacrimosus*, *P. mendax*, *P. olivaceus*, *P. padiali*, *P. pardalinus*, *P. pluvialis*, *P. pseudoacuminatus*, *P. royi*, *P. schultei*, *P. tantanti*, and *P. pulchridormientes* (Ron et al. 2020; Ttito and Catenazzi 2021). However, external morphology may argue for exclusion of *P. deyi* from the *P. lacrimosus* group (see Lehr et al. 2013), whereas *P. rhodostichus* could likely be a member of it (see Duellman and Pramuk 1999; Duellman and Lehr 2009; Chávez and Catenazzi 2016; Shepack et al. 2016).

The *Pristimantis lacrimosus* group has been demonstrated to exhibit great diversity in the montane forests of the Andean slopes (e.g., Rivera-Correa and Daza 2020; Ron et al. 2020; Carrión-Olmedo and Ron 2021), with an apparently high number of unnamed species in the group, as inferred by the presence of genetically highly divergent lineages. It therefore seems obvious that the presence of morphological variability and/or crypsis in combination with scarce molecular and bioacoustic information delayed taxonomic progress in many cases. Here, we contribute to the knowledge of this group by evaluating the relationships and taxonomic status of a population of rain frogs from the southern Cordillera Azul, central Peru, and describing it as a new species.

Materials and methods

Fieldwork was conducted in November 2019 in different areas of the departments Huánuco and Ucayali in central Peru (see also Castillo-Urbina et al. 2021; Köhler et al. 2022, 2023). Specimens were observed and collected during opportunistic searching at night using torches and headlamps, often guided by the sounds emitted by calling males. Geographic position was recorded using handheld GPS receivers set to WGS84 datum.

Collected specimens were euthanized using an overdose of 5% lidocaine gel applied on ventral surfaces of the individuals (McDiarmid 1994). Tissue samples (thigh muscle and tongue pieces) were cut prior to fixation and stored in 99% ethanol, while specimens were fixed us-

ing 96% ethanol and subsequently stored in 70% ethanol. Specimens were deposited at the herpetological collection of the Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM), Lima, Peru, and Hessisches Landesmuseum Darmstadt (HLMD), Germany. FGZC refers to Frank Glaw field numbers.

Morphometric measurements (in millimetres) were taken by the first author with a digital caliper to the nearest 0.1 mm. Measurements taken and used throughout the text are: **SVL**, snout–vent length; **TL**, tibia length; **HW**, head width (at level of angle of jaws); **HL**, head length (from posterior margin of lower jaw to tip of snout); **IOD**, interorbital distance; **ED**, horizontal eye diameter; **E–N**, eye–nostril distance (straight line distance between anterior corner of orbit and posterior margin of external nares); **IND**, inter-narial distance; **TD**, horizontal tympanum diameter; **HandL**, hand length (from proximal border of outer metacarpal tubercle to tip of third finger); **FootL**, foot length (from proximal border of inner metatarsal tubercle to tip of fourth toe). Fingers and toes are numbered preaxially to postaxially from I–IV and I–V, respectively. Lengths of fingers I and II were determined by adpressing the fingers against each other. For character state definitions, we followed Duellman and Lehr (2009). We use the term ‘hyperdistal tubercle’, as proposed by Ospina-Sarria and Duellman (2019) and Ron et al. (2020), for the tubercle underlying the articulation of the last phalange of each finger and toe. Description and diagnostic schemes follow Köhler et al. (2022). Coloration in life was described based on digital photographs.

Vocalizations in the field were recorded using an Olympus LS-05 digital recorder with built-in microphones at 44.1 KHz and 16-bit resolution and saved as uncompressed WAVE format. Recordings were re-sampled at 22.05 kHz and 32-bit resolution and computer-analyzed using the software Cool Edit Pro 2.0. We obtained frequency information through Fast Fourier Transformation (FFT; width 1024 points) at Hanning window function. Spectrograms were produced with Blackman window function at 256 bands resolution. Sensitive filtering was applied to remove background sounds, only to frequencies outside the prevalent bandwidths of calls. Temporal measurements are provided as range with mean \pm standard deviation in parentheses. Analysis, description and terminology follow the recommendations of Köhler et al. (2017), using the call-centered terminological scheme.

Our genetic analysis aimed at identifying lineage divergence among focal lineages of *Pristimantis*. For representative taxon sampling, we largely followed an approach as described by Köhler et al. (2022). We used BLAST searches (Altschul et al. 1990) of newly generated 16S sequences of focal *Pristimantis* against the GenBank nucleotide archive and downloaded sequences identified to species rank with an identity >88%. Subsequently, we manually searched for sequences of nominal species that are currently placed in the *P. lacrimosus* species group (sensu Ron et al. 2020; Carrión-Olmedo and Ron 2021), plus representatives of its sister clade including *P. apendiculatus* and *P. orcesi* (Ron et al. 2020), and added a

selection of sequences of relevant taxa (not the complete set of sequences available for the groups, which include many unidentified samples). For the *P. lacrimosus* species groups, we further added the sequences of two new samples of unidentified specimens collected by us at different localities in central Peru. Three sequences of *Yunganastes* species (*Y. ashkapara*, *Y. mercedesae*, *Y. pluvicanorus*), with *Yunganastes* being sister to *Pristimantis* (Padiál et al. 2009; Canedo and Haddad 2012), were used to root the tree. For details of used samples see the Zenodo.org repository: DOI: 10.5281/zenodo.10048811.

We sequenced a DNA fragment of the mitochondrial 16S rRNA gene (16S) from tissue samples of newly collected specimens. DNA was extracted using a standard salt extraction protocol, Polymerase Chain Reaction (PCR) carried out with primers 16Sar-L (5'-CGCCTGTTTATCAAAAACAT-3') and 16SBr-H (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi et al. 1991), and the PCR products then directly sequenced on automated DNA sequencers by LGC Genomics (Berlin, Germany). All new DNA sequences were submitted to GenBank (accession numbers OR725032–OR725036).

To estimate evolutionary lineage divergence within the *P. lacrimosus* species group we used a Maximum Likelihood (ML) phylogenetic approach. Sequences were exported to AliView v1.26 (Larsson 2014), edited and aligned using MAFFT v7.310 (Katoh and Standley 2013), with the L-INS-i parameter as the iterative refinement method (Katoh et al. 2005). Final alignment length of the 16S rRNA gene was 1637 bp, but many samples, including the focal lineage, were represented by a shorter 3'-terminal fragment of ca. 500 bp. We employed the W-IQ-TREE web server (Trifinopoulos et al. 2016) to infer a molecular phylogeny under ML as optimality criterion. We inferred the best evolution model using ModelFinder under the BIC criterion implemented in IQ-TREE, obtaining TIM2+F+I+G4 as the best fitting substitution model for the data set (-m TEST command; Kalyaanamoorthy et al. 2017). To evaluate branch support, we obtained ultrafast bootstrap values from 2000 pseudoreplicates and 10,000 iterations as the maximum number to stop (commands -bb 2000 and -nm 10000 in IQ-TREE) and Shimodaira–Hasegawa approximate likelihood ratio test (SH-aLRT) with 1000 replicates (-alrt 1000 command; Shimodaira and Hasegawa 1999; Guindon et al. 2010). Genetic divergences were quantified as uncorrected pairwise distances (p-distances) on pairwise alignments using MEGA v11 (Tamura et al. 2021) using default settings (pairwise deletion).

Results

Morphology

Our morphological examination of the focal specimens from the southern Cordillera Azul revealed a combination of qualitative traits being unique within the *P. lacrimosus* species group. The color pattern with a yellow bel-

ly in life, smooth dorsal skin lacking tubercles, coarsely areolate skin on venter, absence of a rostral papilla, absence of dorsolateral folds, presence of a discoidal fold, presence of a tympanum and tympanic annulus, and an almost truncate snout in lateral profile distinguish the focal samples from all nominal species allocated to the *P. lacrimosus* group (see Duellman 1978; Heyer and Hardy 1991; Köhler et al. 1998; McCracken et al. 2007; Morales 2007; Lehr et al. 2006, 2007, 2013; Duellman and Lehr 2009; Moravec et al. 2010; Batallas and Brito 2014; Ortega-Andrade et al. 2015; Chávez and Catenazzi 2016; Shepack et al. 2016; Rivera-Correa and Daza 2020; Ron et al. 2020; Ttito and Catenazzi 2021; Carrión-Olmedo and Ron 2021).

Bioacoustics

Known advertisement calls of species in the *P. lacrimosus* group are often rather similar in general character (e.g., Köhler et al. 1998; Shepack et al. 2016; Chávez and Catenazzi 2016; Rivera-Correa and Daza 2020) and our analysis of calls of the focal lineage from central Peru confirms this observation. However, we were able to find some quantitative differences in call parameters when compared to the closest relatives, supporting lineage divergence. Advertisement calls of the focal lineage differ by longer note duration (98–110 ms) from calls of *P. aureolineatus* (15–42 ms), *P. pluvialis* (23–58 ms), *P. pulchridormientes* (31–75 ms), *P. royi* (24–26 ms), and *P. olivaceus* (39–75 ms) without overlap (Köhler et al. 1998; Köhler 2000; McCracken and Forstner 2006; Morales 2007; Shepack et al. 2016; Chávez and Catenazzi 2016). Furthermore, although similar in body size, calls of *P. olivaceus* exhibit a distinctly higher dominant frequency of 4880 Hz (versus 2744–3021 Hz) with notes exhibiting an upward frequency modulation (Köhler et al. 1998), lacking in calls of the focal lineage. Calls of *P. lacrimosus* differ from those of the focal lineage by longer note duration (139–167 ms) and much longer inter-note intervals (10,191–20,834 ms; Batallas and Brito 2014). The calls of the focal lineage seem to be most similar to calls of *P. zorro* from Colombia, with broad overlap in numerical parameters (note duration 98–110 versus 62–155 ms; inter-note intervals 786–3062 versus 179–3763 ms; dominant frequency 2744–3021 versus 2842–3186 Hz; see Rivera-Correa and Daza 2020). All known calls from this major clade within the *P. lacrimosus* group consist of simple, tonal, single-pulse notes, whereas calls in other major clades within the *P. lacrimosus* group may exhibit more complex pulsatile or multi-pulsed notes (e.g., Carrión-Olmedo and Ron 2021; own unpubl. data).

Molecular genetics

Our Maximum Likelihood tree (Fig. 1) recovered the *Pristimantis lacrimosus* species group as monophyletic with high support. Our tree topology is in general agreement with those published previously (e.g., Ron et al.

2020; Carrión-Olmedo and Ron 2021; Ttito and Catenazzi 2021), with the exception of *P. jaguensis* which in our analysis was placed sister to the clade containing all other *Pristimantis* sequences included (compare Ron et al. 2020). Within the *P. lacrimosus* species group, several major clades are evident, all being supported by moderate to high support. The three focal samples from the southern Cordillera Azul, Peru, herein described as a new species, were grouped with maximum support in a clade containing *P. aureolineatus*, *P. lacrimosus*, *P. pluvialis*, *P. pulchridormientes*, *P. zorro*, and three samples of uncertain taxonomic status (*P. sp.* Panguana, *P. sp.* Chontachaca and *P. cf. olivaceus*). Within this clade, the new species is sister to *Pristimantis sp.* from Panguana, Departamento Huánuco, Peru, a population in need of taxonomic clarification (see Discussion). Together, the new species and *P. sp.* from Panguana are sister to *P. pulchridormientes* from Tingo Maria National Park, with high support values of 96%/99% from bootstrap and SH aLRT analyses. The subclade containing *P. pulchridormientes*, *P. sp.* and the new species is sister to a subclade containing *P. aureolineatus*, *P. pluvialis*, *P. cf. olivaceus* (see Discussion) and *P. sp.* from Chontachaca, Departamento Cuzco, Peru. These mentioned taxa are all sister to *P. zorro* from Departamento Antioquia, Colombia, and together with this species form the sister group to *P. lacrimosus* from Departamento Pastaza, Ecuador. This major clade containing our focal samples is sister to another major clade, containing *P. achupalla*, *P. amaguanae*, *P. bromeliaceus*, *P. cf. mendax*, *P. enigmaticus*, *P. jorgevelosai*, *P. limoncochensis*, *P. moro*, *P. nankints*, *P. omeviridis*, *P. petersi*, *P. petersioides*, *P. romeroae*, and *P. schulzei*, within which the species *P. jorgevelosai*, *P. limoncochensis*, *P. enigmaticus*, and *P. omeviridis* form a remarkably distant subclade. In our analysis, *P. crucifer*, *P. eremitus*, and *P. nyctophylax* form a clade splitting from the most basal node within the *P. lacrimosus* group.

Uncorrected pairwise distances of the focal samples from central Peru when compared to the closest relatives in the same major clade are as follows: 4.7% to *Pristimantis sp.* from Panguana; 7.9% to *P. pulchridormientes*; 11.2% to *P. aureolineatus*; 10.6–10.7% to *P. pluvialis*; 10.2% to *P. cf. olivaceus* from northern Bolivia; 10.9–11.4% to *P. zorro*; and 9.4–14.4% to *P. lacrimosus*.

In summary, our analyses provided different lines of evidence that the focal samples represent a divergent evolutionary lineage. The molecular genetic data reconstruct it (along with the unnamed Panguana population) sister to *P. pulchridormientes*, but it differs from this species by a high genetic distance, longer note duration in advertisement calls, and various morphological characters (see Diagnosis below). We therefore formally describe and name this lineage in the following as new species.

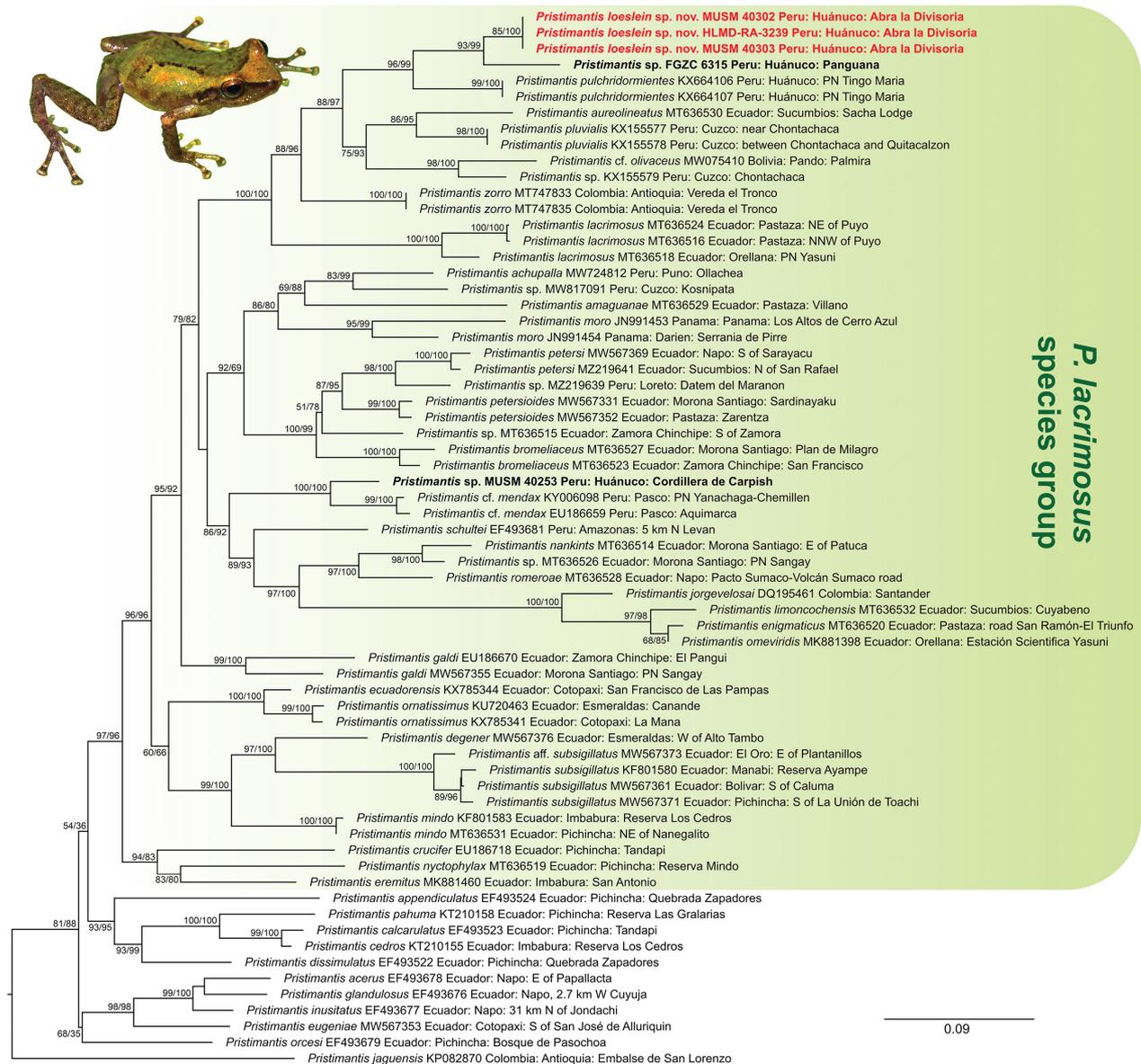


Figure 1. Maximum Likelihood phylogenetic tree of selected samples of *Pristimantis*, representing the *P. lacrimosus* species group and related taxa, inferred from an alignment of 1637 nucleotides of the mitochondrial 16S rRNA gene. Three samples of *Yungasties* were used to root the tree (not shown for better graphical presentation). Numbers at nodes are bootstrap values in percent (2000 pseudoreplicates; not shown if <50%), followed by SH-aLRT values (1000 replicates; not shown if <50) as calculated with IQ-TREE. The taxon name is followed by the GenBank accession number, or voucher number for newly produced sequences (bold terminals), and the sample locality. Inset photo depicts the holotype of *P. loeslein* sp. nov. (MUSM 40302) in life (mirrored).

Taxonomy

Pristimantis loeslein sp. nov.

<https://zoobank.org/C8166BCB-3BA2-46D2-A392-B8A-63F1AC167>

Holotype. MUSM 40302 (FGZC 6257), adult male, from Abra La Divisoria (9°12'01"S, 75°47'43"W, 1650 m above sea level), southern Cordillera Azul, Provincia Leoncio Prado, Departamento Huánuco, Peru, collected on 8 November 2019 by Ernesto Castillo-Urbina, Frank Glaw and Jörn Köhler.

Paratypes. MUSM 40301 (FGZC 6256), MUSM 40303 (FGZC 6258), two adult males, same data as holotype; HLMD-RA-3239 (FGZC 6270), adult male, same data as holotype, except for being collected on 10 November 2019.

Etymology. The species is named after the German family Löslein (from Erlangen and Munich) in recognition of supporting taxonomic research and species conservation in Peru through the BIOPAT initiative. The name is treated as a noun in apposition.

Definition. A species assigned to the genus *Pristimantis* and in particular to the *P. lacrimosus* species group based

on molecular phylogenetic relationships and overall morphological similarities. *Pristimantis loeslein* sp. nov. is characterized by the following combination of characters: (1) Skin on dorsum smooth; skin on flanks with small scattered low subconical tubercles; skin of venter coarsely areolate; discoidal fold present, weakly defined; dorsolateral folds absent; (2) tympanic membrane and tympanic annulus present, round, its length about 2/5 of eye diameter; its upper edge slightly concealed by inconspicuous supratympanic fold; (3) snout acuminate in dorsal view, slightly rounded to almost truncate in profile, lacking distinct rostral papilla; (4) interorbital region flat, broader than upper eyelid; upper eyelid with 1–3 distinct small subconical tubercles; cranial crests absent; (5) dentigerous processes of vomers low, widely separated, posteromedial to choanae; (6) adult males with prominent subgular vocal sac and vocal slits; adult males with inconspicuous nuptial pads on the proximal dorsal part on thumb; (7) first finger shorter than second; all fingers long, discs broadly expanded, rounded to truncate in outline, with circumferential grooves; all fingers bearing a hyperdistal tubercle; fingers with narrow, weakly defined lateral fringes; fingers without webbing; (8) few low ulnar tubercles present or absent; knee and heel tubercles absent; (9) tarsal fold not evident; tarsus bearing row of few elongated low tubercles; (10) inner metatarsal tubercle prominent, oval, approximately 4× the size of round conical outer metatarsal tubercle; few low supernumerary plantar tubercles; (11) all toes with hyperdistal tubercles; toes with narrow lateral fringes; traces of basal webbing present between toes; discs expanded, rounded to truncate in outline, slightly smaller than those on fingers, bearing circumferential grooves; toe V distinctly longer than toe III; (12) in life, predominantly yellowish green to olive-green dorsal coloration, with darker markings; throat, chest and venter yellow; iris copper to bronze with black reticulation; (13) SVL of adult males 20.5–23.3 mm (n = 4); (14) advertisement call consisting of a single tonal note with 98–110 ms duration, repeated in long call series.

Diagnosis. *Pristimantis loeslein* is most closely related to the nominal species *P. aureolineatus*, *P. lacrimosus*, *P. pluvialis*, *P. pulchridormientes*, and *P. zorro* (see Fig. 1). From these, the new species (characters in parentheses) differs as follows: *P. aureolineatus* mainly differs by dorsal skin texture finely shagreened (vs. smooth), snout protruding in lateral profile (vs. slightly rounded to almost truncate), and a creamy-yellow interorbital bar, extending to upper eyelids and posteriorly to the sacrum (vs. interorbital bar blackish without extension); *P. pulchridormientes* mainly differs by absence of tubercles on upper eyelid (vs. 1–3 small subconical tubercles), dentigerous processes of vomers absent (vs. present, low), inner metatarsal tubercle 2× the size of outer metatarsal tubercle (vs. 4×), dorsum yellowish brown in life (vs. yellowish green to olive-green), posterior surfaces of thighs and shanks bright red (vs. brownish to pinkish grey), and note duration of 31–75 ms in advertisement calls (vs. 98–110 ms); *P. pluvialis* mainly differs by skin on flanks smooth (vs.

with small scattered subconical tubercles), snout rounded in profile (vs. slightly rounded to almost truncate), presence of a small rostral tubercle (vs. absence), inner metatarsal tubercle 2.5× the size of outer metatarsal tubercle (vs. 4×), white venter in life (vs. yellow), dorsum orange-brown in life (vs. yellowish green to olive-green), and 23–58 ms note duration in advertisement calls (vs. 98–110 ms); *P. lacrimosus* mainly differs by the presence of a rostral papilla (vs. absence), discoidal fold absent (vs. present, weakly defined), inner metatarsal tubercle 5–6× the size of outer metatarsal tubercle (vs. 4×), yellow, golden-brown, or reddish-brown dorsal coloration in life (vs. yellowish green to olive-green); *P. zorro* mainly differs by the absence of a discoidal fold (vs. present, weakly defined), presence of small rostral papilla (vs. absence), absence of tubercles on upper eyelid (vs. 1–3 small subconical tubercles), cream venter in life (vs. yellow), and presence of dark blotch covering the front of the face, including inter-nostril area forming a facial mask (vs. absence). Furthermore, *P. loeslein* differs from all nominal species in the *P. lacrimosus* group with respective genetic data available by substantial genetic divergence in the 16S rRNA gene (uncorrected p-distances > 7.9%).

Species without analyzed 16S rRNA sequence data allocated to the *P. lacrimosus* group (sensu Carrión-Olmedo and Ron 2021) are: *P. deyi*, *P. latericius*, *P. mendax*, *P. olivaceus* (see Discussion), *P. padiali*, *P. pardalinus*, *P. pseudoacuminatus*, *P. royi*, *P. tantanti*, *P. waorani*, and *P. zimmermanae*. From these, *P. loeslein* differs as follows: *P. deyi* mainly differs by dorsolateral folds present (vs. absent), rostral papillae present as a fleshy process (vs. absent), snout protruding and inclined posteroventrally in profile (vs. slightly rounded to almost truncate), and predominantly brownish dorsal color in life (vs. yellowish green to olive-green); *P. latericius* mainly differs by dorsal skin finely granular (vs. smooth), inner metatarsal tubercle 2× the size of outer metatarsal tubercle (vs. 4×), and dorsal color in life red to light orange (vs. yellowish green to olive-green); *P. mendax* (sensu Morales and Icochea 2000) mainly differs by snout round in dorsal view (vs. acuminate), dorsal skin shagreened bearing conical tubercles (vs. smooth, lacking tubercles), discoidal fold absent (vs. present, weakly defined), dentigerous processes of vomers absent (vs. present, low), tarsal fold evident, sigmoid (vs. not evident), and heel tubercles conspicuous (vs. absent); *P. olivaceus* mainly differs by the presence of a rostral papilla (vs. absence), dorsal skin texture shagreened with scattered tubercles (vs. smooth, lacking tubercles), snout protruding in profile (vs. slightly rounded to almost truncate), and discoidal fold distinct (vs. weakly defined); *P. padiali* mainly differs by ventral skin weakly areolate (vs. coarsely areolate), snout protruding and inclined posteroventrally in profile (vs. slightly rounded to almost truncate), inner metatarsal tubercle 2× the size of outer metatarsal tubercle (vs. 4×), eyelid tubercles absent (vs. 1–3 small subconical tubercles), subgular vocal sacs and vocal slits absent (vs. present), and heel tubercles present (vs. absent); *P. pardalinus* mainly differs by lateral fringes on fingers broad (vs. weakly defined), heel bearing small tubercles and one prominent tubercle (vs.

absent), and white venter in life (vs. yellow); *P. pseudo-acuminatus* mainly differs by snout acutely rounded in profile (vs. slightly rounded to almost truncate), discoidal fold absent (vs. present, weakly defined), inner metatarsal tubercle 5× the size of outer metatarsal tubercle (vs. 4×), tarsus lacking tubercles (vs. bearing a row of few elongated low tubercles), and white venter in life (vs. yellow); *P. royi* differs by snout bluntly rounded in dorsal view (vs. acuminate), nostrils directed dorsolaterally (vs. laterally), supernumerary palmar and plantar tubercles absent (vs. presence of round supernumerary palmar tubercles and few low plantar tubercles), and an advertisement call with note durations of 24–26 ms (Morales 2007) (vs. 98–110 ms); *P. tantanti* mainly differs by absence of a tympanic membrane and annulus (vs. presence), absence of a discoidal fold (vs. presence, weakly defined), and prominent ulnar and tarsal folds (vs. absent, not evident); *P. waorani* mainly differs by absence of tubercles on upper eyelid (vs. 1–3 small subconical tubercles), presence of a tarsal fold (vs. absence), venter translucent white to creamy white in life (vs. yellow), and bright golden-brown dorsal color of males in life (vs. yellowish green to olive-green);

P. zimmermanae mainly differs by snout acute in profile (vs. slightly rounded to almost truncate), dentigerous processes of vomers absent (vs. present, low), and a pulsatile advertisement call (vs. tonal).

Although currently not associated with the *P. lacrimosus* species group, *P. rhodostichus* shares several characters with other species in the group (see Duellman and Lehr 2009; Chávez and Catenazzi 2016; Shepack et al. 2016) and should possibly be considered a member of it. *Pristimantis rhodostichus* mainly differs from the new species by a prominent discoidal fold (vs. weakly defined), dorsum shagreened with numerous scattered conical tubercles (vs. smooth, lacking conical tubercles), snout acutely rounded above and inclined posterolaterally in profile (vs. slightly rounded to almost truncate), and a cream venter in life (vs. yellow).

Description of the holotype. Adult male (SVL 21.0 mm) in good state of preservation (Fig. 2). Body slender; head slightly longer than wide, wider than body; snout acuminate in dorsal view, slightly rounded, almost truncate in lateral profile, rostral papilla absent; canthus ros-

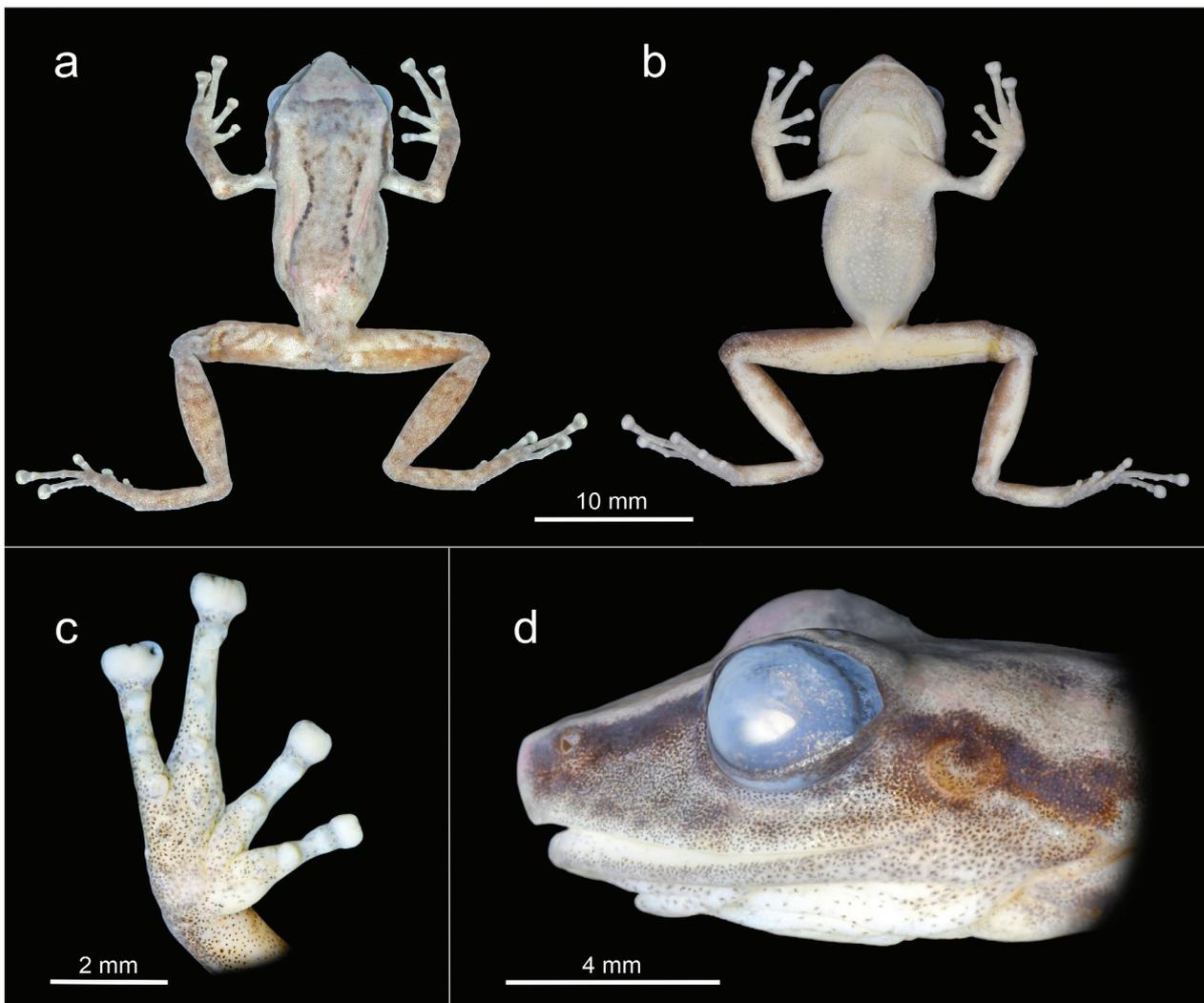


Figure 2. Preserved male holotype of *Pristimantis loeslein* sp. nov. (MUSM 40302, FGZC 6257) from Abra La Divisoria, Cordillera Azul, Departamento Huánuco, Peru, 1650 m a.s.l. (a) Dorsal view, (b) ventral view, (c) palmar surface of right hand, and (d) head in lateral profile.

Table 1. Morphological measurements (in mm) of the male type specimens of *Pristimantis loeslein* sp. nov. For abbreviations see Materials and Methods.

| | Holotype | Paratype | Paratype | Paratype |
|-------|------------|------------|------------|--------------|
| | MUSM 40302 | MUSM 40301 | MUSM 40303 | HLMD-RA-3239 |
| SVL | 21.0 | 20.5 | 22.3 | 23.3 |
| TL | 10.7 | 9.5 | 10.9 | 10.9 |
| HL | 8.0 | 7.6 | 9.5 | 8.2 |
| HW | 7.8 | 7.5 | 8.2 | 8.0 |
| ED | 2.7 | 2.7 | 2.8 | 3.0 |
| TD | 1.1 | 1.1 | 1.2 | 1.3 |
| IOD | 2.9 | 3.0 | 3.2 | 3.0 |
| IND | 1.8 | 1.7 | 2.0 | 2.2 |
| E-N | 2.3 | 2.0 | 2.5 | 2.5 |
| HandL | 6.4 | 6.4 | 6.4 | 6.5 |
| FootL | 9.4 | 8.6 | 9.4 | 9.5 |

tralis distinct, straight in dorsal view, slightly rounded in cross-section; loreal region concave; interorbital region flat, no cranial crests; eye large, protuberant; upper eyelid width about 62% of interorbital distance; right eyelid bearing one subconical tubercle, left eyelid bearing two subconical tubercles. Tympanic membrane and annulus distinct, rounded, with inconspicuous supratympanic fold, slightly obscuring anterodorsal annulus; horizontal diameter of tympanum about 14% of head length, separated from eye by a distance about two thirds of tympanum length; choanae large, rounded, not concealed by palatal shelf of maxillary arc; dentigerous processes of vomers low, posteriomedian to choanae, separated by a distance of about twice their length, each bearing a transverse row of four to five teeth; tongue removed as tissue sample. Skin on dorsum smooth; dorsolateral folds absent; skin on flanks with few minute scattered subconical tubercles; skin on belly coarsely areolate; skin on throat and chest smooth; discoidal fold present, but very weakly defined; skin in upper cloacal region smooth, with few minute rounded tubercles. Forearms slender, each bearing a row of three rounded tubercles at ventral side of lower arm; fingers long and slender, all with expanded discs, those on fingers I and II rounded, those on fingers III and IV truncate in outline; finger discs with circumferential grooves; fingers bearing narrow lateral fringes, basal webbing between fingers absent; relative lengths of fingers I < II < IV < III; three subarticular tubercles on finger III, all tubercles well defined, round in ventral and lateral view; several round supernumerary tubercles present, prominent at the base of the fingers and lower at the palmar surface; palmar tubercle bifid, heart-shaped, about the same length and 130% width of ovoid thenar tubercle; inconspicuous nuptial pad on proximal dorsal part of thumb. Hindlimbs slender; tibia length about 51% of SVL; skin on upper surfaces of hindlimbs smooth, with scattered barely recognizable low rounded tubercles; foot length about 45% of SVL; posterior surfaces of thighs smooth, ventral surfaces of thighs weakly areolate; knee and heel lacking tubercles; no distinct tarsal fold; tarsus bearing a row of three elongated tubercles at median level; toes bearing narrow lateral fringes; trace of basal

webbing between all toes; discs on toes expanded, very slightly smaller than those on fingers, rounded in outline, bearing circumferential grooves; relative lengths of toes I < II < III < V < IV; toe V distinctly longer than toe III, disc on toe V reaches proximal edge of hyperdistal tubercle on toe IV, disc on toe III reaches distal edge of penultimate subarticular tubercle on toe IV; subarticular tubercles rounded, prominent; plantar surface with few low supernumerary tubercles; inner metatarsal tubercle prominent, elliptical, approximately four times the size of rounded and conical outer metatarsal tubercle. For morphological measurements see Table 1.

In life (Fig. 3), dorsal surfaces yellowish green, olive-green to greenish brown. Blackish brown interorbital stripe; anterior head yellowish green with some reddish brown marbling; scapular region with some faint irregular dark flecking; dorsum with two interrupted curved black lines at the border to flanks; flanks bright green with some irregular orange-brown flecking; loreal region dark green to brown, with dark brown canthal stripe; supratympanic fold and region posterior to tympanum dark brown; upper surfaces of limbs dark green to brown with scattered darker blotches and flecks; belly bright yellow; throat and chest transparent yellow with some fine brown spotting; ventral surfaces of limbs brownish to pinkish grey; ventral surfaces of finger and toe discs yellowish green; bones white; iris bronze, with faint horizontal copper streak, irregular black reticulation.

After two years in preservative (Fig. 2), ground color greyish brown to cream, with a brown middorsal area, bordered by fine interrupted black lines; faint pinkish brown fleck in sacral region; black interorbital stripe; flanks greyish cream with some irregular reddish brown markings; dorsal surfaces of limbs greyish brown with dark brown markings; belly, throat and chest greyish cream; ventral surfaces of thighs yellowish cream.

Variation. For variation in measurements among the four males of the type series, see Table 1. Some variation is evident with respect to the dorsal color pattern. Compared to the holotype, paratype MUSM 40301 has a less contrasting dorsal color pattern, lacking dark brown

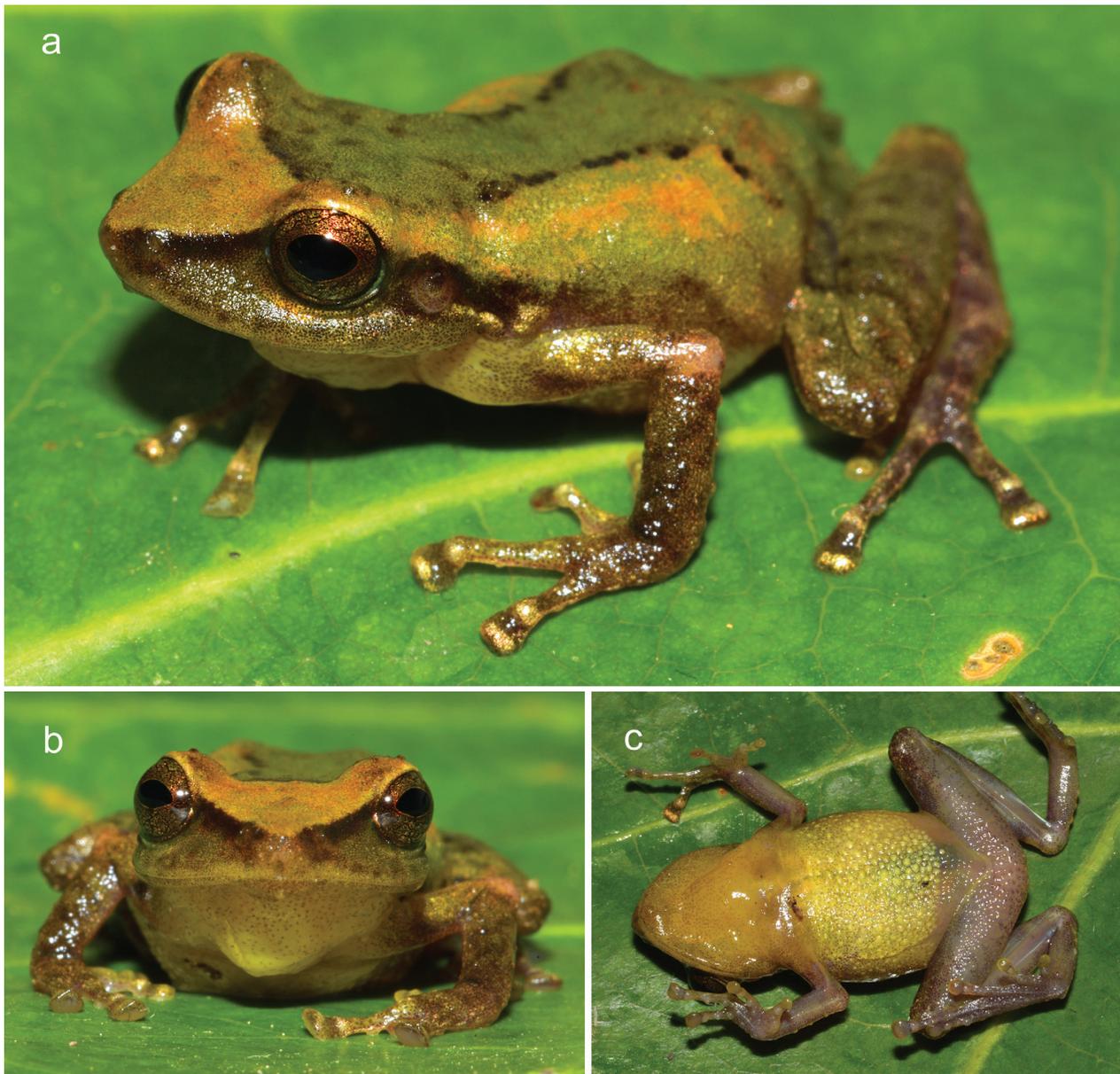


Figure 3. Living male holotype of *Pristimantis loeslein* sp. nov. (MUSM 40302, FGZC 6257) from Abra La Divisoria, Cordillera Azul, Departamento Huánuco, Peru, 1650 m a.s.l., in (a) dorsolateral, (b) frontal, and (c) ventral views (SVL 21.0 mm).

or blackish lines and spots on dorsum, and has a barely recognizable dark interorbital bar. The transverse dark bars on hindlimbs are also very indistinct. Some irregular reddish-brown flecking was evident in life dorsally in the sacral region (Fig. 4a). The paratype MUSM 40303 exhibits a more contrasting dorsal pattern compared to the holotype. Dark interrupted lines on dorsum appeared more blackish in life and additional black flecking is present on the flanks, whereas transverse dark bars on hindlimbs are only evident in the anterior parts (Fig. 4c). The paratype HLMD-RA-3239 is very similar in dorsal color pattern when compared to the holotype, except for a more regularly shaped dark hourglass-pattern on middorsum. Ventral coloration is virtually the same in all four specimens.

Natural history. The type locality is an area of evergreen montane rainforest of moderate height, growing

on moderate to steeply sloped terrain (Fig. 5). The area has partly been altered by different kinds of plantations (see also Köhler et al. 2022). Individuals were observed at night during light rainfall while calling from bushes and low shrub vegetation (secondary growth) at the side slope of a narrow dirt road. The species occurs in syntopy with *Pristimantis nebulosus*, *P. sp.* (aff. *divinae*), *Rhinella sp.* (*margaritifera* group), *Boana lanciformis*, *Dendropsophus aperomeus*, *Scinax cf. garbei*, *S. sp.* (aff. *ruber*), and *Adenomera sp.* (see also Köhler et al. 2022).

Vocalisation. Calls were recorded on 8 November 2019, around 20:00 h, at the type locality, Abra la Divisoria, southern Cordillera Azul, 1650 m a.s.l., Departamento Huánuco, Peru (air temperature estimated by subjective feel app. 18°C). The recorded individuals could not be observed calling, but searching at the spot of sound emission revealed individuals of *P. loeslein*, and other individuals

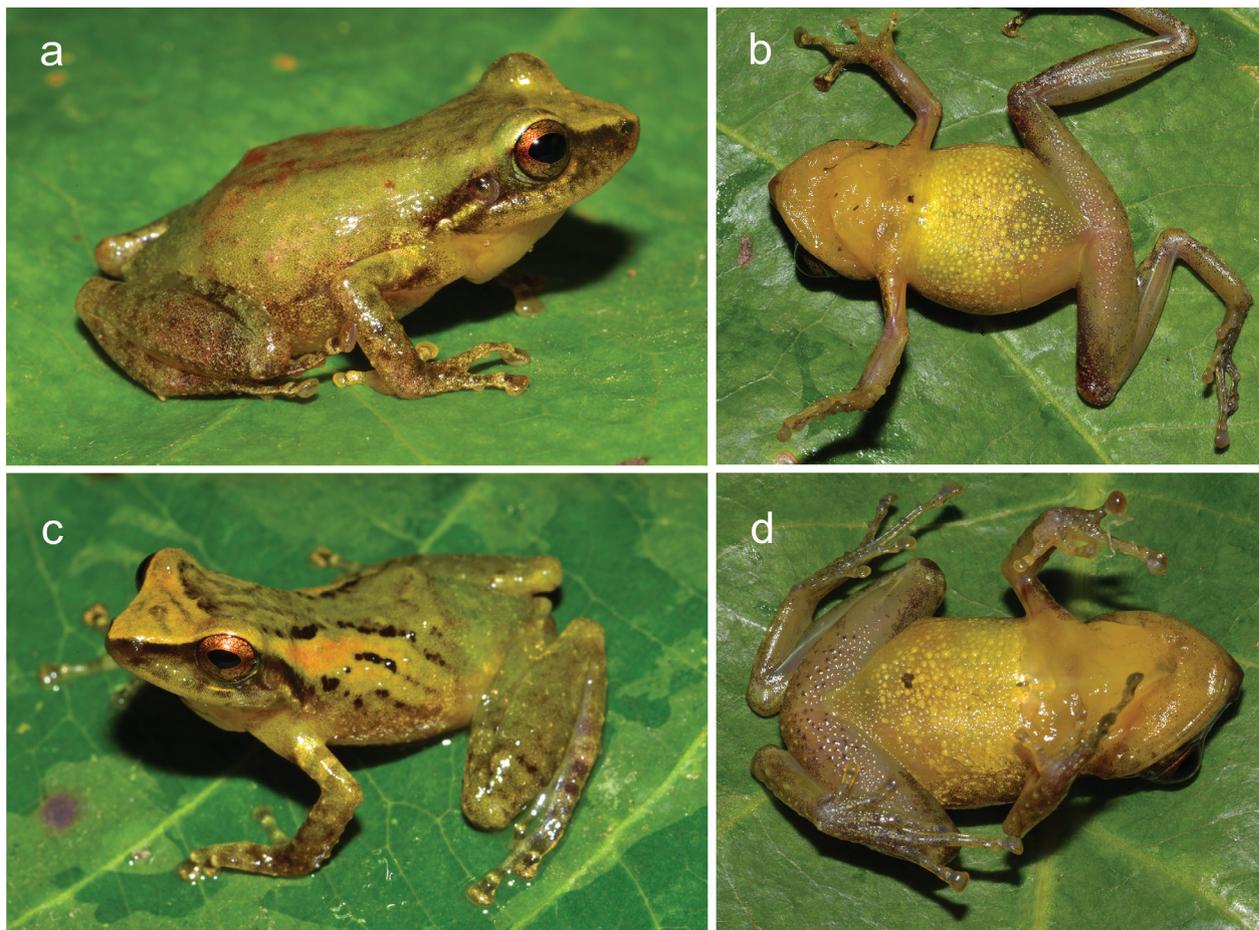


Figure 4. Topotypic male paratypes of *Pristimantis loeslein* sp. nov. in life: MUSM 40301 (FGZC 6256) in (a) dorsolateral and (b) ventral views (SVL 20.5 mm); MUSM 40303 (FGZC 6258) in (c) dorsolateral and (d) ventral views (SVL 22.3 mm).



Figure 5. View of the forest at the type locality of *Pristimantis loeslein* sp. nov. at Abra La Divisoria, southern Cordillera Azul, Departamento Huánuco, Peru, at 1650 m a.s.l.; photo taken on 8 November 2019.

of *P. loeslein* were seen emitting the same call, leaving little doubt that recorded calls actually correspond to this species. The advertisement call consists of a single tonal note of short duration, repeated in long call series at rather long and irregular inter-call intervals (Fig. 6). Call series apparently can continue for several hours. Each call (= note) shows some amplitude modulation with maximum call energy being present at the beginning of the call, then rapidly dropping to about half the maximum call ener-

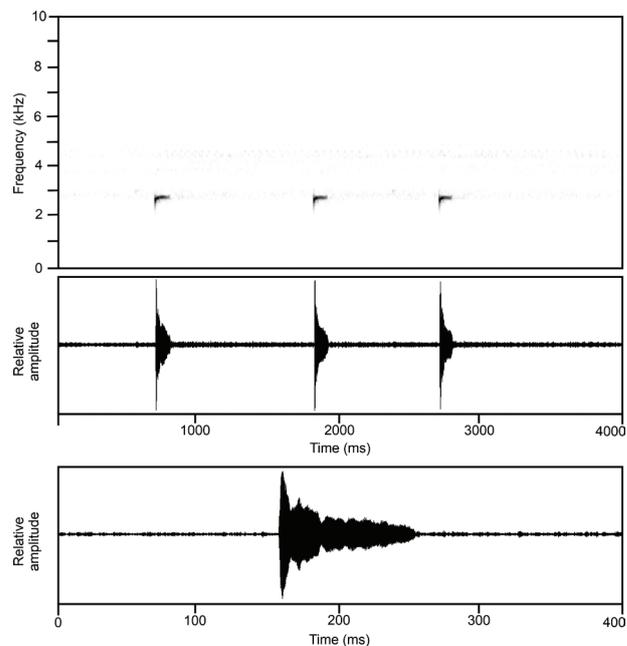


Figure 6. Audiospectrogram and corresponding oscillogram at 4000 ms time scale showing three advertisement calls of *Pristimantis loeslein* sp. nov. recorded on 8 November 2019 at Abra La Divisoria, 1650 m a.s.l., Departamento Huánuco, Peru. Below an oscillogram showing one single call (the center call from oscillogram above) at 400 ms time scale. Recording band-pass filtered at 1500–6000 Hz.

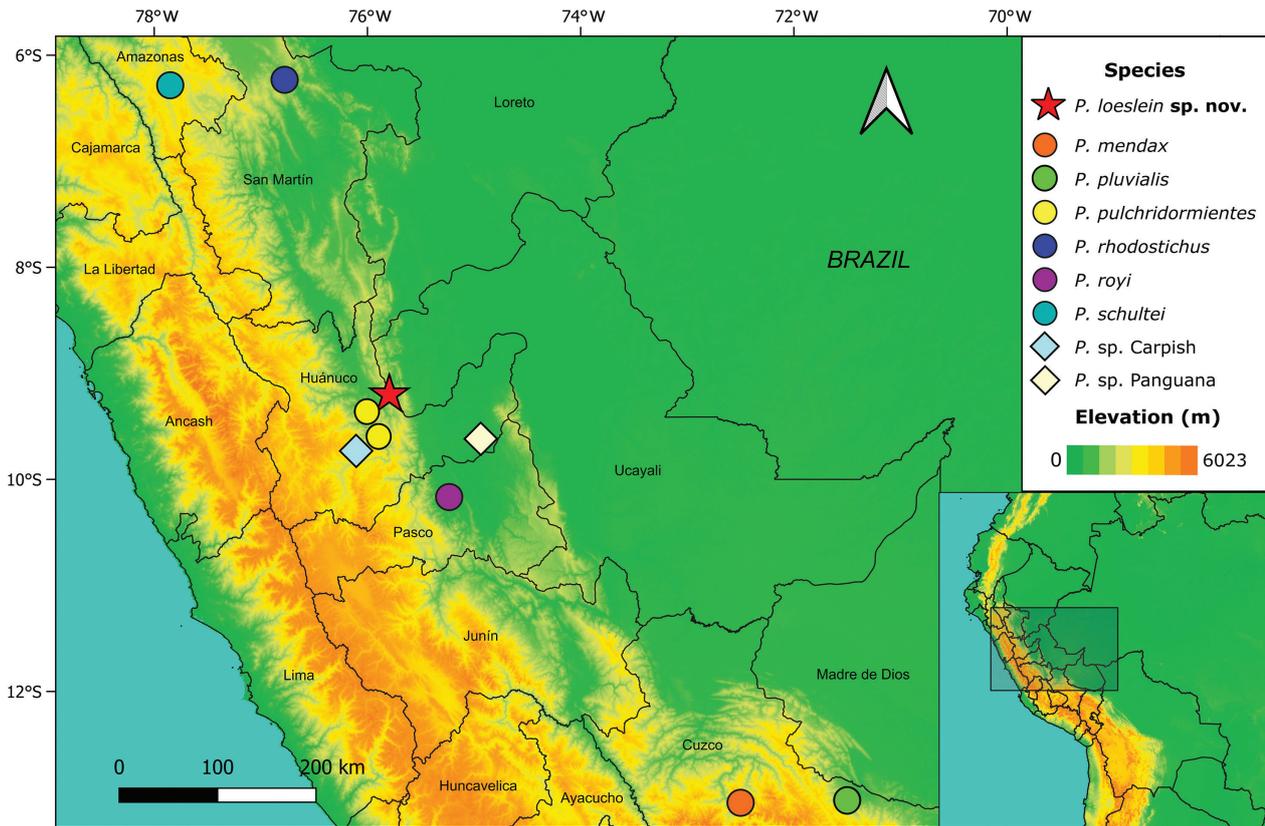


Figure 7. Map of central Peru with adjacent regions in northern and southern Peru, showing the known distribution of seven nominal and two candidate species of the *Pristimantis lacrimosus* species group as discussed in the text. In most cases, symbols refer to the respective type localities. Black lines within Peru do refer to the political borders of respective departments.

gy and continuously decreasing towards the call's end. In some calls, some further subtle amplitude modulation is recognizable (Fig. 6), but clearly it does not qualify for the classification as pulses or pulsatile structures (see Köhler et al. 2017). Frequency is distributed in a very narrow band, harmonic frequencies and frequency modulation are absent. Numerical parameters of 85 analyzed calls, originating at least from four different individuals, are as follows: call duration (= note duration) 98–110 ms (104.2 ± 3.6 ms); inter-call interval 786–3062 ms (1782.3 ± 524.0 ms); dominant frequency 2744–3021 Hz (2840 ± 109 Hz); prevalent bandwidth 2500–3300 Hz.

Distribution. Known so far only from the type locality in the southern Cordillera Azul (Fig. 7). However, the species is likely to occur in other areas of montane rainforests in central Peru. Possibly, some records formerly referred to other nominal species in the *P. lacrimosus* group from that area (e.g., *P. cf. mendax*, *P. lacrimosus*) may actually refer to this new species.

Discussion

As apparent from most recent publications (e.g., Ron et al. 2020; Rivera-Correa and Daza 2020; Carrión-Olmedo and Ron 2021; Tito and Catenazzi 2021), the *Pris-*

timantis lacrimosus species group forms a diverse and species-rich clade within Pristimantinae. With the description of *P. loeslein*, we added a further taxon to this clade of frogs by providing different lines of evidence for its distinctiveness (morphology, molecular genetics, and bioacoustics). However, taxonomic resolution within this species group is far from being complete. Prior to the application of molecular analyses and inclusion of topotypic samples, there have been numerous documented instances of misidentifications and confusion among species, as a distinction between intra-specific morphological variation on the one hand, and inter-specific morphological crypsis on the other hand is rather difficult (e.g., Morales and Icochea 2000; Ortega-Andrade et al. 2015; Ron et al. 2020; Carrión-Olmedo and Ron 2021). In light of the great species diversity in the *P. lacrimosus* group, particularly in central and southern Peru (unpublished data), uncovered by molecular analyses, it is paramount to assign existing names to living populations and clarify the taxonomic status of nominal species with uncertain identity. In most cases, this can only be achieved by sampling the type localities of respective taxa, with the goal of obtaining tissue samples for molecular analyses and ideally recordings of advertisement calls. With respect to Peru, certain nominal species deserve comments in this context.

The identity and relationships of *Pristimantis mendax*, with its type locality in southern Peru (Colonia Pistipata, SE of Huyro, 1820 m a.s.l., Departamento Cuzco) and



Figure 8. Amplexant couple of *Pristimantis* sp. (FGZC 6314–6315) in situ at Panguana, Departamento Huánuco, Peru, 260 m a.s.l. This species is the closest known relative of *P. loeslein* sp. nov. (uncorrected p-distance 4.7%), with its identity yet to be clarified.



Figure 9. Living individual (FGZC 6300) of a second unidentified species of the *Pristimantis lacrimosus* group occurring at Panguana, Departamento Huánuco, Peru, 260 m a.s.l. (no sequence data available). Morphologically, this species is apparently most similar to *P. royi*, described from a nearby locality (Morales 2007).

paratypes from different low and high elevation localities in the Departamentos Ayacucho, Cuzco, and Huánuco (Duellman 1978) remains uncertain (see Introduction). By detailed morphological investigation, Morales and Icochea (2000) suggested that the type series of *P. mendax* is composed of at least three different species. However, their conclusion that paratypes from lowland Panguana, Departamento Huánuco, correspond to the two nominal species *P. bromeliaceus* and *P. lacrimosus* was later indirectly falsified by Ron et al. (2020), who for the first time studied molecular samples of both taxa, which apparently have a much more restricted distribution than previously suggested. One of the unidentified species from Panguana contained in the paratype series of *P. mendax* likely corresponds to the population included in our molecular analysis as *Pristimantis* sp. Panguana (Fig. 8), being the closest known relative of *P. loeslein* (Fig. 1; uncorrected p-distance 4.7%). Likely, this candidate species, with its taxonomic status yet to be clarified, has been identified as *P. bromeliaceus* by Morales and Icochea (2000), but is actually only distantly related to it (Fig. 1). Based on morphological examination of our Panguana material, we here also confirm that a second species of the *P. lacrimosus* group (voucher specimen FGZC 6300; Fig. 9) occurs at Panguana (likely the species identified as *P. lacrimosus* by Morales and Icochea 2000), that differs morphologically from *P. mendax* and shows greatest similarities with *P. royi* described by Morales (2007) from a locality only approximately 68 km southwest of Panguana (Fig. 7). To our knowledge, so far no sample reliably assignable to nominal *P. mendax* has been included in any molecular phylogenetic study. Our attempts to find *P. mendax* at the type locality in 2022 were unsuccessful.

Another taxonomic uncertainty concerns *Pristimantis olivaceus*, with its type locality situated at the lower Andean slopes of central Bolivia (Chapare region, Departamento Cochabamba), that has also been recorded from southern Peru (Pakitza, PN Manu, Departamento Madre

de Dios) in the original description (Köhler et al. 1998). Although several southern Peruvian populations likely correspond to *P. olivaceus* (J.C. Chaparro, pers. comm.), the identity of many populations flagged as it or as *P. cf. olivaceus* (e.g., Villacampa-Ortega et al. 2017; Moravec et al. 2020; Carrión-Olmedo and Ron 2021) should be regarded with reservation. For example, the population from northern Bolivia (Palmira, Departamento Pando) included in our analysis as *P. cf. olivaceus* (Fig. 1) likely does not represent nominal *P. olivaceus*, and the same is true for the record from San Antonio, Departamento Pando, northern Bolivia, by Moravec et al. (2011), as is apparent from the photo provided by these authors (their fig. 2F), showing a species that differs from *P. olivaceus* by dorsal color pattern, iris color, and snout shape. Our attempts to obtain DNA sequences from topotypical samples of *P. olivaceus* unfortunately failed.

In addition to these difficulties, the presumed loss of the type specimens of *P. royi* (MUSM 11186–11187; Morales 2007), which are not present in the MUSM collection (absence of any information about their whereabouts), hampers the clarification of its identity and morphologically very similar populations in the same general area (e.g., Panguana; see above, Fig. 8).

These cases, together with the fact that other nominal species lacking phylogenetic information may actually be part of the *P. lacrimosus* group (e.g., *P. rhodostichus*), exemplify the challenging taxonomy of these frogs in Peru and highlight the need for future integrative taxonomic studies, including topotypic samples.

The closest nominal relative of the new species described herein, *Pristimantis loeslein*, was revealed by our analysis to be *P. pulchridormientes* occurring at Tingo Maria National Park (uncorrected p-distance 7.9%). Notably, these two species share a similar habitat, altitudinal distribution, and occur in close geographical proximity, with their respective type localities being only around 28 km airline distance apart (see Chávez and Catenazzi

2016). However, the Tingo Maria National Park and the southern Cordillera Azul are separated by the Huallaga river valley, which at Tingo Maria is at an elevation of approximately 640 m a.s.l., and thus about 1000 m lower in elevation than the habitats of both species. The Huallaga valley may constitute a boundary of biogeographic areas and act as a dispersal barrier that significantly influenced the evolution of *Pristimantis*, leading to speciation through vicariance (Cracraft 1985; Smith et al. 2014). It is easily imaginable that the lower river valley hinders gene flow for montane forest species which are adapted to moister and cooler environments, compared to their relatives in the lowlands. This hypothesis is supported by another species-pair in the *Pristimantis conspicillatus* species group, namely *P. nebulosus*, which shares its type locality with *P. loeslein* in the southern Cordillera Azul and its sister species *P. symptosus* occurs west of the Huallaga river valley in the Cordillera de Carpish (Köhler et al. 2022).

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