A new cryptic species of the *Pristimantis lacrimosus* group (Anura, Strabomantidae) from the eastern slopes of the Ecuadorian Andes

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Abstract

With 566 species, the neotropical genus *Pristimantis* is the most speciose vertebrate genus. As a result of its striking diversity, taxonomic reviews remain a challenge. Herein, we present an updated phylogeny of the *Pristimantis lacrimosus* group and describe a new species from Llanganates and Sangay National Parks. We also report, for the first time, the phylogenetic position of *Pristimantis degener*, *P.* eugeniae, *P.* katoptroides, and *P.* petersi. Based on our phylogeny, we add two species to the *Pristimantis lacrimosus* group. Through the integration of molecular and bioacoustic evidence, we describe a new species which was hidden under “*Pristimantis petersi*”. *Pristimantis petersioides* sp. nov. is most closely related to *Pristimantis petersi* and an undescribed species from Peru. It can be distinguished from *P. petersi* by its advertisement call and large genetic differences (uncorrected *p*-genetic distances 7.9% to 8.4% for gene 16S). Moreover, the new species and *P. petersi* are not sister species. We suggest assigning the new species to the Endangered Red List category because it has a small distribution range with deforestation as result of agriculture and other anthropogenic influences.

Key Words

Amphibia, Bioacoustics, Conservation, Diversity, National Parks, Phylogeny, Taxonomy

Introduction

The genus *Pristimantis* Jiménez de la Espada, 1870 has bewildered scientists for its striking diversity. Comprising 566 Neotropical species it is the most speciose vertebrate genus (Hedges et al. 2008; Frost 2021). In Ecuador, this genus encompasses more than one third of all anuran species, with 233 out of 640 species (Ron et al. 2019).

The astounding species richness of *Pristimantis* has been attributed to terrestrial breeding – direct embryonic development without a tadpole stage (Padial et al. 2014) – and the appearance of geographic barriers as result of the Andean uplift (Lynch and Duellman 1997; Mendoza et al. 2015). However, other sympatric Andean genera with similar reproductive mode (e.g., *Strabomantis* Peters, 1863) and age are not as diverse suggesting that terrestrial breeding and Andean uplift are not the only factors explaining the high diversity of *Pristimantis*.

The number of described species of *Pristimantis* is increasing rapidly as result of the use of DNA sequences allowing the discovery of a large number of cryptic species. DNA sequences helps to achieve better informed taxonomic decisions and speed up species discovery with more than 40 species of *Pristimantis* (e.g. Ortega et al. 2015; Guayasamin et al. 2017; Páez and Ron 2019) described in Ecuador in the last five years. In some clades, the number of undescribed species outnumbers described species (e.g., Ortega et al. 2015; Páez and Ron 2019) suggesting that there could be hundreds of undescribed species of *Pristimantis*. 

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Taxonomy within this group have been problematic and unstable because most species descriptions have been based on morphological traits (Lynch and Duellman 1997), which can be highly variable and homoplastic (e.g., Guayasamin et al. 2015; Páez and Ron 2019). Taxonomic problems in Pristimantis are still pervasive and are still far from being fully resolved (e.g., Páez and Ron 2019; Reyes-Puig et al. 2019; Moravec et al. 2020).

One clade of Pristimantis containing undescribed species is the Pristimantis lacrimosus species group (Ron et al. 2020). Hedges et al. (2008) proposed it as monophyletic based on molecular data from only three species. However, more recent phylogenies with better species sampling showed that this group was paraphyletic (Padial et al. 2014; Rivera-Correa and Daza 2016). Rivera-Correa and Daza (2016) identified two non-sister clades within the Pristimantis lacrimosus species group, “clade A” endemic to Colombia and “clade B” composed by species from Central America, Ecuador, and Peru. González-Durán et al. (2017) proposed the Pristimantis boulengeri group for “clade A” and hypothesized that “clade B” corresponds to the P. lacrimosus species group. That assignment was questioned by Rivera-Correa and Daza (2020) but was demonstrated as correct by Ron et al. (2020) who included in their phylogeny, for the first time, the type species for the group, P. lacrimosus (Jiménez de la Espada, 1875). Ron et al. (2020) also described three new species and redefined the group to include all descendant species of the most recent common ancestor of P. eremitus (Lynch, 1980) and P. lacrimosus for a total of 36 formally described species.

Despite these advances, there still are species of the Pristimantis lacrimosus group which have never been included in molecular phylogenies. One of them is Pristimantis petersi (Lynch & Duellman, 1980). Since its description, this species suffered several taxonomic changes. Lynch (1991) changed its name to Eleutherodactylus petersorum to avoid homonymy with a Mexican species with the same epithet, which now is known as Eleutherodactylus nitidus (Peters 1870). Additionally, Lynch (1996) created the name Eleutherodactylus johnwighti, as an amend to the previous epithet petersorum. Afterwards, Frost (2009) applied Article 59.4 of the International Code of Zoological Nomenclature making Eleutherodactylus johnwighti and Eleutherodactylus petersorum invalid replacement names because the taxa in question are not congeneric and recovered the first epithet used for this species.

Pristimantis petersi has been usually assigned to the P. lacrimosus species group. Lynch and Duellman (1980) placed it in the P. unistrigatus group, P. lacrimosus assembly. Later, Lynch and Duellman (1997) placed it in the unistrigatus group, martincensis series. Hedges et al. (2008) proposed this species as a member of the Pristimantis lacrimosus group and Padial et al. (2014) and Rivera-Correa and Daza (2016) followed this proposal but without including Pristimantis petersi in their phylogenies.
Materials and methods

Nomenclature

Taxon names follow Duellman and Lehr (2009) and AmphibiaWeb (2019).

DNA extraction, amplification and sequencing

We inferred the phylogenetic relationships of the new species and closely related taxa based on DNA sequences of one nuclear gene: Recombination activating gene 1 (RAG-1) and three mitochondrial genes: 12S rRNA (12S), 16S rRNA (16S), NADH dehydrogenase subunit 1 (ND1) and their flanking tRNAs. DNA was extracted from muscle or liver tissue preserved in 95% ethanol using standard Guanidine thiocyanate extraction protocols. We used polymerase chain reaction (PCR) to amplify DNA fragments. Primers used for amplification of 12S were t-Phe-frog and t-Val-frog (Wiens et al. 2005), 12SZ-L and 12SK-H (Goebel et al. 1999), for 16S, primers were 12SL13 (Feller and Hedg 1998), 16L19 and 16H36E (Heinicke et al. 2007), for ND1, primers were WL379, WL384, t-Met-frog and 16S-frog (Moen and Wiens 2009), for RAG1, primers were R182, R270, Rag1FF2, Rag1FR2 (Heinicke et al. 2007). PCR amplification was performed under standard protocols and sequenced in both directions by the Macrogen Sequencing Team (Macrogen Inc., Seoul, Korea). All sequences were assembled in Genoic 7.1.7. and then exported to Mesquite version 3.40 where each genomic region was aligned separately using default parameters in Muscle (Edgar 2004). Unambiguous alignment errors were corrected manually in Mesquite (Maddison and Maddison 2018). The aligned matrix is available in https://doi.org/10.5281/zenodo.3785738. To calculate the uncorrected pairwise genetic p-distances of 16S we used MEGA7 on a fragment of 653 pb (Kumar et al. 2016).

We included 156 GenBank sequences of congeneric species. To find relevant sequences in GenBank, we made a 16S BLASTn search with the sequences of the new species (Table 1). These sequences showed that the most similar sequences belong to species from the P. lacrimosus group: P. schultei (Duellman, 1990) (identity 88.62%, accession EF493681), P. bromeliaceus (Lynch, 1979) (identity 86.92%, accession EF493351.1) and others such as P. galdi Jiménez de la Espada, 1870 (identity 87.76%, accession EU186670.1), and P. cf. mendax (Duellman, 1978) (identity 87.07%, accession EU186659.1). Therefore, we included sequences used in previous studies on P. lacrimosus group (e.g. Arteaga et al. 2013; Palial et al. 2014; Ortega-Andrade et al. 2015; Rivera-Correa and Daza 2016; Chávez and Catenazzi 2016; Shepack et al. 2016; Guayasamin et al. 2017; Ron et al. 2020; Rivera-Correa and Daza 2020). Samples of Niceforonia nigrovittata (Andersson, 1945), N. elassodisca (Lynch, 1973), and P. w-nigrum (Boettger, 1892) were set as outgroups. The combined DNA matrix had up to 4067 bp and 140 terminals.

Phylogeny

The phylogeny was inferred using Maximum Likelihood as optimality criterion. To choose the substitution models that best adjusted to our sequences, we used Model Finder under the command MFP+MERGE (Kalyaanamoorthy et al. 2017; Chernomor et al. 2016) as implemented in IQ-TREE 1.6.8 (Nguyen et al. 2015). We partitioned the sequences by gene and by codon position in coding genes. For the ML search we used IQ-TREE 1.6.8 (Nguyen et al. 2015) under default values. To assess branch support we obtained ultrafast bootstrap values from 2000 pseudoreplicates and 10000 iterations as maximum number to stop (commands -bb 2000 and -nm 10000 in IQ-TREE) and SH-like approximate likelihood ratio test (SH-aLRT ) with 1000 replicates (-alrt 1000 command, Guindon et al. 2010). We considered that branches with bootstrap values > 95 and SH-aLRT values > 80 had strong support. Additionally, we inferred phylogenies from mitochondrial DNA and the nuclear gene RAG1 separately to compare the topology of the phylogenetic tree derived from DNA regions with independent segregation.

Morphology

Diagnostic characters and comparisons are based on preserved specimens from Museo de Zoología at Pontificia Universidad Católica del Ecuador, Quito (QCAZ) and, when available digital photographs. Examined specimens are listed as Suppl. material 1. Character definitions and terminology follow Duellman and Lehr (2009). For subarticlar tubercle terminology we follow Ron et al. (2020). Sex was determined by presence of nuptial pads or vocal slits, and direct inspection of gonads. Descriptions of coloration and variation in life are based on digital photographs. We examined the following qualitative characters: dorsal and ventral skin texture, presence of tympanic membrane and annulus, snout shape, presence of rostral papilla, presence of vomerine odontophores, presence of vocal slits and gular sac in males, relative length of fingers and toes, disc shape, presence of dorsolateral, discoidal and supratympanic folds, presence of lateral fringes on fingers and toes, presence of palmar, ulnar, tarsal, metatarsal, subarticular, supernumerary, knee, heel, and eyelid tubercles, and webbing on fingers and toes. We follow the name “hyperdistal tubercle” proposed in Ospina-Sarria and Duellman (2019) to refer to the most distal tubercle in Fingers and Toes.

Adults were measured with digital calipers (to the nearest ± 0.01 mm) for eleven morphological variables, following Duellman and Lehr (2009): (1) snout-vent length; (2) tibia length; (3) foot length; (4) head length;

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Table 1. Genbank accession numbers for DNA sequences used for phylogenetic analyses.

| Species                  | Voucher       | 12S Accession Numbers | 16S Accession Numbers | RAG1 Accession Numbers | ND1 Accession Numbers |
|--------------------------|---------------|-----------------------|-----------------------|------------------------|-----------------------|
| Niceforonia elassodisca  | QCAZ52495     | MW567328              | MW567375              | MW451754               | MW567403              |
| Niceforonia nigrovittata | QCAZ59410     | NA                    | MW567340              | NA                     | NA                    |
| Pristimantis acerus      | KU 217876     | EF493678.1             | NA                    | EF493696.1             | EF493432.1            |
| P. amatum               | KU 218703     | NA                    | NA                    | NA                     | NA                    |
| P. acuminatus           | CORBIDI 4769  | MN128391              | NA                    | NA                     | NA                    |
| P. aff. subsigillatus    | QCAZ58017     | MW567360              | MW567373              | MW451755               | MW567411              |
| P. altamazonicus         | KU 215460     | EF493670.1             | NA                    | EF493670.1             | NA                    |
| P. angulifer           | KU 177673     | EF493523.1             | NA                    | EF493523.1             | NA                    |
| P. annulineatus         | KU 177673     | EF493523.1             | NA                    | EF493523.1             | NA                    |
| P. appendiculatus       | KU 177673     | EF493523.1             | NA                    | EF493523.1             | NA                    |
| P. baccalis             | QCAZ58017     | MW567360              | MW567373              | MW451755               | MW567411              |
| P. boulengeri           | MHIUA 8951    | NA                    | MW567357              | MW451727               | NA                    |
| P. brevirostris         | nrps 0059     | JN991498.1             | NA                    | JN991443.1             | NA                    |
| P. brocheli            | QCAZ16699     | MT636505              | MT636527              | MT635618               | MT635659              |
| P. calcurations         | KU 177658     | EF493523.1             | NA                    | EF493523.1             | NA                    |
| P. celator             | QCAZ66230     | MW567326              | MW567368              | MW451723               | MW567406              |
| P. cf. mendax           | MTD 45080     | EU186659.1             | NA                    | EU186669.1             | NA                    |
| P. convolutus           | QCAZ5349      | NA                    | MW567358              | MW451746               | MW567394              |
| P. crucifer            | KU 177733     | EU186736.1             | NA                    | EU186718.1             | NA                    |
| P. curtipes            | QCAZ40722     | MW567323              | MW567363              | MW451750               | MW567416              |
| P. degener             | QCAZ40304     | MW567305              | MW567376              | MW451761               | MW567379              |
| P. diadematus           | KU 221999     | EU186688.1             | NA                    | EU186668.1             | NA                    |
| P. dissimulatus         | QCAZ59442     | MW567354              | MW567375              | MW451735               | MW567419              |
| P. doronotus            | QCAZ166738    | KP082864.1             | NA                    | KP082874.1             | NA                    |
| P. ecaudoviris          | KU 5530       | KX785339               | NA                    | KX785343               | NA                    |
| P. ecrizicrineri        | KU 5531       | KX785340               | KX785344              | NA                    | KX785348              |
| P. enigmaticus          | QCAZ40918     | MT636513               | MT636520              | MT635636               | MT635670              |
| P. erinaceus            | QCAZ49002     | NA                    | NA                    | NA                     | NA                    |
| P. frogi                | QCAZ49652     | NA                    | MW567366              | MW451729               | MW567391              |
| P. galeifer            | QCAZ43392     | NA                    | MW567333              | NA                    | NA                    |
| P. eucalixa             | DHEMCN8809    | M853052                | MT853039              | NA                    | NA                    |
| P. gaudii               | QCAZ52367     | MW567322               | MW567353              | MW451743               | MW567380              |
| P. glandulosus          | KU 218002     | EF493567.1             | NA                    | EF493567.1             | NA                    |
| P. imitatrix            | KU 215476     | EF493584.1             | NA                    | EF493584.1             | NA                    |
| P. imitator            | KU 218015     | EF493577.1             | NA                    | EF493577.1             | NA                    |
| P. jahuapitak          | MHIUA 7249    | KP082862.1             | KP082870.1            | NA                    | NA                    |
| P. kathoproides         | QCAZ46360     | NA                    | MW567378              | MW451753               | MW567408              |
| P. lacrimosus           | QCAZ52538     | MW567322               | MW567353              | MW451743               | MW567380              |
| P. lecythidomystax      | KU 210802     | EF493567.1             | NA                    | EF493567.1             | NA                    |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| P. limoncochensis       | QCAZ43794     | NA                    | MT635625              | MT635627               | MT635665              |
| Species              | Voucher               | 12S    | 16S    | RAG1   | ND1  |
|---------------------|-----------------------|--------|--------|--------|------|
| *P. nankints*       | QCAZ69137             | NA     | MT63514| MT63535| NA   |
| *P. nyctophylax*    | KU 177812             | EF493526.1 | EF493526.1 | NA | NA |
| *P. olivaceus*      | QCAZ49637             | NA     | MT63561 | MT63562 | MT63566 |
| *P. omeviridis*     | QCAZ55392             | MN82140 | NA     | NA     | NA   |
| *P. orcesi*         | KU 218021             | EF493526.1 | EF493526.1 | NA | NA |
| *P. ornaticusimus*  | MZUTI 4798            | KU720464 | NA     | NA     | NA   |
| *P. olivaceus*      | QCAZ69137             | NA     | MT63514| MT63535| NA   |
| *P. nyctophylax*    | KU 177812             | EF493526.1 | EF493526.1 | NA | NA |
| *P. olivaceus*      | MZUTI 32288           | NA     | MT63561 | MT63562 | MT63566 |
| *P. omeviridis*     | QCAZ55392             | MN82140 | NA     | NA     | NA   |
| *P. orcesi*         | KU 218021             | EF493526.1 | EF493526.1 | NA | NA |
| *P. ornaticusimus*  | MZUTI 4798            | KU720464 | NA     | NA     | NA   |
| *P. olivaceus*      | QCAZ69137             | NA     | MT63514| MT63535| NA   |
| *P. nyctophylax*    | KU 177812             | EF493526.1 | EF493526.1 | NA | NA |
| *P. olivaceus*      | MZUTI 32288           | NA     | MT63561 | MT63562 | MT63566 |
| *P. omeviridis*     | QCAZ55392             | MN82140 | NA     | NA     | NA   |
| *P. orcesi*         | KU 218021             | EF493526.1 | EF493526.1 | NA | NA |
| *P. ornaticusimus*  | MZUTI 4798            | KU720464 | NA     | NA     | NA   |
| *P. olivaceus*      | QCAZ69137             | NA     | MT63514| MT63535| NA   |
| *P. nyctophylax*    | KU 177812             | EF493526.1 | EF493526.1 | NA | NA |
| *P. olivaceus*      | MZUTI 32288           | NA     | MT63561 | MT63562 | MT63566 |
| *P. omeviridis*     | QCAZ55392             | MN82140 | NA     | NA     | NA   |
| *P. orcesi*         | KU 218021             | EF493526.1 | EF493526.1 | NA | NA |
| *P. ornaticusimus*  | MZUTI 4798            | KU720464 | NA     | NA     | NA   |
| *P. olivaceus*      | QCAZ69137             | NA     | MT63514| MT63535| NA   |
| *P. nyctophylax*    | KU 177812             | EF493526.1 | EF493526.1 | NA | NA |
| *P. olivaceus*      | MZUTI 32288           | NA     | MT63561 | MT63562 | MT63566 |
| *P. omeviridis*     | QCAZ55392             | MN82140 | NA     | NA     | NA   |
| *P. orcesi*         | KU 218021             | EF493526.1 | EF493526.1 | NA | NA |
| *P. ornaticusimus*  | MZUTI 4798            | KU720464 | NA     | NA     | NA   |
| *P. olivaceus*      | QCAZ69137             | NA     | MT63514| MT63535| NA   |
| *P. nyctophylax*    | KU 177812             | EF493526.1 | EF493526.1 | NA | NA |
| *P. olivaceus*      | MZUTI 32288           | NA     | MT63561 | MT63562 | MT63566 |
| *P. omeviridis*     | QCAZ55392             | MN82140 | NA     | NA     | NA   |
| *P. orcesi*         | KU 218021             | EF493526.1 | EF493526.1 | NA | NA |
| *P. ornaticusimus*  | MZUTI 4798            | KU720464 | NA     | NA     | NA   |
| *P. olivaceus*      | QCAZ69137             | NA     | MT63514| MT63535| NA   |
| *P. nyctophylax*    | KU 177812             | EF493526.1 | EF493526.1 | NA | NA |
| *P. olivaceus*      | MZUTI 32288           | NA     | MT63561 | MT63562 | MT63566 |
| *P. omeviridis*     | QCAZ55392             | MN82140 | NA     | NA     | NA   |
| *P. orcesi*         | KU 218021             | EF493526.1 | EF493526.1 | NA | NA |
| *P. ornaticusimus*  | MZUTI 4798            | KU720464 | NA     | NA     | NA   |
| *P. olivaceus*      | QCAZ69137             | NA     | MT63514| MT63535| NA   |
| *P. nyctophylax*    | KU 177812             | EF493526.1 | EF493526.1 | NA | NA |
(5) head width; (6) eye diameter; (7) tympanum diameter; (8) interorbital distance; (9) eye width; (10) internarial distance; and (11) eye-nostril distance. Morphometric analyses were performed based on measurements of adults (number of specimens in parenthesis): *P. petersi* (12), *P. aff. petersi* (new species; 54).

To explore morphometric differentiation between species, we applied a Principal Components Analysis (PCA). To remove the effect of size covariation, we carried out linear regressions between the morphometric variables and SVL. The PCA was applied to the residuals of the regressions. Morphometric variables associated with eyes (i.e., eye diameter, interorbital distance, eyelid width, internarial distance and eye-nostril distance) had weak correlation with snout-vent length. Low correlation appears to be a result of the difficulty of defining the eye edge on preserved specimens. Therefore, were removed those variables from the analysis. Prior to the PCA, we ran a MANOVA on the residuals to test for morphometric sexual dimorphism independent of size differences. Because the MANOVA was non-significant, we pooled the measurements of both sexes on a single PCA.

**Bioacoustics**

To assess species limits between the new species and the closely related *P. petersi*, we analyzed calls from three males of the new species: QCAZ58940, SVL = 19.3 mm, from Refuge 1, Sardinayacu, Sangay National Park, Morona Santiago Province (2.0983°S, 78.1555°W, 1406 m) collected on 21 January 2015, air temperature 19 °C, recorded in situ by Daniel Rivadeneira, and QCAZ59466, SVL = 19.1 mm, from the ravines of Yurugyacu river, Zarentza community, Llanganates National Park, Pastaza Province, (1.3524°S, 78.0597°W, 1419 m) collected on 24 February 2015 and recorded in captivity on 6 March 2015 by Santiago R. Ron and one male not collected from Sardinayacu, Sangay National Park, Morona Santiago Province, recorded by Diego Batallas. Advertisement calls of *P. petersi* were analyzed from two adult males (not collected) from near its type locality, Cocodrilos, Napo Province, (0.66812°S, 77.7975°W, 1725 m) recorded on 22 June 2016 by Santiago R. Ron. We did not have size data for the recorded individuals of *P. petersi*. However, we collected other adult males on the same night and chorus and used the size of those individuals to assess interpopulation size differences. For two of the recorded males of the new species, SVL was 19.1 and 19.3 mm; for *Pristimantis petersi* average SVL was 17.2 mm (16.5–17.8 mm; n = 3). Recordings were made in WAV format, with a sample rate of 44100 Hz and 16-bits. Call variables were measured with RAVEN PRO 1.5 (Charif et al. 2010), under a Hanning function, 2048 DFT, sample rate of 46 kHz and a grid spacing of 20 kHz.

Most of our recordings lacked temperature information. However, the variables that allowed us to differentiate the new species from the closely related *P. petersi*, where call duration and call frequency which are static and are not strongly influenced by ambient temperature (Köhler et al. 2017). Moreover, equatorial Andean forests have low seasonality and with low temperature variation, so it is unlikely that our results are biased by temperature differences between localities or seasons. At Zarentza, for example, in 2015, the monthly average of the minimum daily temperature varied between 14.7 (January) and 15.9 °C (May) while at Cocodrilos (where recordings for *P. petersi* were made) it had a range between 15.1 (January) and 16.0 °C (November). Temperature data were obtained from the WorldClim database (https://www.worldclim.org/).

For call measurements and terminology, we followed the call-centered approach by Köhler et al. (2017). We followed the step-by-step guide of Köhler et al. (2017) for measuring variables: we measured temporal variables such as call duration, call rate, call interval, call rise time and amplitude modulation in the oscillogram and the spectral variables such as frequency band, fundamental frequency, and dominant frequency in the spectrogram. Due to call structure, parameters such as notes, notes rates, note series, pulses and pulse rates were absent and only eight acoustic parameters (modified from Köhler et al. 2017) were measured: (1) Call duration = time from beginning to end of the call, measured from oscillogram; (2) Call rate = number of calls per minute; (3) Call interval = time from end of call to beginning of next call; (4) Call rise time = time from beginning of call to point of maximum amplitude; (5) Amplitude modulation = change in the amplitude level of a sound wave over time; (6) Frequency band = difference between upper and lower frequencies measured visually along the entire call; (7) Fundamental frequency = frequency with highest energy on 1st harmonic in the call; (8) Dominant frequency = frequency with highest energy along entire call. Recordings are deposited in the Sound Archive of Museo de Zoología QCAZof Pontificia Universidad Católica del Ecuador and are available at the Anfibios del Ecuador website, https://bioweb.bio/faunaweb/amphibiaweb/).

**Results**

**Phylogeny and genetic distances**

The Maximum Likelihood tree (Fig. 1) is similar in topology to Rivera-Correa and Daza (2016), Rivera-Correa and Daza (2020), and Ron et al. (2020). Support values for the *Pristimantis lacrimosus* group is strong (bootstrap = 100); this clade includes the species reported in Ron et al. (2020) as well as species not included in previous phylogenies as *P. degener* (Lynch and Duellman, 1997), *P. petersi*, and the new species. Additionally, we report for the first time the phylogetic position of *Pristimantis eugeniae* (Lynch and Duellman, 1997) and *P. katoptroides* (Flores, 1988). *Pristimantis eugeniae* is the sister species of a clade formed by *P. glandulosus* (Boulenger,
Pristimantis katoptroides is sister to *P. quaquaversus* (Lynch, 1974) + *P. melanogaster* (Duellman and Pramuk, 1999). *Pristimantis subsigillatus* (Boulenger, 1902) was polyphyletic because two samples (KU 218147 and QCAZ49637) were more closely related to *P. nyctophylax* (Lynch, 1976) than to the other samples of *P. subsigillatus*. After reviewing the morphology of QCAZ49637 we concluded that it belongs to *P. nyctophylax*. Because KU 218147 is genetically identical, we also assigned it to *P. nyctophylax*.

The ML tree from mitochondrial DNA shows similar topology to the ML tree from all genes. The best-fit models of DNA evolution for each partition are available as Suppl. material 2.

The mtDNA tree shows strong support for the *Pristimantis lacrimosus* group (bootstrap = 99), for *Pristimantis petersi* (bootstrap = 100) and for the new species (bootstrap = 99). The ML tree inferred from RAG1 shows lower support values but is congruent in showing a monophyletic *Pristimantis lacrimosus* group (bootstrap = 91) and in confirming a close relationship between *P. petersi* and the new species. The mtDNA and RAG1 phylogenies do not show strongly supported incongruences. Mitochondrial DNA and RAG1 phylogenetic trees are available as Suppl. material 3.

The new species is the sister to *P. petersi* and an undescribed species from Cordillera Escalera, Peru. The uncorrected pairwise p-genetic distances for 16S between *P. petersi* and the new species range from 7.9% to 8.4%. The clade comprising these three species is sister to an

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**Figure 1.** Maximum likelihood tree of the *Pristimantis lacrimosus* group inferred from a partitioned analysis of 4026 aligned sites of DNA sequences of the mitochondrial genes 12S, 16S, and ND-1 and the nuclear gene RAG-1. Bold characters highlight individuals included for the first time in a phylogeny, red taxa highlight previously misidentified species. *Pristimantis petersioides* sp. nov. is highlighted in blue. SH-aLRT support (above branch) and ultrafast bootstrap support (below) are shown as percentages; asterisks denote 100% for both measures. Outgroups are not shown. Number for voucher museum specimens are shown to the left of the species name; locality is shown to the right and country abbreviation at the end, as follows: **ECU** Ecuador, **PER** Peru, **COL** Colombia, **VEN** Venezuela, **GUY** Guyana, **PAN** Panama.
undescribed species from Bombuscaro, Podocarpus National Park, Zamora Province, Ecuador. Samples of the new species separate in two sister clades, a northern clade in Zarentza, Pastaza Province and a southern clade from Sardinayacu and Bosque Protector Abanico, Morona Santiago Province; the uncorrected pairwise p-genetic distances between these clades range from 1.2% to 1.4%.

Morphometric analysis

MANOVA results showed no sexual dimorphism (after removing size effects) on the new species and *P. petersi*. The PCA show broad overlap in morphometric space between both species (Fig. 2). PC I (49.5%) had high loadings on head width, head length, and tibia length while PC II (21.5%) had high loading on tympanum diameter. Both principal components explained 71.0% of the morphometric variation (Table 2).

Systematic account

The differences in advertisement calls (see Comparisons with other Species section), the branch lengths in the phylogeny, and genetic distances indicate that the new species, in fact, represents a different species from *P. petersi* (see below). In the following section, we update the species content of the *Pristimantis lacrimosus* group and describe the new species.
*P. degener* (Boulenger, 1899), *P. deyi* Pardalinus, Caminer, Sagredo, Navarrete, Ortega, Varela-Jaramillo, Maldonado-Castro & Terán, 2020, *P. reolineatus* rela-Jaramillo, Maldonado-Castro & Terán, 2020, *P. tayrona* Suárez-Segovia, 2007), *P. tromeraniae* Ron, Carrión, Caminer, Sagredo, Navarrete, Ortega, Varela-Jaramillo, 2017, *P. tenigmaticus* Guayasamin, Hutter, Tapia, Culebras, Peñafiel, Pyron, 2020, *P. petersioides* & Duellman, 1980), *P. petersi* Lundberg, Aguilar and von May, 2006), *P. ornattissimus* (Despax, 1911), *P. omeviridis* Ortega-Andrade, Rojas-Soto, Valencia, Espinosa de los Monteros, Morrone, Ron & Cannatella, 2015, *P. pardinus* di-Urrutia & Arista-Tuanama, 2010, *P. padiali* Ron & Cannatella, 2015, *P. tayrona* Suárez-Segovia, 2007), *P. tazorami* Jiménez de la Espada, 1870, *P. tamaguanae* Pardalinus, Caminer, Sagredo, Navarrete, Ortega, Varela-Jaramillo, Maldonado-Castro & Terán, 2020, *P. petersioides* & Duellman, 1980), *P. petersi* Lundberg, Aguilar and von May, 2006), *P. ornattissimus* (Despax, 1911), *P. omeviridis* Ortega-Andrade, Rojas-Soto, Valencia, Espinosa de los Monteros, Morrone, Ron & Cannatella, 2015, *P. pardinus* di-Urrutia & Arista-Tuanama, 2010, *P. padiali* Ron & Cannatella, 2015, *P. tayrona* Suárez-Segovia, 2007), *P. tazorami* Jiménez de la Espada, 1870, *P. tamaguanae* Pardalinus, Caminer, Sagredo, Navarrete, Ortega, Varela-Jaramillo, Maldonado-Castro & Terán, 2020, *P. petersioides* & Duellman, 1980), *P. petersioides* sp. nov. and *P. petersi* sp. nov. by Daniel Rivadeneira, David Velalcázar, Javier Pinto, Francy Mora, Darwin Nuñez, Juan Sanchez, and Andrea Correa; QCAZ58936, 58939, QCAZ58941, females, QCAZ58940, 58942–43, males from Refuge

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**Figure 2.** Principal components 1 and 2 from analysis of five size-corrected morphological variables. See Table 2 for character loadings on each component.

**Table 2.** Character loadings, eigenvalues, and percentage of explained variance for Principal Components (PC) I–II. The analysis was based on the residuals of morphometric variables of adult *P. petersioides* sp. nov. and *P. petersi*. Bold figures indicate highest loadings.

| Variable               | Character loading | PC I | PC II |
|------------------------|-------------------|------|-------|
| Tibia length           | 0.7855            | -0.3038 |
| Foot length            | 0.7066            | 0.0834 |
| Head length            | 0.7873            | 0.2181 |
| Head width             | 0.8332            | 0.2434 |
| Tympanum               | -0.2087           | 0.9334 |
| Eigenvalues            | 4.9483            | 2.1547 |
| %                      | 49.48             | 21.54 |

**Pristimantis lacrimosus species group**

**Content.** We include all the descendants from the most recent common ancestor of *P. eremitus* and *P. lacrimosus* according to Ron et al. 2020. We exclude *Pristimantis eugeniae* from this group because it belongs to the sister clade of *P. lacrimosus* species group. We also include *P. degener* which is sister to *P. subgilissatus* and the new species.

According to our findings and those of Ron et al. 2020, the *Pristimantis lacrimosus* group comprises 39 species (species included in the phylogeny are marked with an asterisk): *P. acuminatus* (Shreve, 1935), *P. amaguanae* Ron, Carrión, Caminer, Sagredo, Navarrete, Ortega, Varela-Jaramillo, Maldonado-Castro & Terán, 2020, *P. aureolineatus* (Guayasamin, Ron, Cisneros-Heredia, Lamar & McCracken, 2006), *P. bromeliaceus* (Lynch, 1979), *P. calina* Ospina-Sarria & Duellman, 2019, *P. crucifer* (Boulenger, 1899), *P. degener* (Lynch & Duellman, 1997), *P. deyi* Lehr, Gregory & Catenazzi, 2013, *P. ecuadorensis* Guayasamin, Hutter, Tapia, Culebras, Peñafiel, Pyron, Morochz, Funk & Artieaga-Navarro, 2017, *P. enigmaticus* Ortega-Andrade, Rojas-Soto, Valencia, Espinosa de los Monteros, Morrone, Ron & Cannatella, 2015, *P. eremitus* (Lynch, 1980), *P. galdi* Jiménez de la Espada, 1870, *P. lacrimosus* (Jiménez de la Espada, 1875), *P. latericus* Batallas & Brito, 2014, *P. limoncochensis* Ortega-Andrade, Rojas-Soto, Valencia, Espinosa de los Monteros, Morrone, Ron & Cannatella, 2015, *P. mendax* (Duellman, 1978), *P. minno Arteaga, Yanez-Munoz & Guayasamin, 2013, *P. moro* (Savage, 1965), *P. nankints* Ron, Carrión, Caminer, Sagredo, Navarrete, Ortega, Varela-Jaramillo, Maldonado-Castro & Terán, 2020, *P. nycliphyllax* (Lynch, 1976), *P. olivaceus* (Köhler, Morales, Lötters, Reichle & Aparicio, 1998), *P. omeviridis* Ortega-Andrade, Rojas-Soto, Valencia, Espinosa de los Monteros, Morrone, Ron & Cannatella, 2015, *P. ornattissimus* (Despax, 1911), *P. padiali* Moravec, Lehr, Pérez-Peña, López, Gagliardi-Urrutia & Arista-Tuamana, 2010, *P. pardinus* (Lehr, Lundberg, Aguilar and von May, 2006), *P. petersi* (Lynch & Duellman, 1980), *P. petersioides* sp. nov. (herein), *P. pluvialis* Shepack, von May, Titto, & Catenazzi, 2016, *P. pseudoacuminatus* (Shreve, 1935), *P. romeroae* Ron, Carrión, Caminer, Sagredo, Navarrete, Ortega, Varela-Jaramillo, Maldonado-Castro & Terán, 2020, *P. royi* (Morales, 2007), *P. pulchridormientes* Chávez & Catenazzi, 2016, *P. schuelei* (Duellman, 1990), *P. subgilissatus* (Boulenger, 1902), *P. tantanti* (Lehr, Torres-Gastello & Suárez-Segovia, 2007), *P. taxrona* (Lynch & Ruiz-Carranza, 1985), *P. wauranti* (McCracken, Forstner & Dixon, 2007), *P. zimmermanae* (Heyer & Hardy, 1991), and *P. zorro* Rivera-Correa & Daza 2020.

**Distribution.** The *Pristimantis lacrimosus* group is distributed in Central America, the Guianan Shield, Pacific Basin of Ecuador, and the Amazon Basin. Its species richness peaks in the Ecuadorian Andes (n = 19) and Amazon basin of Ecuador and Peru (n = 14).

**Remarks.** We refrain from assigning *Pristimantis sneiderni* (Ospina-Sarria and Duellman 2019) to the *Pristimantis lacrimosus* group due to the lack of molecular evidence and following Ron et al. (2020).

**Pristimantis petersioides** sp. nov.

http://zoobank.org/F2204639-4B59-4071-A033-5BE637144BEE

Eleutherodactyly petersi Lynch & Duellman 1980 (in part)

Pristimantis petersi Bathallas & Brito 2016

Pristimantis petersi Brito et al. 2017

**Holotype.** (Figs 3, 4) QCAZ58939, adult female from Ecuador, Morona Santiago Province, Sangay National Park, Sardinayacu (2.0983°S, 78.1555°W), 1406 m. Found in amplexus with QCAZ58940. collected by Daniel Rivadeneira, David Velalcázar, Javier Pinto, Francy Mora, Darwin Nuñez, Juan Sanchez, and Andrea Correa; QCAZ58936, 58939, QCAZ58941, females, QCAZ58940, 58942–43, males from Refuge
Figure 3. Holotype of *Pristimantis petersiooides* sp. nov. QCAZ58939, adult female, SVL = 22.02 mm. Sangay National Park, Sardínayacu, Ecuador. A lateral view of live individual, B dorsal view of live individual, C dorsal view of preserved individual, D ventral view of preserved individual. Photographs A, B by Juan Carlos Sánchez, C, D by Julio C. Carrión-Olmedo

1 (2.0988°S, 78.1561°W, 1406 m), QCAZ58937–38, males from Chimereilla lagoon (2.0885°S, 78.2069°W, 1650 m) collected by Daniel Rivadeneira, Francy Mora, Juan Sánchez and Andrea Correa; QCAZ58881, 58950, females, QCAZ58949 male from the proximities of Cormorant lagoon (2.0738°S, 78.2195°W, 1835 m) collected by Javier Pinto, David Velalcázar and Darwin Nuñez, QCAZ58880, 58951, males from El Enmascarado lagoon (2.0600°S, 78.2207°W, 1796 m) collected by Javier Pinto, David Velalcázar and Darwin Nuñez. in January 2015. QCAZ59166, female, QCAZ59167, 58945–48, males from Refuge 3 (2.0757°S, 78.2157°W, 1724 m), collected by Santiago Ron, Diego Paucar, Pablo Venegas, Pamela Baldeón, Marcel Caminer and Kunam Nuñirquia; QCAZ59169–71, males from Cormorant lagoon (2.0738°S, 78.2195°W, 1835 m), collected by Santiago Ron, Diego Paucar, Pablo Venegas, Pamela Baldeón, Marcel Caminer and Kunam Nuñirquia, in February 2015. Pastaza Province: QCAZ53227, female, from Anzu river (1.4177°S, 78.0485°W, 1272 m a.s.l), col-

Evolution of Life, (74x796)
lected by Mauricio Ortega in May 2012. Llanganates National Park: QCAZ45846–50, 45892, 45898, males, from Challuwa Yacu river, Ankaku Reserve (1.2792°S, 78.0779°W, 2300 m) collected by Elicio Tapia and Silvia Aldás in October 2009; QCAZ66553, male, from Ankaku Reserve (1.2770°S, 78.0698°W, 2216 m) collected by Diego Almeida, Santiago Guamán, Darwin Nuñez, María Navarrete, Verónica Andrade, Angel Alvarado, Fernando Alvarado in January 2017, QCAZ59456, male, from Yurugyacu river (1.3523°S, 78.0597°W, 1419 m); QCAZ59451, 59467–68, 59479, males, from Zarentza community (1.3556°S, 78.0597°W, 1363 m); QCAZ59458–59, females from near Yurugyacu river (1.3523°S, 78.0597°W, 1419 m) collected by Daniel Rivadeneira, Francy Mora, Juan Carlos Sánchez, David Velalcázar, Darwin Nuñez and Javier Pinto in February 2015.

Referred specimens. Napo Province: QCAZ46159, male, from Salcedo-Tena highway, km 60 (0.9847°S, 78.1928°W, 2253 m), collected by Elicio Tapia and Fernando Nuñez in November 2009. Pastaza Province: QCAZ59452–53, 59460, 59464, juveniles from the ravines of Yurugyacu river (1.3523°S, 78.0597°W, 1419 m) collected by Daniel Rivadeneira, Franca Mora, Juan Carlos Sánchez, David Velalcázar, Darwin Nuñez and Javier Pinto in February 2015.

Suggested common name. English: Sardinayacu’s Rain Frog. Spanish: Cutín de Sardinayacu

Diagnosis. The assignment of the new species to the genus Pristimantis is based on the phylogeny (Fig. 1). Pristimantis petersioides sp. nov. is characterized by the following combination of characters: (1) Skin on dorsum smooth to shagreen with or without scattered small tubercles, head with or without one interorbital small tubercle, skin of venter shagreened to weakly areolate; discoidal fold present, ill-defined; dorsolateral folds absent; (2) tympanic membrane and tympanic annulus present, round, its

Figure 4. Hand and foot of the holotype of Pristimantis petersioides sp. nov. QCAZ58939, adult female. Ventral views of right foot A and right hand B. Abbreviations: B = basal, D = distal, HD = hyperdistal. Photographs by Julio C. Carrion-Olmedo.
length 2/5 to 1/2 of eye diameter; its upper border weakly concealed by inconspicuous supratympanic fold; (3) snout rounded to truncate in dorsal view; truncate in lateral view, bearing a small rostral papilla; (4) interorbital space flat, broader than upper eyelid; upper eyelid with one distinct subconical tubercle surrounded by lower, indistinct rounded tubercles; cranial crests absent; (5) vomerine odontophores low to prominent, oblique, moderately separated, posteromedia to choanae; (6) males with prominent, sub- 
gular vocal sac and vocal slits; (7) first finger shorter than second; all fingers long, discs broadly expanded, rounded to truncate; all fingers bearing a hyperdistal tubercle (Fig. 
4B); (8) fingers with narrow lateral fringes; (9) few ulnar tubercles; (10) no knee and heel tubercles, outer tarsal fold 
bearing one to three indistinct tubercles; (11) two metatarsal 
tubercles, inner oval, 3x the size of outer conical and elliptical metatarsal tubercle; supernumerary plantar tu- 
bercles numerous; (12) all toes with hyperdistal tubercles; toes with narrow lateral fringes; basal toe webbing absent, 
discs broadly expanded, Toe IV much longer than Toe III (disc on Toe III reaches proximal edge of penultimate sub-
tarticulare tubercle on Toe IV, disc on Toe V exceeds the dis-
tal edge of penultimate subarticular tubercle on Toe IV), 
discs as expanded as those on fingers (Fig. 4A); (13) SVL 
22.8 ± 1.4 mm (20.4–24.8 mm; n = 22) in females, 18.5 ± 
1.5 mm (15.8–23.9 mm; n = 39) in males.

Comparison with other species. Color comparisons are 
based on digital photos of live specimens, unless oth-
erwise noted. Pristimantis petersioides sp. nov. is most similar to other species of the Pristimantis group, especially P. petersi (Lynch & Duellman, 1980), P. bro-
meliaecus (Lynch, 1979), P. lacrimosus (Jiménez de la 
Espada, 1875), P. schultei (Duellman, 1990), P. pastaze-
sis (Andersson, 1945), and P. rhodostichus (Duellman & 
Pramuk, 1999) (Fig. 5). Pristimantis petersioides sp. nov. 
can only be distinguished from P. petersi by differences in 
advertisement calls. Call duration is shorter in P. pe-
tersioides sp. nov. 0.25 s (Table 3; 0.19–0.32 s; n = 3) 
than in P. petersi, 0.42 s (0.37–0.46 s; n = 2). Dominant 
frequencies also differ: 4430.79 Hz (4122–4837.22 Hz; n 
= 3) in P. petersioides sp. nov. and 3956.75 Hz (3836.67– 
4076.84 Hz; n = 2) in P. petersi. Call duration and domi-
nant frequency are static call traits and, therefore, are less 
variable within species and most reliable to define spe-
cies boundaries (Köhler et al. 2017). Crucially, the new 
species and P. petersi are not sister species and are separated by large genetic distances: uncorrected pairwise p-
genetic distances for gene 16S range from 7.9% to 8.4%. Pris-
timantis petersioides sp. nov. can be distinguished from 
P. bromeliuscus by snout shape (rounded to truncate in 
P. petersioides sp. nov. vs. subacuminate in P. bromel-
leicus), texture of ventral skin (weakly areolate in P. pe-
tersioides sp. nov. vs. coarsely areolate in P. bromeliuscus), 
iris coloration (reddish coppery in P. petersioides sp. nov. 
vs. brown flecked with gold or bronze in P. bromeliuscus), 
and by having an eyelid with one conical tubercle 
surrounded by lower tubercles (two to three non-conical 
tubercles in P. bromeliuscus). Pristimantis petersioides 
sp. nov. differs from P. lacrimosus (Jiménez de la Espa-
da, 1875) in dorsal coloration (dark greenish brown to 
pale yellowish green in P. petersioides sp. nov. vs. gold-
en brown in P. lacrimosus), presence of eyelid tubercles 
and narrow lateral fringes (both absent in P. lacrimosus), 
and size of outer metatarsal tubercle (3× bigger than the 
inner metatarsal tubercle in P. petersioides sp. nov. vs. 
5–6× bigger in P. lacrimosus). Pristimantis petersioides 
sp. nov. is also similar to P. rhodostichus and P. schul-
tei from Peru and Ecuador. It can be distinguished from 
both by snout shape in dorsal view (rounded to truncate 
in P. petersioides sp. nov. vs. long acuminate in P. rho-
dostichus [Duellman & Pramuk, 1999] and acuminate in 
P. schultei). It can be further distinguished from P. schul-
tei by lacking heel tubercles (present in P. schultei), and 
from P. rhodostichus by lacking red markings on the dor-
sum (present in P. rhodostichus, Duellman & Pramuk, 
1999). Additionally, P. petersioides sp. nov. differs from 
P. pastazensis (Andersson, 1945) by snout shape in dorsal 
view (rounded to truncate in P. petersioides sp. nov. vs. 
subacuminate in P. pastazensis, Andersson, 1945), tux-
terles on upper eyelid (one distinct conical tubercle sur-
rrounded by lower, indistinct rounded tubercles in P. pe-
tersioides sp. nov. vs. several minute rounded tubercles 
in P. pastazensis, Andersson 1945), and skin of venter 
(weakly areolate in P. petersioides sp. nov. vs. coarsely 
granular in P. pastazensis, Andersson 1945). For further 
comparison see Table 4.

Description of the holotype. Adult female 
(QCAZ58939). Measurements (in mm): SVL 22.02; 
tibia length 12.07; foot length 10.72; head length 8.82; 
head width 9.09; eye diameter 2.96; tympanum diamete-ner 1.35; interorbital distance 2.52; upper eyelid width 
2.44; internarial distance 1.59; eye-nostril distance 2.59; 
tympanum-eye distance 0.71. Body slender; head slight-
ly wider than long, wider than body; snout rounded to 
truncate with rostral papilla in dorsal view, truncate in 
Lateral profile; canthus rostralis distinct, slightly curved 
in dorsal view; loreal region concave; interorbital space 
flat, no cranial crests; eye large, protuberant; upper eyelid 
about 97% of interorbital distance, bearing one subcon-
ic tubercle. Tympanic membrane and annulus distinct, 
rounded, with inconspicuous supratympanic fold, par- 
tially obscuring anterodorsal edge; horizontal diameter of 
tympanum about 13% of head length, separated from eye 
by a distance about one half tympanum length; choanae 
large, rounded, not concealed by palatal shelf of maxil-
lary arc; dentigerous processes of vomers prominent, 
oblique, bearing a transverse row of five teeth; tongue 
big, elliptical, posterior border slightly notched, 40% of 
the anterior surface adherent to floor of mouth. Skin on 
dorsum smooth to shagreen; dorsolateral folds absent; 
skin on upper flanks bearing scattered low tubercles; skin 
on belly weakly areolate; skin on throat and chest smooth; 
discoidal fold ill-defined; skin in upper cloacal region 
shagreen. Forearms slender bearing low antebra
cicial tubercle and one subconical ulnar tubercle at the dis
tal half of the forearm; fingers large and slender, all with broadly
Figure 5. Live specimens of *Pristimantis petersioides* sp. nov. and most similar species. **A** *Pristimantis petersioides* sp. nov., QCAZ58938, adult male (SVL 17.99 mm). **B** *Pristimantis petersi*, QCAZ63455, adult male (SVL 16.49 mm). **C** *Pristimantis* sp., QCAZ62940, adult male (SVL 23.45 mm). **D** *Pristimantis bromeliaceus*, QCAZ56454, adult male (SVL 21.93 mm). **E** *Pristimantis schultei*, QCAZ51551, adult male (SVL 24.60 mm). Photographs by Juan Carlos Sánchez **A**, by David Velalcázar **B**, by Valeria Chasiluisa **C**, by Jorge Brito **D**, by Diego Paucar **E**.

expanded pads, all fingers with discs; fingers bearing narrow lateral fringes; relative lengths of fingers I < II < IV < III; three subarticular tubercles on finger III (Fig. 4B), the most distal we refer as hyperdistal, all the tubercles well defined, round in ventral and lateral view; several supernumerary tubercles present, prominent at the base of the fingers and lower, indistinct at the palmar surface; palmar tubercle bifid, heart-shaped, about the same length and twice the width of elliptical thenar tubercle (Fig. 4B).

Hindlimbs slender; tibia length about 55% of SVL; upper surfaces of hindlimbs smooth; foot length about 48% of SVL, posterior surfaces of thighs smooth, ventral surfaces of thighs slightly areolate; knee and heel lacking tubercles; outer surface of tarsus bearing three low, inconspicuous tubercles, equally distributed along tarsus; toes bearing narrow lateral fringes; webbing between toes absent; discs on toes broadly expanded as those on fingers, rounded; relative lengths of toes: I < II < III < V < IV; Toe V much longer than Toe III (disc on Toe III reaches proximal edge of penultimate subarticular tubercle on Toe IV; disc on Toe V exceeds the distal edge of penultimate subarticular tubercle on Toe IV), subarticular tubercles rounded, simple, elevated; plantar surface with low supernumerary tubercles, bearing four subarticular tubercles (Fig. 4A), inner metatarsal tubercle prominent, elliptical, approximately 3x size of oval and conical outer metatarsal tubercle (Fig. 4A).

Color of holotype in preservative. (Fig. 3C, D) Background color pale grayish cream with scattered, irregular dark brown chevrons, head bearing dark brown supratympanic and canthal stripe, upper lip bearing ill-defined stripe formed by irregular dark brown dots; upper flanks bearing dark brown, irregular flecks and blotches densely distributed; venter, ventral surfaces of forearms and hindlimbs pale creamy white, chest and throat with diminutive dark brown dots uniformly distributed (visible under magnification); ventral surfaces of hands and foot with dense minute dark brown dots, posterior surfaces
of thighs pale cream to dark brown; iris reddish coppery with fine, dense, black reticulation. 

Color of holotype in life. (Fig. 3A, B) Dorsal surfaces yellowish green with scattered, irregular dark brown chevrons; canthal stripe and supratympanic fold black, upper flanks pale cream with dark brown irregular flecks and blotches; venter creamy white; axils pinkish white; ventral surfaces of limbs, thighs yellowish green; iris reddish copper with dark bronze faint horizontal streak and thin irregular black reticulations.

Variation in preservative. (Fig. 6) Adult males (15.79–23.93 mm) are smaller than adult females (20.42–24.81 mm). See Table 5 for measurements of the type series. Males bearing vocal slits and prominent subgular sac, lacking nuptial pads. Skin tuberculation is less noticeable than in live specimens, it can vary from dorsum completely smooth (e.g., QCAZ58943, 59171) with or without dark interorbital bar. Variation in life. (Fig. 7). Tuberculation pattern varies from dorsum completely smooth (e.g., QCAZ58943, 58951) to dorsum shagreen (e.g., QCAZ58938, 58939), some individuals bear scattered small tubercles on anterior half of dorsum (e.g., QCAZ58880) or have the dorsum densely tuberculated (e.g., QCAZ59463). When dorsum is tuberculated, flanks and limbs usually bear scattered tubercles more conspicuous than those in the dorsum. Similarly, the interorbital tubercle and upper eyelid tubercles are more prominent when the dorsum is tuberculated. There is extensive variation in dorsal coloration (Fig. 7). Dorsum varies from dark greenish brown (e.g., QCAZ59471), bright orange (e.g., QCAZ58943), olive green (e.g., QCAZ58938), to pale yellowish green (e.g., QCAZ58941). Dark marks on dorsum vary from scattered dark brown flecks to irregular brown chevrons that form a triangle that extends from the ilium to the scapula, to ill-defined, dark brown flecks and spots (e.g., QCAZ58948, 59472), pale cream middorsal bar from the snout to the cloaca (e.g., QCAZ59456), to black dorsolateral stripes suffused with supratympanic stripes (e.g., QCAZ58943, 59171), with or without dark interorbital bar.
Figure 6. Color variation in preserved individuals of *Pristimantis petersioides* sp. nov. A Dorsal view (left to right): QCAZ59461 (SVL 22.95 mm), QCAZ59470 (SVL 22.94 mm), QCAZ58939 (SVL 22.02 mm), QCAZ58951 (SVL 19.75 mm); B Dorsal view (left to right): QCAZ59171 (SVL 19.50 mm), QCAZ59456 (SVL 19.05 mm), QCAZ59462 (SVL 19.00 mm), QCAZ59468 (SVL 18.35 mm); C Ventral view of specimens in (A), D Ventral view of specimens in (B). Photographs by Julio C. Carrión-Olmedo.

ish brown, pale yellowish green to bright orange (e.g., QCAZ59455, 59466, 59471).

**Advertisement call.** Quantitative measurements of the advertisement call of *Pristimantis petersioides* sp. nov. (QCAZ58940) are shown in Table 3. The call is a metallic click with an average duration of 0.25 s (0.19–0.32 s; n = 3; Fig. 8). The amplitude peak occurs at 20–30 ms and then decreases gradually towards the end (Fig. 8). The calls are repeated at a mean rate of 19.89 calls per minute (11.26–25.78; n = 3). Three or four harmonics are visible, but most of the energy is located on the first one. The dominant frequency (= fundamental frequency) is 4430.79 Hz (4122–4837.22 Hz; n = 3).
Table 5. Morphometric variables of *P. petersioides* sp. nov. and *P. petersi*. Mean ± SD is given with range in parentheses. All measurements are in millimeters.

| Variable              | *P. petersioides* sp. nov. | *P. petersi* |
|-----------------------|-----------------------------|--------------|
|                      | male | female | male | female |
| Snout-vent length     | 18.5 ± 1.5 (15.8–23.9)      | 22.8 ± 1.4 (20.4–24.8) | 18.3 ± 1.7 (16.5–22.7) | 20.1 ± 1.4 (19.1–21.1) |
| Tibia length          | 9.8 ± 0.8 (8.3–11.9)        | 11.7 ± 1.0 (8.5–12.6) | 9.6 ± 0.6 (8.6–11.0) | 10.7 ± 1.6 (9.6–11.8) |
| Foot length           | 8.6 ± 0.9 (6.9–10.7)        | 10.6 ± 0.7 (8.8–11.6) | 8.6 ± 0.7 (7.8–10.1) | 10.0 ± 0.3 (9.8–10.2) |
| Head length           | 6.5 ± 0.6 (5.3–7.8)         | 8.0 ± 0.6 (7.2–9.1) | 6.1 ± 0.5 (5.3–7.1) | 7.1 ± 0.5 (6.8–7.5) |
| Head width            | 7.0 ± 0.6 (5.9–8.5)         | 8.8 ± 0.5 (8.0–9.5) | 6.9 ± 0.5 (6.3–8.1) | 7.7 ± 0.4 (7.5–8.0) |
| Eye diameter          | 2.6 ± 0.2 (2.2–3.1)         | 3.0 ± 0.3 (2.4–3.5) | 2.6 ± 0.2 (2.4–3.1) | 2.8 |
| Tympanum diameter     | 0.9 ± 0.1 (0.7–1.1)         | 1.1 ± 0.2 (0.8–1.4) | 0.9 ± 0.1 (0.7–1.0) | 1.1 ± 0.1 (1.0–1.2) |
| Interorbital distance | 2.2 ± 0.2 (1.9–2.6)         | 2.6 ± 0.2 (2.4–3.0) | 2.2 ± 0.1 (2.0–2.5) | 2.6 ± 0.2 (2.4–2.7) |
| Upper eyelid width    | 2.2 ± 0.3 (1.6–2.6)         | 2.6 ± 0.2 (2.3–3.0) | 2.1 ± 0.4 (1.7–2.9) | 2.27 |
| Internarial distance  | 1.4 ± 0.1 (1.2–1.7)         | 1.7 ± 0.1 (1.5–2.0) | 1.5 ± 0.2 (1.3–1.7) | 1.6 ± 0.2 (1.4–1.7) |
| Eye–nostril distance  | 1.9 ± 0.2 (1.6–2.5)         | 2.5 ± 0.2 (2.2–2.8) | 2.1 ± 0.3 (1.8–2.9) | 2.3 ± 0.4 (2.0–2.6) |

Distribution and natural history. *Pristimantis petersioides* sp. nov. is known from six localities in the eastern Andean slopes of central Ecuador between 1221–2300 m (Fig. 9). It inhabits the Eastern Andean Foothills Forest and Eastern Montane Forest natural regions (as defined by Ron et al. 2019). It has been recorded in primary forest and, less frequently, in secondary forest. Individuals were found during nocturnal surveys, usually perching on ferns, herbs, or *Heliconia* leaves, branches, or inside bromeliads up to 350 cm above the ground, usually near water bodies. Three amplexant pairs were found on January and February 2015 in Sar dinayacu and Zarentza.

Etymology. The specific epithet is a masculine noun in apposition. The suffix *oides* is derived from the Greek *eidos* meaning similar. The name makes reference to the
Figure 8. Advertisement calls of *Pristimantis petersioides* sp. nov. and *Pristimantis petersi*. (A) oscillogram and spectrogram of a call series of *Pristimantis petersioides* sp. nov., (B) oscillogram (top), spectrogram (middle), and power spectrum (bottom) of a single call of *Pristimantis petersioides* sp. nov. and (C) oscillogram (top), spectrogram (middle), and power spectrum (bottom) of a single call of *Pristimantis petersi*. 
similarity between the new species and its sister species, *Pristimantis petersi*.

**Conservation status.** Four out of six known localities are inside National Parks (Sardinayacu in Parque Nacional Sangay and Ankaku, Zarentza and Salcedo-Tena road in Parque Nacional Llanganates); nonetheless, based on a vegetation cover map (Ministerio del Ambiente 2018a) and a deforestation map 2016–2018 (Ministerio del Ambiente 2018b), Zarentza is < 1 km from deforested areas for agriculture. At the year of collection (2009) the locality at Salcedo-Tena highway was in a forested region with small, deforested patches at distances > 2.5 km (based on a 2008 deforestation map by Ministerio de Ambiente). Sardinayacu, refuge 3 occur > 6 km from pastures, while Sardinayacu, refuge 1 is < 0.5 km from deforested areas for agriculture.

In Sardinayacu, this species was one of the most common during surveys (24 individuals found in 9 days by 13 people) which suggest it can be locally abundant. Brito et al. (2017) also reported abundant populations in the upper basin of the Upano river, Sangay National Park, Morona Santiago Province (referred both as “*Pristimantis petersi*” and also “*P. aff. petersi*”). Its extent of occurrence is

![Map of species distribution](image)

**Figure 9.** Records of *Pristimantis petersioides* sp. nov. (yellow) and *P. petersi* (red). Confirmed records are based on specimens deposited at the Museum of Zoology, Pontificia Universidad Católica del Ecuador. Unconfirmed records (diamonds) from Brito et al. (2017), Lynch and Duellman (1980), Mueses-Cisneros (2005), and Stuart et al. (2008).
1402 km² (based on a minimum convex polygon). Despite being locally abundant, we consider *Pristimantis petersioides* sp. nov. to be in the Red List category Vulnerable (VU) following B1, B2ab(iii) IUCN criteria because: (i) it is only known from six localities (sensu IUCN 2017), (ii) its Extent of Occurrence is less than 5000 km² (1433 km²); and approximately 9% of its Extent of Occurrence has been affected by deforestation, human settlements and agriculture (Fig. 10).

**Remarks.** *Pristimantis petersioides* sp. nov. differs from *P. sp.* (QCAZ 60398, from Bombuscaro) by the snout shape (in dorsal view, rounded in *P. petersioides* sp. nov., subacuminate in *P. sp.* QCAZ60398), venter texture (weakly areolate in *P. petersioides* sp. nov.; coarsely areolate in *P. sp.* QCAZ60398), presence of small rostral papilla (absent in *P. sp.* QCAZ60398); furthermore, *P. petersioides* sp. nov. bears a complete, rounded tympanic annulus, weakly obscured posterodorsally by a thin supratympanic fold (tympanic annulus concealed posterodorsally by a thick supratympanic fold in *P. sp.* QCAZ60398). It differs from *P. nankints* by snout shape in dorsal view (rounded to truncate in *P. petersioides* sp. nov. vs. acuminate in *P. nankints*).

![Figure 10. Records of Pristimantis petersioides sp. nov. (blue circles) and its Extent of Occurrence. Colors indicate vegetation cover based on Ministerio de Ambiente (2018a).](evolsyst.pensoft.net)
Discussion

On the identity of *Pristimantis petersi*

*Pristimantis petersi* was considered to have a wide distribution from the central Andes of Colombia in Caquetá, Huila, and Putumayo (Lynch and Duellman 1980; Mueses-Cisneros 2005; Stuart et al. 2008), to the eastern slopes of the Ecuadorian Andes, from Sucumbíos to Morona Santiago Provinces (Brito et al. 2017; Ron et al. 2019). Herein, we show that it was composed of two species which appear to be allopatric, south and north of the Quilindaña paramos in Napo Province.

Lynch and Duellman (1980) remark of size differences between populations from the north and south of “*P. petersi*” was not supported in our data but their suspicion of the distinctiveness of the populations from the Pastaza trench was correct. Based in our review, we tentatively consider *Pristimantis petersi* as distributed from the central Andes of Colombia to Napo Province (Fig. 9). We recommend verifying the identity of Colombian populations using genetic data. Recent reviews of Andean *Pristimantis* indicate that species usually have a restricted distribution (e.g., Páez and Ron 2019). The geographic distance of Colombian populations (up to 320 km from the type locality) suggest that, at least some of them, could represent a separate species.

Guayasamín and Funk (2009) reported an abundant population of “*Pristimantis cf. petersi*” at Yanayacu Biological Station. Examination of voucher specimens deposited at the QCAZcollection indicate that they are not conspecific with *P. petersi* nor *P. petersioides* sp. nov.

Our results and those of previous systematic reviews (show that eastern montane forests still harbor many undescribed species of *Pristimantis*. Similar findings have been previously reported by Ortega et al. (2015), Páez and Ron (2019), and Ron et al. (2020). As in previous reviews (e.g., Restrepo et al. 2017; Páez and Ron 2019), we also found broad inspecific and intrapopulation variation in dorsal color within *P. petersioides* sp. nov. (Figs 6, 7) and *P. petersi* (Fig. 11). This large inspecific and intrapopulation variation hinders the use of dorsal coloration for diagnosis between both species. Most individuals have greenish dorsal color which is characteristic of several species of the *P. lacrimosus* group. We did not find diagnostically morphological characters to distinguish the new species from *Pristimantis petersi*, which highlights the importance of including molecular and bioacoustic data to clarify species identity.

Use of bioacoustics for species delimitation

Similar to Páez and Ron (2019), our morphometric analysis was of little help to distinguish closely related species of *Pristimantis*. In contrast, advertisement calls and genetic data indicate that *P. petersioides* sp. nov. represents a lineage independent from *P. petersi*. We found differences with little or no overlap in two static call traits, call duration and dominant frequency (Köhler et al. 2017). Moreover, differences in call frequency are likely an underestimate because the calling males of *P. petersioides* sp. nov. were larger than those from the recorded chorus of *P. petersi*. Because there is an inverse relationship between body size and call frequency (Gerhardt and Huber 2002), the higher frequency of the call of *P. petersioides* sp. nov. would be likely higher after a size correction.

Bioacoustic comparisons are of importance for taxonomy because advertisement calls mediate species recognition and mate choice (e.g., Ryan and Rand 1995). It has been widely accepted that calls are among the most useful characters differentiating closely related anuran species (Vences and Wake 2007). However, calls have been of limited use in the taxonomy of *Pristimantis* (Duellman and Lehr 2009). Our study and some recent works (Hutter and Guayasamin 2015; Páez and Ron 2019; Székely et al. 2020) highlight the usefulness of bioacoustic characters in *Pristimantis* taxonomy. Future taxonomic reviews will benefit from a more comprehensive knowledge of advertisement calls in *Pristimantis*.

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Figure 11. Variation in live adult individuals of Pristimantis petersi. A QCAZ63452 (SVL 17.42 mm), B QCAZ63453 (SVL 17.75 mm), C QCAZ63454 (SVL 21.11 mm), D QCAZ63456 (SVL 19.11 mm). Photographs by Santiago R. Ron A, by David Velalcázar B–D.
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Supplementary material 1

**Collection data for Museum specimens examined in the morphological comparisons**

Authors: Julio C. Carrión-Olmedo, Santiago R. Ron
Data type: Collection data
Explanation note: All specimens are deposited at the amphibian collection of the Zoological Museum (QCAZ), Pontificia Universidad Católica del Ecuador in Quito, Ecuador.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/evolsyst.5.62661.suppl1

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Supplementary material 2

**Best-fit models of DNA evolution for partitions of the phylogenetic analyses**

Authors: Julio C. Carrión-Olmedo, Santiago R. Ron
Data type: Phylogenetic
Explanation note: Models were chosen according to the BIC criterion.
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Link: https://doi.org/10.3897/evolsyst.5.62661.suppl2

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Supplementary material 3

**Mitochondrial DNA and RAG1 phylogenetic trees**

Authors: Julio C. Carrión-Olmedo, Santiago R. Ron
Data type: Phylogenetic
Explanation note: The *Pristimantis lacrimosus* species group is shown in green.
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