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One hundred years of solitude: The rediscovery of *Pristimantis ruidus* (Anura, Strabomantidae) in the southern Andes, Ecuador and its phylogenetic relationships

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Abstract

We report the rediscovery, a century after its last record, of two specimens of *Pristimantis ruidus* from approximately 6 km northeast of its type locality, in a montane forest from the western Andes of southern Ecuador. This species, described by John D. Lynch in 1979, based on specimens collected in 1922 by George H. Tate, was previously known only from the type series, all specimens originating from a single locality (Molleturo, Provincia Azuay, Ecuador, 2317 m elev.). The rediscovery occurred in November 2022 in the Reserva de Conservación Quitahuaycu, Molleturo Parish, Azuay Province. The two specimens, both females, are characterised by rough skin with numerous rounded low warts and tubercles on their dorsum, a W-shaped occipital fold, tympanum and tympanic ring not externally visible and by the lack of cranial crests, characters that correspond with the description of the holotype. We evaluate the phylogenetic relationships, based on two mitochondrial genes (*12S* and *16S* rRNA) and one nuclear gene (*RAG-1*). These analyses reveal *Pristimantis ruidus* as a member of the subgenus *Huicundomantis*, being most closely related to *P. philipi*, a species with a distribution restricted to the paramo of the same mountain region (Macizo del Cajas). *Pristimantis ruidus* co-exists with *Pristimantis jimenezi*, three additional species of *Pristimantis* and one *Noblella* with uncertain taxonomic status. This rediscovery underscores the urgency of implementing effective conservation and monitoring measures for the few remnants of the original ecosystems from western Ecuador, which face ongoing anthropogenic threats and harbour a diversity that remains insufficiently studied.

Resumen

Reportamos el redescubrimiento, un siglo después de su último registro, de dos especímenes de *Pristimantis ruidus* en aproximadamente 6 km al noreste de su localidad tipo, en un bosque montano en los Andes occidentales del sur de Ecuador. Esta especie, descrita por John D. Lynch en 1979, con base en especímenes recolectados en 1922 por George H. Tate, se conocía solo por la serie tipo con todos los especímenes originarios de la misma localidad (Molleturo, Provincia Azuay, Ecuador, 2317 m.). El redescubrimiento ocurrió en noviembre de 2022 en la Reserva de Conservación Quitahuaycu, parroquia Molleturo, provincia de Azuay. Los dos ejemplares son hembras y se caracterizan por presentar una piel rugosa con numerosas verrugas bajas y tubérculos redondeados

en su textura dérmica dorsal, un pliegue occipital en forma de W, tímpano y anillo timpánico no visibles externamente y carecen de crestas craneales, caracteres que concuerdan con el holotipo. Evaluamos relaciones filogenéticas basadas en dos genes mitocondriales (*12S* y *16S* rRNA) y un gen nuclear (*RAG-1*). Estos análisis revelan a *Pristimantis ruidus* como miembro del subgénero *Huicundomantis*, estando más estrechamente relacionado con *P. philipi*, especie de distribución restringida que habita el páramo de la misma región montañosa (Macizo del Cajas). *Pristimantis ruidus* coexiste con *Pristimantis jimenezi*, tres especies adicionales de *Pristimantis* y una *Noblella* con estatus taxonómico incierto. Este redescubrimiento subraya la urgencia de implementar planes efectivos de conservación y monitoreo para todos los remanentes de los ecosistemas del oeste de Ecuador, que enfrentan amenazas antropogénicas continuas y albergan una diversidad que aún no está suficientemente estudiada.

Key Words

Amphibia, biodiversity, conservation, *Huicundomantis*, molecular genetics, morphology, new record, phylogeny, systematics, taxonomy

Palabras clave

Anfibios, biodiversidad, conservación, filogenia, genética molecular, *Huicundomantis*, morfología, nuevo registro, sistemática, taxonomía

Introduction

The direct-developing frogs of the genus *Pristimantis* Jiménez de la Espada, 1870 are the most species-rich group of all amphibians, with more than 600 formally recognised species (Frost 2023). The high diversity of the genus is also accompanied by exceptional levels of ecological specialisation, many species presenting small ranges of distribution, especially at higher elevations in the Andes (Ron et al. 2022). With respect to southern Ecuador, numerous studies of the *Pristimantis* have revealed an extraordinary diversity and endemism, but also massive threats (Lynch 1979; Lynch and Duellman 1997; Urgiles et al. 2014, 2017, 2019; Yánez-Muñoz et al. 2016, 2019; Sánchez-Nivicela et al. 2018, 2021; Székely et al. 2018, 2020, 2021; Páez and Ron 2019; Ortega-Andrade et al. 2021).

Pristimantis ruidus (Lynch, 1979) has eluded field researchers for over a hundred years. The 16 known specimens used in the description were collected by the naturalist George Henry Hamilton Tate (1894–1953) during his 1922 expedition for the American Museum of Natural History. The specimens were collected in south-western Ecuador, in Molleturo, Azuay Province, on the Pacific slopes of the Cordillera Occidental, at an elevation of 2317 m (Lynch 1979; Lynch and Duellman 1997).

In 2022, during an expedition to the Reserva de Conservación Quitahuaycu, led by Fundación y Bioparque AMARU, members of the exploration team collected two specimens of *Pristimantis*, which, after comparison with the original description and holotype photographs, were identified as *P. ruidus*. Based on the newly-acquired specimens, we present a re-description of this species, provide for the first time images of living specimens, as well as a genetic assessment and evaluation of phylogenetic relationships of *P. ruidus*.

Methods

Specimen collection and study site

Fieldwork was carried out at Reserva de Conservación Quitahuaycu (-2.74047, -79.33532 [WGS84]), Molleturo Parish, Azuay Province, Ecuador (Fig. 1). The study area is a montane forest, situated at an elevation between 2400 and 2900 m, approximately 6 km southwest from the type locality of *P. ruidus*. For the species habitat description, we follow the ecosystem classification system proposed by the Ministerio del Ambiente del Ecuador (MAE 2012).

We used the complete species inventory methodology (Angulo et al. 2006), with diurnal and nocturnal samplings, visits being conducted in November 2022. The collected specimens were deposited at the División de Herpetología (DHMECN) of the Instituto Nacional de Biodiversidad (INABIO), Quito, Ecuador and Museo de Zoología, Universidad Técnica Particular de Loja (MUTPL), Loja, Ecuador. Specimens were manipulated in accordance with Beaupre et al. (2004), photographed alive and euthanised with a 5% Roxicain solution. Liver tissue samples were extracted and preserved in 90% ethanol, after which the specimens were fixed with 10% formalin and preserved in 70% ethanol, following the methods proposed by Heyer et al. (2001) and Simmons and Muñoz-Saba (2005). Research permits were issued by the Ecuadorian Ministry of Environment (019-2018-IC-FAU-DNB/MAE, MAE-DNB-CM-2018-0106 and MAATE-DBI-CM-2021-0181).

Morphological analysis

The collected specimens were identified, based on the original description (Lynch 1979) and compared with photographs of the holotype AMNH 17590 (**AMNH** = American Museum of Natural History). The definition follows the

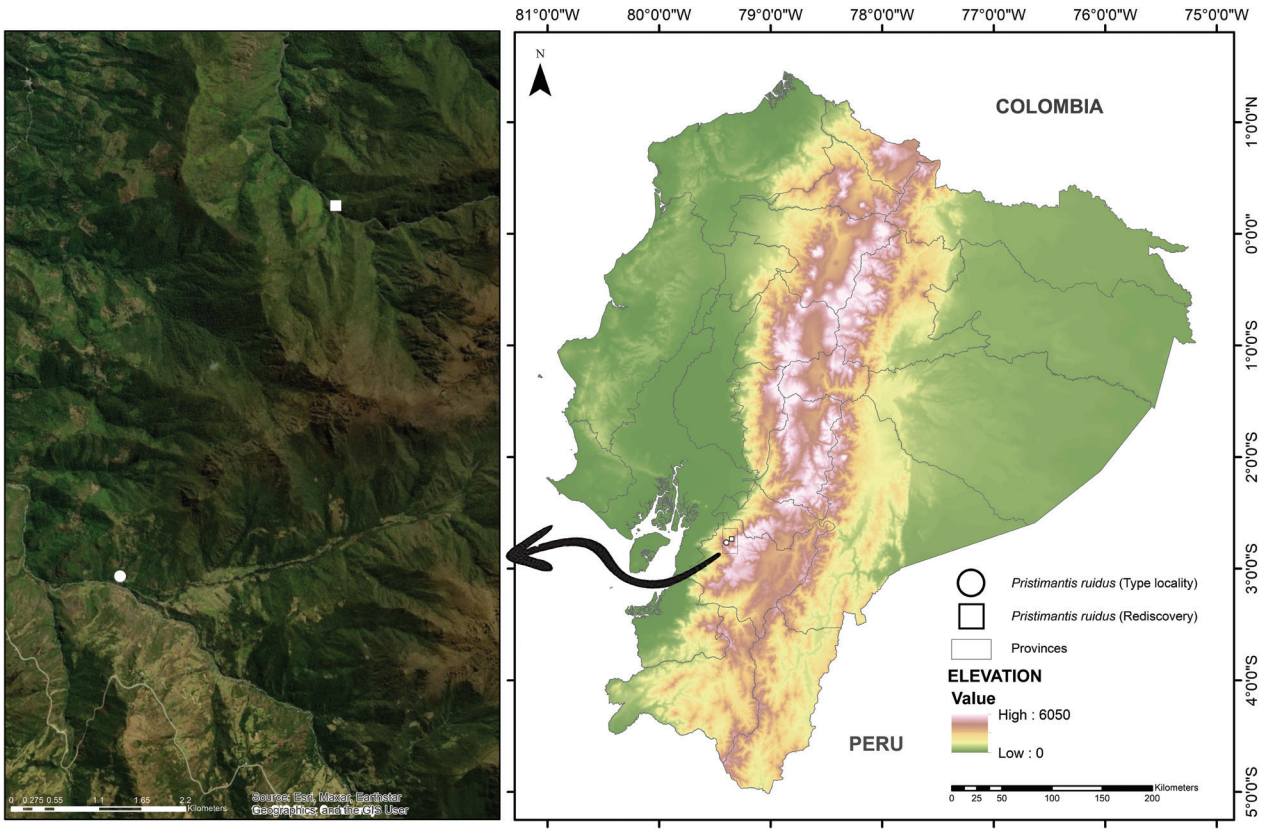

Figure 1. Map of Ecuador showing the distribution of *Pristimantis ruidus* in Azuay Province, Ecuador. On the left, an enlarged view of the area. Source: Esri, Maxar, Earthstar geographics and the GIS User Community.

characters and diagnostic terms proposed by Duellman and Lehr (2009). The sex and maturity of the specimens were determined by inspecting the sexual organs through a dorsolateral incision and the external characteristics. Fingers and Toes are numbered pre-axially to postaxially from I to IV and I to V, respectively. The relative lengths of Toes III and V were determined by adpressing them against Toe IV and Fingers I and II were compared by adpressing them against each other.

Morphological measurements were taken with a precision caliper and rounded to the nearest 0.1 mm. The abbreviations used follow the format of Duellman and Lehr (2009): **SVL** = Snout-vent length (distance from the tip of snout to the vent); **TL** = Tibia length (distance from the knee to the distal tibia end); **FL** = Foot length (distance from the proximal margin of inner metatarsal tubercle to tip of Toe IV); **HL** = Head length (distance from angle of the jaw to the tip of the snout); **HW** = Head width (distance between the angle of the jaws); **IOD** = Interorbital distance (between the orbits); **EW** = Width of upper eyelid (perpendicular distance to the outer edge of the upper eyelid); **IND** = Internarial distance (distance between the inner edges of the narial openings); **EN** = Eye-nostril distance (between the anterior corner of orbit and posterior margin of the narial opening); **ED** = Eye diameter (horizontal length of the orbit).

Colour patterns in life and other important external characteristics were based on field notes and photographs taken in the field and laboratory. The colour identification in life follows Köhler (2012). The photographs were taken by Ernesto Arbeláez, Jaime Culebras and Juan Sánchez.

Molecular analysis

Genomic extraction, amplification and sequencing were done as described in Székely et al. (2020); the six newly-generated DNA sequences were deposited in GenBank (Appendix 1). We analysed the molecular data by using sequences of two mitochondrial genes (*12S* and *16S* rRNA) and one nuclear gene (*RAG-1*) from 61 specimens (60 from Ecuador and one from Peru) of 35 species or candidate species available in GenBank, representing all the currently confirmed species of the subgenus *Huicundomantis* of *Pristimantis* (*sensu* Ortega et al. (2022)). As outgroups, we used the species from Székely et al. (2021): *Pristimantis unistrigatus* (Günther, 1859), *P. ceuthospilus* (Duellman & Wild, 1993), *P. imitatrix* (Duellman, 1978), *P. diadematus* (Jiménez de la Espada, 1875), *P. rhodoplichus* (Duellman & Wild, 1993), *P. melanogaster* (Duellman & Pramuk, 1999), *P. wiensi* (Duellman & Wild, 1993), *P. simonsii* (Boulenger, 1900), *P. orestes* (Lynch, 1979), *P. colodactylus* (Lynch, 1979) and *P. orcesi* (Lynch, 1972), the trees being rooted with *P. galdi* Jiménez de la Espada, 1870. We conducted BLAST searches to identify similar *12S*, *16S* and *RAG-1* sequences in GenBank and used FastTree v.2.1 (Price et al. 2010), implemented in the programme Geneious Prime (v.2023.1.2, Biomatters Ltd., Auckland, New Zealand) to build an exploratory tree with most of the available Ecuadorian *Pristimantis* sequences in order to determine the identity and relationships of our species.

The sequences were edited, assembled and aligned (MAFFT algorithm with the G-INS-i iterative refinement

method; Katoh and Standley (2013) in Geneious Prime; the *12S*, *16S* and *RAG-1* aligned sequences were visually inspected to correct alignment errors in PhyDE (Müller et al. 2010), concatenated into a single matrix and then used for the phylogenetic analyses. The analyses were based on a 2413-base pair (bp) dataset (924 bp for *12S*, 877 bp for *16S* and 612 bp for *RAG-1*); the matrix is available at https://doi.org/10.5281/zenodo.11099783.

Phylogenetic relationships were inferred using both Bayesian Inference (BI) and Maximum Likelihood (ML). We used PartitionFinder v. 2.1.1 (Lanfear et al. 2017) to select the best partition scheme with the Bayesian Information Criterion (BIC) as a model of selection; PartitionFinder identified three partition schemes (best model in parentheses): *12S* and *16S* (GTR+I+G), *RAG-1* 1st position (K80+G) and *RAG-1* 2nd and 3rd position (F81+I+G). The BI analysis was implemented in MrBayes v.3.2.6 (Ronquist et al. 2012), the Markov Chain Monte Carlo runs being performed twice, independently, for 40 million generations, with trees sampled every 1,000 generations; convergence of the runs was assessed from the average split frequency of standard deviations ($p < 0.001$) and by checking the potential scale reduction factors (PSRF ~ 1.0) for all model parameters; consensus trees were summarised after discarding the initial 25% as burn-in. The ML analyses were conducted in IQ-TREE 2 (Minh et al. 2020). We performed four different runs, in order to test the tree's topologies: one with IQ-TREE's default settings, in which the programme determined the best-fit model for our alignment (TIM2+F+I+R4 under BIC), with 100,000 bootstrap replicates for the SH-like approximate likelihood ratio branch test (SH-aLRT; Guindon et al. (2010)) and with 100,000 ultrafast bootstrap replicates (UFBoot; Hoang et al. (2018)) to assess the branch support; a second run with the three partition schemes identified by PartitionFinder and with the same number of SH-aLRT and UFBoot replicates (100,000); a third run with the default settings, but with the -bnni option (optimised, hill-climbing nearest neighbour interchange, NNI, search) in order to reduce the risk of overestimating the branch supports of the UFBoot, with 10,000 replicates for both SH-aLRT and UFBoot; and finally, a fourth run with the default settings, but the branch support evaluated with 500 standard non-parametric bootstrap (Boot; Felsenstein (1985)) searches. The phylograms were visualised and edited with FigTree (Rambaut 2014) and the uncorrected genetic p-distances were calculated for *16S* with MEGA6 (Tamura et al. 2013).

Results

Morphological identification of *Pristimantis ruidus*

Using the diagnosis and description from Lynch (1979), the two specimens (DHMECN 19106 and MUTPL 1613) were recognised as *P. ruidus*, based on the following characters: dorsal skin warty, W-shaped ridge on occiput; some larger warts on flanks; no dorsolateral folds; venter coarsely areolate without discoidal folds; head wider than body, wider than long; tympanum, tympanic membrane and annulus absent; two large postrictal tubercles; snout rounded in dorsal and lateral view; cranial crest absent; vomerine odontophores triangular in contour; canthus rostralis rounded; loreal region concave, sloping to non-flared lips; upper eyelid warty (not bearing enlarged tubercles); supratympanic fold prominent, extending from corner of eye to base of arm; Finger I shorter than Finger II, digits bearing broad discs on dilated pads; fingers with lateral fringes; non-conical tubercles on heel; inner tarsal fold present; two metatarsal tubercles, inner oval, twice the size of round outer; supernumerary plantar tubercles only at bases of Toes II–IV; toes with lateral fringes, internal toe pads smaller than those of outer fingers (Figs 2–5).

Additionally, the photographs of holotype (AMNH 17590, Fig. 6) show that *P. ruidus* has a little tubercle in the interorbital space; digital discs are elliptical; ulnar tubercles are present, low, and rounded, present on the outer border of the forearm; basal web between toes is absent, the membrane appearance corresponds to dilated lateral fringes (all these characteristics being present also in the specimens DHMECN 19106 and MUTPL 1613).

Phylogeny

The Bayesian Inference and the four Maximum Likelihood phylogenetic trees showed almost the same topology, with only minor differences in the position of some of the unresolved branches; the BI tree presented fewer unresolved branches and relatively higher branch support (Fig. 7). In the case of the four ML trees, values of the branch support differed slightly between the runs; the non-parametric bootstrap run (fourth run) proved to be the most conservative (having overall the smallest support values), followed by the NNI tree (third run).

Similar to Ortega et al. (2022), we recovered the subgenus *Huicundomantis* of *Pristimantis* as monophyletic, with strong support (SH-aLRT = 99.8–99.9%; UFBoot = 100%; Boot = 97%; posterior probabilities (PP) = 1). However, in our tree (Fig. 7), the basal terminals are those represented by *P. philipi* (Lynch & Duellman, 1995) and *P. ruidus* and not the samples including *P. miktos* Ortego-Andrade & Venegas, 2014 (Ortega et al. 2022), although with strong support only in the BI and the SH-like approximate likelihood ratio branch test (SH-aLRT = 80.4–85.5%; UFBoot = 81–93%; Boot = 69%; PP = 0.98); this difference is most likely a consequence of the different gene sampling scheme and further analyses (with matrices that contain longer sequences and more genes) are needed to clarify the relationships of the species in this subgenus. However, our tree has a topology very similar to the one presented in Székely et al. (2021).

The initial exploratory FastTree, as well as the BI and ML phylogenetic trees, recovered *P. ruidus* as a species in the subgenus *Huicundomantis*. *Pristimantis ruidus* is revealed as the sister species of *P. philipi* and both together

form a basal group in *Huicundomantis* (Fig. 7). Uncorrected genetic p-distances for the *16S* gene between *P. ruidus* and its sister species, *P. philipi*, range from 4.1% to 5.3% and between them and the other species of the subgenus *Huicundomantis* from 4.6% to 11.4%.

Taxonomy

Pristimantis ruidus (Lynch, 1979)

Figs 2–6

Common English name: Molleturo Robber Frog

Common Spanish name: Cutín de Molleturo

Etymology. Latin, meaning rough, in reference to the skin texture (Lynch 1979).

Types and type locality. ***Holotype*** (AMNH 17590), adult female obtained at Molleturo, Provincia Azuay, Ecuador, 2317 m elev., on 5–19 June 1922 by G. H. Tate. Paratypes (AMNH 17588, 89, 17591–96, 17598–601, 17603), topoparatypes (Lynch 1979).

Newly-collected material. Two females: DHMECN 19106 (field series: JCS-2081) and MUTPL 1613 (field series: JCS-2084). The first individual was found amongst the remains of a felled tree (75 cm from the ground), along a stream edge (-2.73185, -79.34213, elevation 2677 m); the second individual, was on the leaf of a low shrub (40 cm from the ground), also by a stream edge (-2.73387, -79.34182, elevation 2525 m); both specimens were collected in the Reserva de Conservación Quitahuaycu, Molleturo Parish, Azuay Province, Ecuador, by Luis Salagaje, Ernesto Arbeláez, Jaime Culebras and Nicolás Astudillo, on 27 November 2022 (Fig. 1).

Definition. A moderate-sized species of *Pristimantis* in the subgenus *Huicundomantis* as inferred from molecular phylogenetic relationships, characterised by the following combination of traits: (1) skin on head, dorsum, flanks and extremities shagreen with scattered tubercles, pustules and W-shaped scapular fold; venter coarsely areolate; discoidal fold absent; ventral texture of thighs areolate; (2) tympanic membrane and tympanic annulus absent; (3) snout rounded in dorsal and profile views; (4) upper eyelid with small rounded tubercles, 85.2% of the IOD; interocular space with rounded tubercle; cranial crests absent; (5) dentigerous processes of vomers rounded, with four and five teeth each, triangular in contour; (6) males lacking vocal sac and slits; (7) Finger I shorter than Finger II; disc expanded, elliptical; (8) fingers with narrow lateral fringes; (9) ulnar tubercles present, rounded, on the outer border of the forearm; (10) heel with several small rounded tubercles; tarsal tubercles rounded; inner tarsal fold present; (11) inner metatarsal tubercle oval, twice the size of round outer metatarsal tubercle; supernumerary plantar tubercles few in number; (12) toes with narrow lateral fringes; webbing absent; Toe V longer than Toe III; discs slightly shorter in size than those on outer fingers; (13) dorsum dark olive or brown with dark brown spots; dark W-shaped mark in scapular region, with orange spots in the higher portions; interorbital blotch, post ocular stripe and labial bars dark brown; venter dusty brown with white spots; (14) SVL females 20.1 and 30.0 mm (type series: males = 25.8–31.1 mm, females = 37.1–39.8 mm).

Diagnosis. *Pristimantis ruidus* is distinguished from its congeners in the subgenus *Huicundomantis* by lacking a tympanic membrane and a tympanic annulus, head wider than long, short snout, cranial crest absent, thick supratympanic fold and by the presence of subacuminated postrictal tubercles. *Pristimantis philipi* is the only species lacking the middle ear and vocal apparatus and cranial crests (Lynch and Duellman 1997). *Pristimantis ruidus* differs from *P. philipi* by the broadly expanded discs of the digits (discs barely expanded in *P. philipi*), the evident scapular fold (lack of scapular fold in *P. philipi*) and the colouration pattern composed by dark olive or brown with dark brown spots, dark W-shaped marks in the scapular region, with orange spots in the higher portions (two colour patterns in dorsum, black and pale yellow blotches or irregular flecks and blotches green, black, yellowish-green and grey in *P. philipi*).

Description of female DHMECN 19106. Head wider than long, short snout (HL = 33% of SVL), rounded in dorsal and lateral profile, nostrils orientated posterodorsally; *canthus rostralis* concave; cranial crest absent; one rounded interocular tubercle; upper eyelid with rounded tubercles (three and four each) and few lower; supratympanic fold thick and discontinuous; four large subacuminated postrictal tubercles; tympanic annulus and membrane absent; few small and subconical maxillary tubercles; choana barely visible in the palatal shelf, posteriorly to level of choana and broadly separated, dentigerous process of vomers conspicuous, triangular, with four and five vomerine teeth each; tongue wider than long, 30% adhering to the floor mouth; scapular fold W-shaped. Dorsum skin shagreen with numerous low warts; dorsolateral and medial fold absent; flanks shagreen with low warts and scarcely subconical and low tubercles; venter areolate with large and scattered warts; discoidal and pectoral fold absent; cloacal vent with rounded warts. Arms with low and oblique ridges and small subconical tubercles; subconical ulnar tubercles; U-shaped palmar tubercle, same length to thenar tubercle; thenar tubercle oval; low and few palmar supernumerary tubercles; subarticular tubercles rounded; fingers with lateral fringes, without interdigital membranes; Finger I shorter than Finger II; all digits with ventral pads, narrow and rounded in Finger I, barely expanded and elliptical in Finger II, expanded and elliptical in Fingers III and IV; discs in Fingers II and IV with circumferential grooves. Legs with low and oblique ridges, more noticeable in hind limbs and foot and small rounded tubercles; TL = 51% of SVL; heel and tarsus with subconical tubercles; inner tarsal fold low and extending along the tarsus; inner metatarsal tubercle oval, three times larger than external which is round; few low plantar supernumerary tubercles; subarticular tubercles rounded; toes with lateral fringes, without interdigital membranes; all digits with ventral pads and circumferential grooves,

barely expanded and elliptical in Toes I–V; relative length of Toes I < II < III < V < IV (Figs 2–5).

In preservative, dorsum brown with dark brown blotches, interocular bar dark brown, scapular fold dark brown with yellowish-cream blotches in the higher portions. Flanks greyish-cream with white spots in tubercles tip or warts, postocular stripe black, labial bars dark brown with thin white lines or blotches. Venter yellowish-cream with white spots in warts. Extremities yellowish-cream in concealed surfaces, dorsally brown with diagonal dark brown bars with white spots in tubercles tip, ventrally yellowish-cream with some ash shading and white spots in tip tubercles or warts (Fig. 4).

In life, dorsally Olive Clay Colour (85), with Verona Brown (37) blotches including scapular fold, and diagonal bars in extremities; snout Olive Sulphur Yellow (90), tip of snout and upper eyelids Lime Green (105); tip of tubercles in scapular fold Brunt Orange (10). In lateral view, flanks Olive Clay Colour (85) with Russet (44) botches in the upper middle portion, change to Medium Greenish-Yellow (88) and ashen Ferruginous (35) in the lower middle portion; russet labial bars, canthal blotch and postocular stripe; Smoky White (261) blotches and lines in labials. Ventrally, ashen Cream Colour (12) with Smoky White (261) spots. Interior surfaces of digits, Spectrum Yellow (79) with Verona Brown (37) splashes. Iris Light Chrome Orange (76) with black reticulations and sclera (Figs 2, 3).

Variation. In the case of the MUTPL 1613 specimen, the Olive Clay Colour (85) of the dorsum and other olive or green colour tones are replaced with Salmon Colour (83) (Fig. 2). The two female specimens (MUTPL 1613 and DHMECN 19106) are smaller in size (SVL 20.1 and 30.0 mm, respectively) than the females of the type series (37.1–39.8 mm), reported by Lynch (1979). Morphometric variation is presented in Table 1.

Distribution and natural history. *Pristimantis ruidus* was known only from its type locality Molleturo (Lynch 1979). The new record is approximately 6 km from the town of Molleturo, from the Western Andean montane evergreen forest (MAE 2012), on the same western slope of the Macizo del Cajas (Fig. 1).

Both individuals were found during the night, with full moon lighting (21:58 h–00:09 h), close to the ground (< 70 cm), in forested areas with abundant shrubby and herbaceous vegetation, soil covered by leaf litter and close to streams (Fig. 8). *Pristimantis ruidus* was observed in sympatry with *P. jimenezi* Páez & Ron, 2019 and three additional *Pristimantis* species and one *Noblella* Barbour, 1930 species with uncertain taxonomic status.

Conservation status. Currently, the forests on the Andean foothills in western Ecuador, which include the Molleturo Forest, retain only 30% of their original extent, this being one of the most degraded and fragmented ecosystems in Ecuador (Sierra et al. 2021). Molleturo is part of the Área de Vegetación y Bosques Protectores (AVBP) Molleturo-Mollepungo, a form of community reserve that covers over 140,000 hectares; however, currently it does not have a management plan and is not included in the National Environmental Protection Policy (Política Ambiental Nacional del Ecuador); this makes the entire area highly vulnerable to loss and destruction of natural vegetation cover.

Various significant threats have been identified in the region, mostly linked to activities of anthropogenic origin, such as deforestation for the expansion of grazing areas and the extraction of wood for fire. The situation is aggravated by the presence of numerous mining concessions throughout the area, as well as the proliferation of illegal mining. The Molleturo Parish has one of the highest rates of poverty and inequality in the country (Molina et al. 2015), making the area even more susceptible to rapid destruction and consequent loss of environmental quality.

The Reserva de Conservación Quitahuaycu comprises approximately 900 hectares (0.6% of the total area of Molleturo-Mollepungo AVBP), protecting ecosystems of high montane and montane evergreen forests of the western mountain of the Andean cordillera (0.14%) and the Páramo grassland (0.02%) (MAE 2012). This Reserve is the result of conservation efforts made by the community coalition of Arquillo, San José de Huigra, San Antonio and Migüir, together with the Fundación y Bioparque AMARU, aiming to preserve threatened species of amphibians which are present here, such as *Atelopus nanay* Coloma, 2002 (CR), *Pristimantis verrucolatus* Páez & Ron, 2019 (CR), *Pristimantis jimenezi* (CR), in addition to species with taxonomic uncertainty that are probably new species to science.

Pristimantis ruidus has a very low detectability. Despite intense search efforts carried out by different research groups in the area, its presence was missed over the last century and we were only able to encounter two individuals. We suspect that it only inhabits densely-forested areas with ravines and humid areas, so that the species is restricted to the best conserved parts of the ecosystem, Bosque siempreverde montano de Cordillera Occidental de Los Andes (MAE 2012), between elevations of 2317 and 2677 m.

Pristimantis ruidus is categorised as "Critically Endangered (Possibly Extinct) D" according to a recent assessment, because "given survey efforts and lack of records since 1922, it was inferred that it is either extinct or if there is still an extant population, that it is very small (< 50 mature individuals)" (IUCN SSC Amphibian Specialist Group 2023). We consider the Critically Endangered status to be

Table 1. Morphological measurements (in mm) of the two newly-collected *Pristimantis ruidus* specimens and for the holotype as provided by Lynch (1979). Abbreviations: SVL = Snout-vent length, TL = Tibia length, FL = Foot length, HL = Head length, HW = Head width, IOD = Interorbital distance, EW = Width of upper eyelid, IND = Internarial distance, EN = Eye-nostril distance, ED = Eye diameter.

Museum Code	SVL	TL	FL	HL	HW	IOD	EW	IND	EN	ED	Sex	State
DHMECN 19106	30.0	15.5	15.3	10.0	10.9	3.0	2.6	2.5	2.6	3.3	Female	adult
MUTPL 1613	20.1	10.2	9.6	6.7	7.5	2.0	1.8	1.7	1.6	2.3	Female	adult
AMNH 17590 (Holotype)	38.4	16.3	–	11.4	13.4	2.9	3.6	–	3.2	4.5	Female	adult

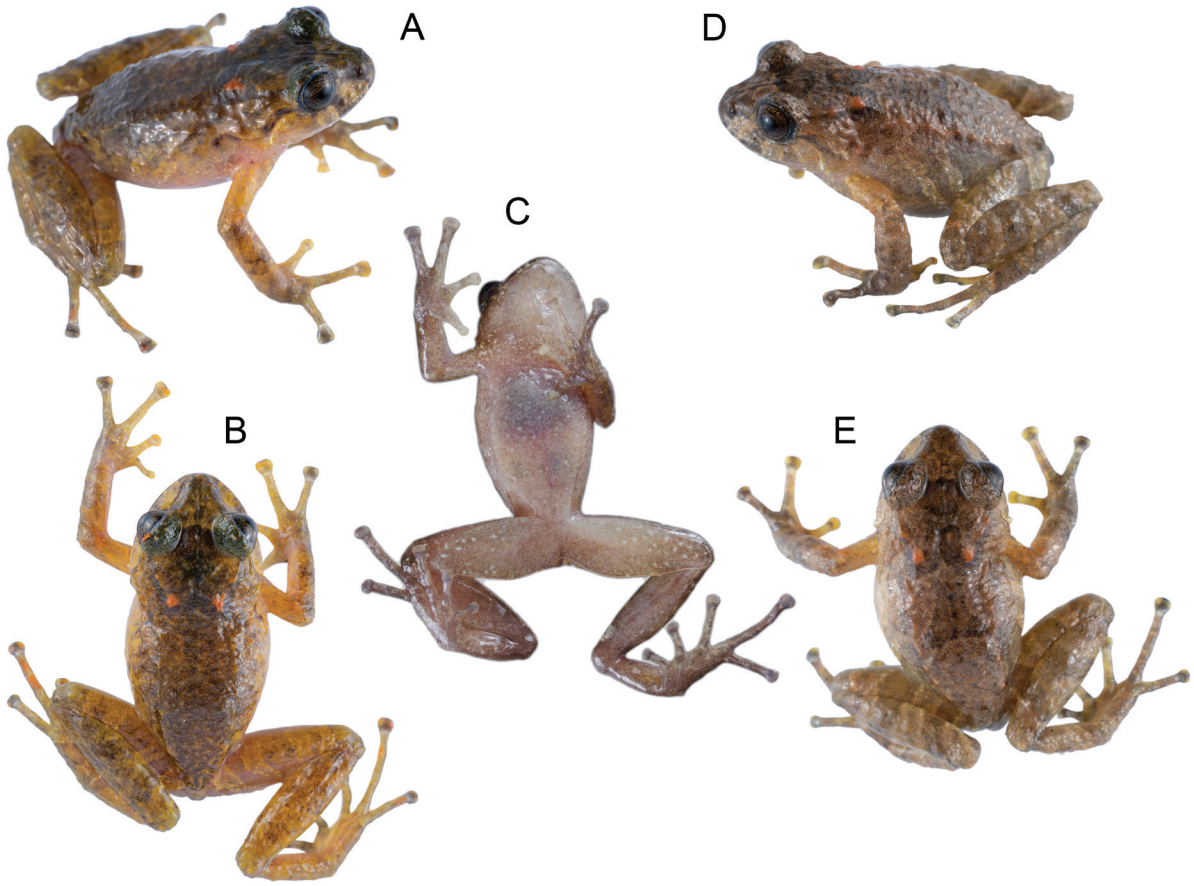

Figure 2. *Pristimantis ruidus* in life. Female DHMECN 19106: **A.** Dorso-lateral view; **B.** Dorsal view; **C.** Ventral view; SVL = 30.0 mm. Female MUTPL 1613; **D.** Dorso-lateral view; **E.** Dorsal view; SVL = 20.1 mm. Photographs: Jaime Culebras, Juan Sánchez.

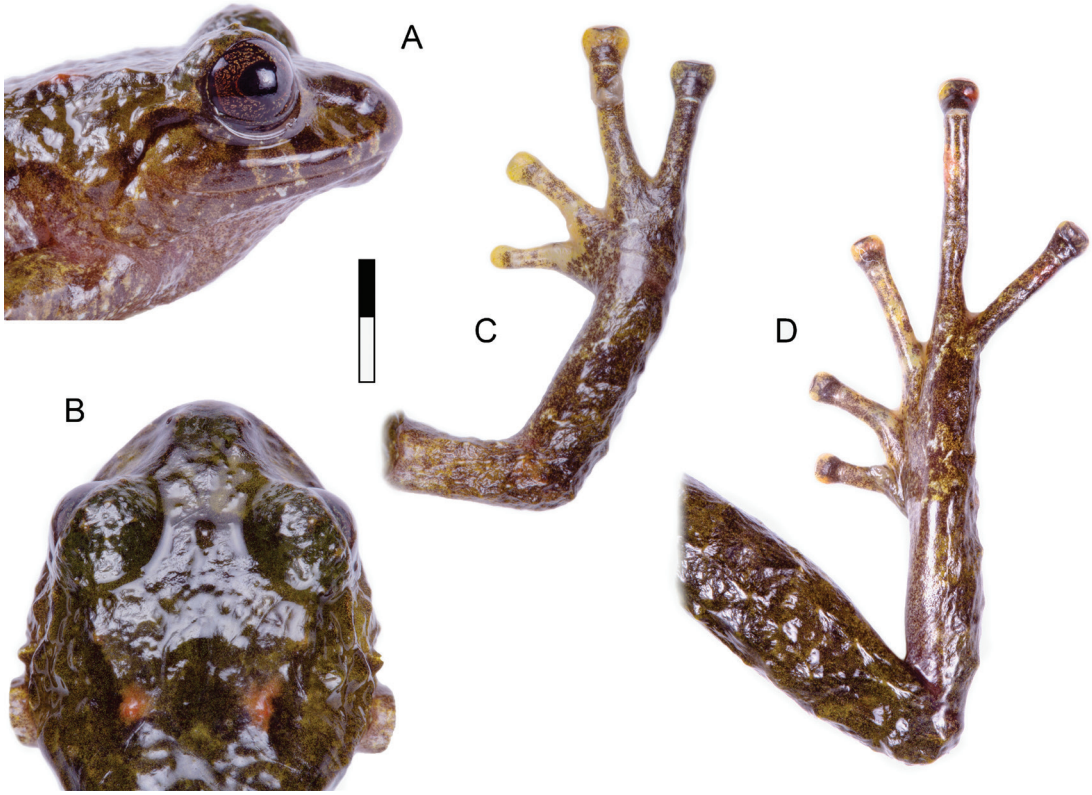

Figure 3. *Pristimantis ruidus*, adult female DHMECN 19106 in life: **A.** Lateral view of head; **B.** Dorsal view of head; **C.** Dorsal view of hand; **D.** Dorsal view of foot. Scale bar: 4 mm. Photographs: Juan Sánchez.

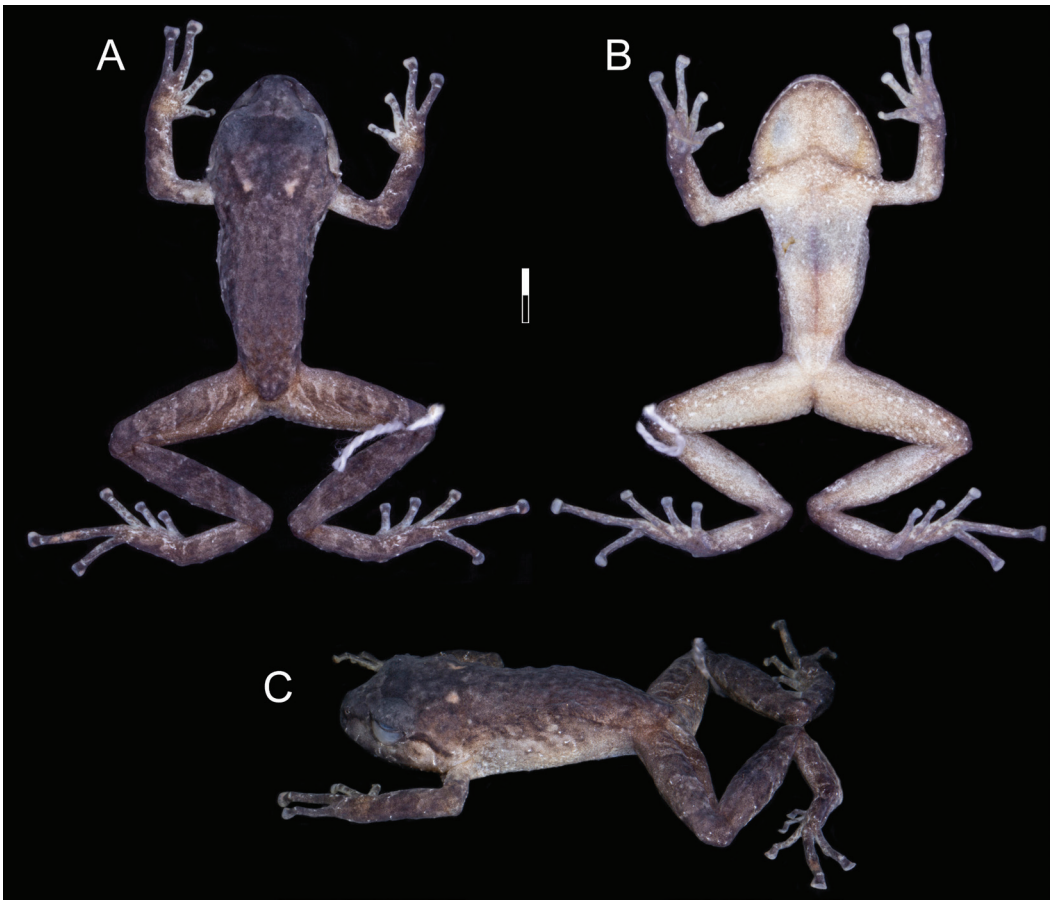

Figure 4. *Pristimantis ruidus*, adult female DHMECN 19106 in preservative: **A.** Dorsal view; **B.** Ventral view; **C.** Lateral view. Scale bar: 4 mm. Photographs: Juan Sánchez.

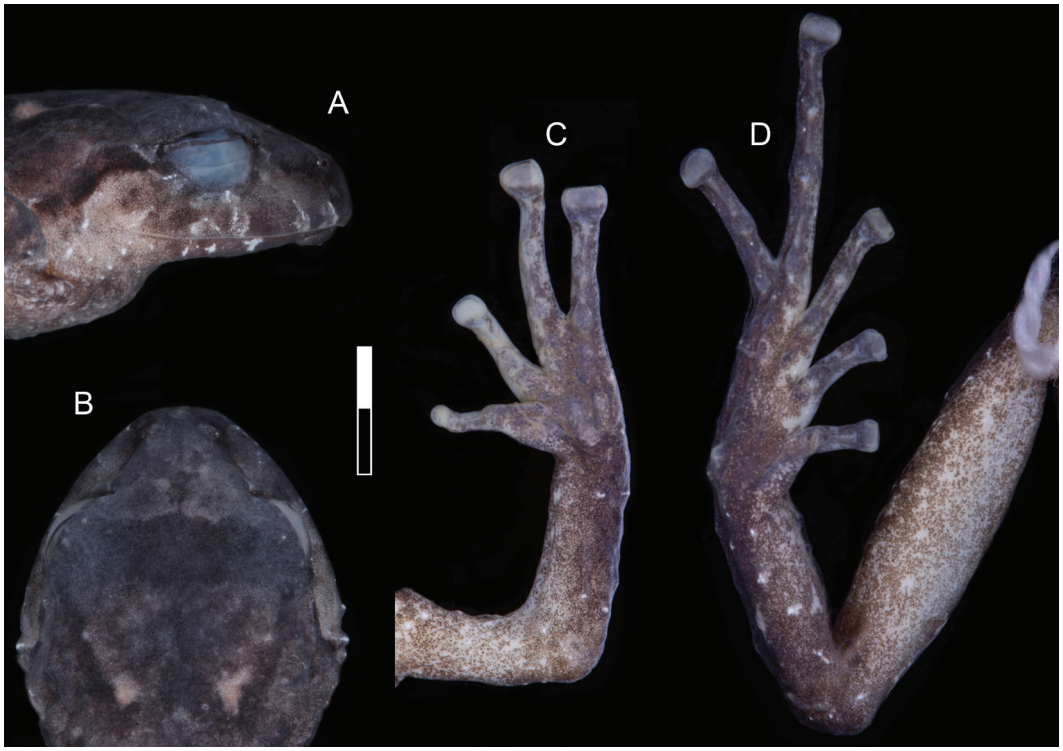

Figure 5. *Pristimantis ruidus*, adult female DHMECN 19106, details in preservative: **A.** Lateral view of head; **B.** Dorsal view of head; **C.** Ventral view of hand; **D.** Ventral view of foot. Scale bar: 4 mm. Photographs: Juan Sánchez.

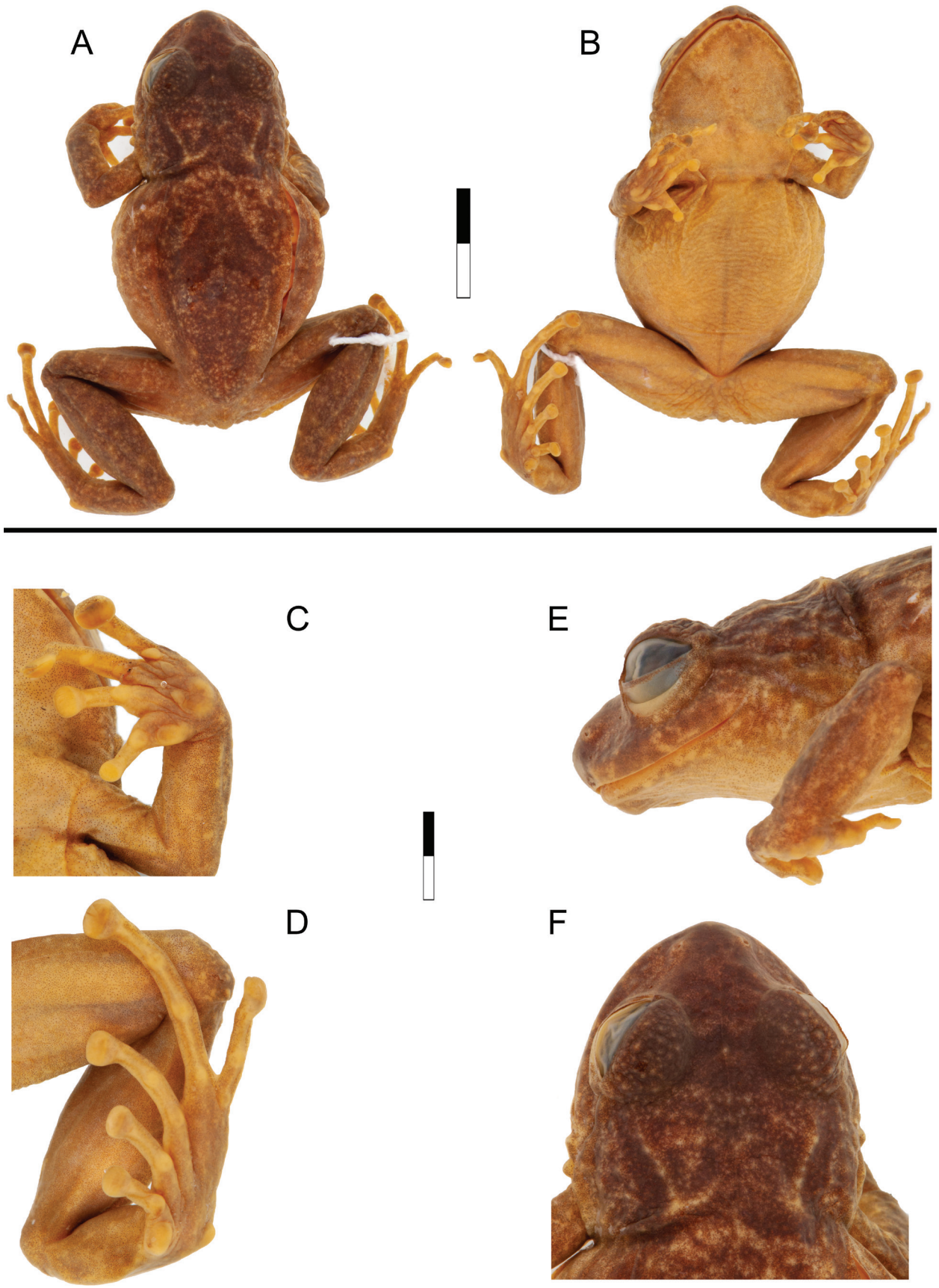

Figure 6. Holotype of *Pristimantis ruidus* AMNH 17590: **A.** Dorsal view; **B.** Ventral view; **C.** Ventral view of hand; **D.** Ventral view of foot; **E.** Lateral view of head **F.** Dorsal view of head; SVL = 38.4 mm. Scale bar: 10 mm (Above the black line); 4 mm (Below the black line). Photographs: Lauren Vonnahme.

Figure 7. Bayesian phylogenetic tree of the *Huicundomantis* subgenus of *Pristimantis*. The analysis is based on 2413 base-pairs of concatenated mitochondrial DNA from *12S* and *16S* and nuclear DNA *RAG-1* gene fragments. Outgroup is not shown. The catalogue number, species name, province and short locality name of the vouchers (in the case of Ecuadorian specimens) are shown next to each terminal (associated data are listed in Appendix 1). Abbreviations: SH-aLRT = SH-like approximate likelihood ratio branch test, UFBoot = ultrafast bootstrap, Boot = standard non-parametric bootstrap, PP = Bayesian posterior probabilities.

accurate, as the Extent of Occurrence (EOO) is less than 20 km^2, the Area of Occupancy (AOO) is less than 10 km^2, probably only one viable population and a low number of individuals. However, the complement "Possibly Extinct" should be removed from the IUCN assessment.

Remarks. We identified contradictions between the *Diagnosis* and *Description* sections from Lynch (1979), as well as characteristics that could have been lost due to the preservation of the original specimens and which are easily recognisable by examining the living specimens and recently preserved specimens. In the case of the condition of digits without rounded pads, we recognised them as elliptical. The interorbital space originally described as flat, but without mention of the presence of the one evident rounded tubercle, as it is noticeable in the photographs of the holotype and the fig. 18 in Lynch (1979). The discoidal fold is absent in our

Figure 8. Above: Panoramic view of Reserva de Conservación Quitahuaycu (Amaru Foundation). Below: Montane evergreen forest habitat of *Pristimantis ruidus*. Photographs: Ernesto Arbeláez.

specimens and the holotype photographs in concordance with *Diagnosis* (Lynch 1979) but differs from the *Description* in the same publication and is misidentified as relatively prominent. In Lynch and Duellman (1997), the authors mentioned that Toe V was much longer than Toe III; however, when examining the holotype photographs, we can observe that, in reality, the size relationship between Toe V and Toe III is similar to that of our specimens (Toe V extends slightly beyond subarticular tubercle II and Toe III does not exceed subarticular tubercle II) (Fig. 6). According to Lynch (1979), AMNH 17597 (SVL = 29.0 mm) is not a mature female, having moderate convolutions and small eggs. However, our specimen DHMECN 19106 (SVL = 30.0 mm) is an adult female because it has developed ovaries.

Discussion

The rediscovery of *P. ruidus* after one hundred years shows that there still exist montane forests that preserve conditions suitable for the survival of rare and threatened species. Despite several unsuccessful efforts to record the species in the type locality, leading to an evaluation of possibly extinct (IUCN 2023), we found that a population still persists in the area, emphasising the importance of conserving this habitat, one of the last remnants of West Andean Montane Forest in southern Ecuador (Sierra et al. 2021). For any effective conservation measures, efforts should focus on the protection and monitoring of montane forests and paramos in the region, along with the collection of basic ecological data, as well as intensive searches for additional populations.

In the original description of this species, Lynch (1979) reports that the specimens were found under rocks. This might be because, in the past, most searches for amphibians were done during the day, due to complicated logistics of night-time fieldwork. In several species of *Pristimantis* whose activity is nocturnal, they take refuge on the ground, under rocks or logs during the day (Arroyo et al. 2008). Based on our observations, *P. ruidus* similarly displays nocturnal activity. Additionally, both specimens of *P. ruidus* were found on shrubs or vegetation at the edge of streams, a microhabitat preference which we have also noticed in other *Pristimantis* species that lack an external tympanum, such as *P. philipi* and *P. cryophilius* (personal observation).

The phylogenetic closeness between *P. ruidus* and *P. philipi* and their basal position within the entire clade of the subgenus *Huicundomantis* (Páez and Ron 2019) implies an allopatric speciation process associated with the Macizo del Cajas. This region is isolated due to its high mountain ecosystems (Barnett 1999) and houses endemic species of amphibians, such as *Atelopus nanay*, *Atelopus onorei* Coloma, Lötters, Duellman & Miranda-Leiva, 2007, *P. erythros* Sánchez-Nivicela, Celi-Piedra, Posse-Sarmiento, Urgilés, Yánez-Muñoz & Cisneros-Heredia, 2018, *P. philipi* and *P. ruidus* (Lynch 1979; Lynch and Duellman 1997; Coloma 2002; Coloma et al. 2007; Sánchez-Nivicela et al. 2018).

Our comprehensive description of the morphological characters of *P. ruidus*, along with the additional information regarding its phylogenetic position, distribution and natural history, enhances our understanding of a species that has eluded researchers for decades. This will enable future taxonomic and systematic research of this group of frogs and assist in conservation efforts for a threatened fauna.

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Appendix 1

Table A1. Voucher number, GenBank accession number and locality for the *Pristimantis* (*Huicundomantis*) specimens used in the phylogenetic analysis. Bold letters mark the sequences generated by the present study.

Species	Voucher number	GenBank accession no.			Locality
		12S	*16S*	*RAG1*	
Pristimantis andinogigas	MUTPL359	MT764339	MT756022	MT810305	Ecuador: Loja, Parque Nacional Podocarpus, Cajanuma
Pristimantis atillo	QCAZ42488	-	MK881440	MK881340	Ecuador: Morona Santiago, Parque Nacional Sangay, Lagunas de Atillo
Pristimantis atillo	QCAZ42498	-	MK881444	MK881344	Ecuador: Morona Santiago, Parque Nacional Sangay
Pristimantis atratus	QCAZ45580	-	MK881471	MK881364	Ecuador: Loja, Cajanuma
Pristimantis atratus	QCAZ45645	-	MK881473	MK881366	Ecuador: Loja, Parque Nacional Podocarpus, Cajanuma
Pristimantis balionotus	MUTPL180	MT778069	MT756023	MT810306	Ecuador: Loja, Reserva Madrigal del Podocarpus
Pristimantis balionotus	MUTPL392	MT778071	MT756025	MT810308	Ecuador: Loja, Abra de Zamora
Pristimantis ceuthospilus	KU212216	EF493520	EF493520	-	Peru: Cajamarca, Chota, 12 km W Llama
Pristimantis chomskyi	MUTPL524	MZ678943	MZ678934	MZ700220	Ecuador: Loja, Parque Nacional Podocarpus, Cerro Toledo
Pristimantis chomskyi	QCAZ45666	-	MK881476	MK881369	Ecuador: Zamora Chinchipe, Reserva Tapichalaca
Pristimantis colodactylus	MUTPL388	MT778072	MT762200	MT810310	Loja, Abra de Zamora
Pristimantis cryptomelas	MUTPL135	MT778073	MT756026	MT810311	Ecuador: Loja, Abra de Zamora
Pristimantis cryptomelas	MUTPL168	MT778075	MT756028	MT810313	Ecuador: Loja, Bosque Protector Washapamba
Pristimantis diadematus	KU221999	EU186668	EU186668	-	Peru: Loreto, Teniente Lopez
Pristimantis gagliardoi	QCAZ42575	-	MK881456	MK881355	Ecuador: Morona Santiago, Parque Nacional Sangay, Ranger Station, Tinguichaca river
Pristimantis gagliardoi	QCAZ46738	-	MK881480	MK881372	Ecuador: Cañar, Reserva Mazar
Pristimantis galdi	QCAZ32368	EU186670	EU186670	EU186746	Ecuador: Zamora Chinchipe, El Pangui
Pristimantis gloria	KU218035	EF493348	EF493348	-	Ecuador: Azuay, 8.1 km W Morona Santiago border, Gualaceo-Limón road
Pristimantis gloria	MUTPL223	MT778079	MT756032	MT810317	Ecuador: Loja, 21 km E Urdaneta
Pristimantis gloria	QCAZ16448	-	MK881402	MK881316	Ecuador: Azuay, Gualaceo-Macas road
Pristimantis hampatusami	QCAZ58042	-	MK881504	MK881387	Ecuador: El Oro, Reserva Buenaventura
Pristimantis hampatusami	QCAZ58044	-	KX525478	KX525472	Ecuador: El Oro, Reserva Buenaventura

Species	Voucher number	GenBank accession no.			Locality
		12S	*16S*	*RAG1*	
Pristimantis imitatrix	KU215476	EF493824	EF493667	-	Peru: Madre de Dios, Cuzco Amazonico, 15 km E Puerto Maldonado
Pristimantis jimenezi	QCAZ45178	-	MK881468	MK881362	Ecuador: Azuay, San Antonio, Parque Nacional Cajas border
Pristimantis jimenezi	QCAZ46978	-	MK881482	MK881374	Ecuador: Azuay, Molleturo, Zadracay river
Pristimantis lojanus	MUTPL178	MZ678945	MZ678936	MZ700222	Ecuador: Loja, Loja, Quebrada San Simon
Pristimantis lojanus	MUTPL191	MZ678946	MZ678937	MZ700223	Ecuador: Loja, Cristal
Pristimantis lutzae	QCAZ32785	-	MK881421	MK881326	Ecuador: Azuay, Bosque Protector Yanuncay-Irquis, Páramo de Quimsacocha
Pristimantis lutzae	QCAZ53728	-	MK881495	-	Ecuador: Azuay, Parque Nacional Cajas, El Capo, Laguna Toreadora
Pristimantis mallii	QCAZ45744	MZ330729	MZ241492	-	Ecuador: Pastaza, Reserva Comunitaria Ankaku
Pristimantis melanogaster	MHNSM56846	EF493826	EF493664	-	Peru: Amazonas, N. Slobe Abra Barro Negro, 28 km SSW Leimebambe
Pristimantis mallii	QCAZ45770	MZ330730	MZ241496	MZ332932	Ecuador: Pastaza, Reserva Comunitaria Ankaku
Pristimantis miktos	GGU807	-	KP064151	-	Peru: Loreto, Nanay, Lote 123
Pristimantis miktos	QCAZ55445	-	MZ241510	MK391383	Ecuador: Orellana, Parque Nacional Yasuní, Tambococha
Pristimantis multicolor	QCAZ47213	-	MK881488	-	Ecuador: Loja, Parque Nacional Yacuri, Laguna Negra
Pristimantis multicolor	QCAZ47214	-	MK881489	-	Ecuador: Loja, Parque Nacional Yacuri, Laguna Negra
Pristimantis muscosus	QCAZ54857	-	MK881501	MK881386	Ecuador: Zamora Chinchipe, Reserva Tapichalaca
Pristimantis nangaritza	QCAZ41710	-	MK881436	MK881336	Ecuador: Zamora Chinchipe, Alto Nangaritza PF, Las Orquídeas, Tepuy forest
Pristimantis orcesi	KU218021	EF493679	EF493679	-	Ecuador: Pichincha, Bosque de Pasochoa
Pristimantis orestes	MUTPL242	MT778087	MK604538	MK602185	Ecuador: Loja, 11 km NE Urdaneta
Pristimantis percultus	MUTPL810	MT778088	MT756034	MT810325	Ecuador: Loja, Parque Nacional Podocarpus, Cajanuma
Pristimantis percultus	MUTPL812	MT778089	MT756035	MT810326	Ecuador: Loja, Parque Nacional Podocarpus, Cajanuma
Pristimantis philipi	KU217863	EF493672	EF493672	-	Ecuador: Azuay, 4 km W Laguna Toreadora, nearby Parque Nacional Cajas
Pristimantis philipi	QCAZ37537	-	MK881426	MK881331	Ecuador: Azuay, Parque Nacional Cajas
Pristimantis phoxocephalus	QCAZ58463	-	MK881507	MK881390	Ecuador: Cotopaxi, Pilaló surroundings
Pristimantis rhodoplichus	KU219788	EF493674	EF493674	-	Peru: Piura, El Tambo
Pristimantis ruidus	DHMECN19106	**PP725379**	**PP723736**	**PP731015**	Ecuador: Azuay, Quitahuaycu reserve
Pristimantis ruidus	MUTPL1613	**PP725380**	**PP723737**	**PP731016**	Ecuador: Azuay, Quitahuaycu reserve
Pristimantis simonsii	KU212350	EU186665	EU186665	-	Peru: Cajamarca, S slope Abra Quilsh, 28 km NNW Cajamarca
Pristimantis spinosus	KU218052	EF493673	EF493673	-	Ecuador: Morona Santiago, 10.6 km W Plan de Milagro
Pristimantis tamia	QCAZ59630	MZ330735	MZ241526	MZ332958	Ecuador: Pastaza, Parque Nacional Llanganates, Comunidad Zarentza
Pristimantis tamia	QCAZ59643	MZ330736	MZ241528	MZ332960	Ecuador: Pastaza, Parque Nacional Llanganates, Comunidad Zarentza
Pristimantis teslai	QCAZ46213	-	MK881478	-	Ecuador: Tungurahua, Llanganatillo, Parque Nacional Llanganates border
Pristimantis tinguichaca	QCAZ31945	-	MK881418	MK881323	Ecuador: Morona Santiago, Parque Nacional Sangay, San Vicente
Pristimantis tinguichaca	QCAZ40582	-	MK881433	MK881334	Ecuador: Morona Santiago, Parque Nacional Sangay, Lagunas de Atillo
Pristimantis torresi	MUTPL996	MZ678947	MZ678938	MZ700224	Ecuador: Loja, Guachanamá, El Apretadero
Pristimantis torresi	QCAZ47397	-	MK881492	MK881380	Ecuador: Loja, Celica-Alamor road
Pristimantis totoroi	KU218025	EF493349	EF493349	-	Ecuador: Chimborazo, 70 km W Riobamba via Pallatanga
Pristimantis totoroi	QCAZ58425	-	MK881505	MK881388	Ecuador: Cotopaxi, Pilaló surroundings
Pristimantis unistrigatus	KU218057	EF493387	EF493387	EF493444	Ecuador: Imbabura, 35 km E Pquela
Pristimantis verrucolatus	QCAZ46982	-	MK881483	MK881375	Ecuador: Azuay, Yumate, Shoupshe
Pristimantis verrucolatus	QCAZ46993	-	MK881485	MK881377	Ecuador: Azuay, Cochapamba
Pristimantis versicolor	KU218096	EF493389	EF493389	EF493431	Ecuador: Loja, Abra de Zamora
Pristimantis versicolor	MUTPL494	MT778095	MT756038	MT810332	Ecuador: Loja, Reserva Madrigal del Podocarpus
Pristimantis wiensi	KU219796	EF493377	EF493668	-	Peru: Piura, 12.7 km E Canchaque
Pristimantis sp.	QCAZ26642	-	MK881409	-	Ecuador: Azuay, San Antonio de Chaucha
Pristimantis sp.	QCAZ32790	-	MK881423	MK881328	Ecuador: Azuay, Bosque Protector Yanuncay-Irquis, Páramo de Quimsacocha
Pristimantis sp.	QCAZ45029	-	MK881461	-	Ecuador: Morona Santiago, Parque Nacional Sangay, Etén, Rio Culebrillas
Pristimantis sp.	QCAZ45129	-	MK881462	MK881358	Ecuador: El Oro, Chillacocha
Pristimantis sp.	QCAZ45720	-	MZ241490	MZ332927	Ecuador: Pastaza, Reserva Comunitaria Ankaku
Pristimantis sp.	QCAZ45945	-	MZ241502	MZ332938	Ecuador: Pastaza, Reserva Comunitaria Ankaku
Pristimantis sp.	QCAZ53999	-	MK881496	-	Ecuador: Zamora Chinchipe, Yacuambi, Romerillos
Pristimantis sp.	QCAZ58855	-	MZ241514	MZ332949	Ecuador: Morona Santiago, Parque Nacional Sangay, Sardinayacu