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# Similar looking sisters: A new sibling species in the *Pristimantis danae* group from the southwestern Amazon basin (Anura, Strabomantidae)

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## Abstract

We describe a new frog species that is the sibling of *Pristimantis reichlei*. These two sister species inhabit the Amazonian lowlands and adjacent foothills of the Andes, from central Bolivia to central Peru. *Pristimantis reichlei* occurs from central Bolivia to southern Peru (Alto Purús National Park), while the new species occurs from northern Bolivia (Departamento Pando) to Panguana in central Peru (Departamento Huánuco), at elevations between 220 and 470 m a.s.l. In spite of their morphological crypsis, these siblings occur in syntopy without evidence of interbreeding (in the Alto Purús area) and are recovered as reciprocally monophyletic. Their uncorrected pairwise genetic distances in the 16S rRNA gene range from 9.5–13.5%, and their advertisement calls differ in both qualitative and quantitative traits. Moreover, our study found uncorrected pairwise distances within the new species of up to 5.0% and up to 9.3% within *P. reichlei*. We therefore cannot rule out the possible existence of hybrids or additional species-level lineages hidden in this complex. Furthermore, we found another potential pair of sibling species composed of *P. danae* and an unnamed lineage, with divergences of 9.4% in the 16S gene, whose in-depth analysis and taxonomic treatment are pending future revision. With the new nominal species, the *Pristimantis danae* species group now includes 20 species, distributed across the upper Amazon basin and in the eastern Andes, from western Brazil to Bolivia and Peru. Our study, together with an increasing number of other studies, indicates that sibling species are far from being rare among Amazonian amphibians and that species resolution remains low even for groups that have received considerable attention in recent years.

## Key Words

Amphibia, bioacoustics, integrative taxonomy, morphology, molecular genetics, systematics, Bolivia, Brazil, Peru

# Introduction

The genus *Pristimantis* Jiménez de la Espada, 1870 represents an extremely species-rich and ecologically diverse clade of Neotropical frogs. It is considered challenging for researchers with respect to their systematics because of the many genealogically distant species co-occurring in sympatry yet showing highly similar phenotypes, and due to the numerous geographically and phylogenetically distant yet also similar-looking species (e.g., Elmer et al. 2007; Padial and De la Riva 2009; García et al. 2012; Pinto-Sánchez et al. 2012). These patterns of variation and distribution led to the erection of many non-monophyletic species groups (see Padial et al. 2014). Although our knowledge of species diversity and relationships remains rudimentary, the application of molecular phylogenetics has improved the systematics of the genus considerably over the last two decades (e.g., Heinicke et al. 2007; Hedges et al. 2008; Canedo and Haddad 2012; Pinto-Sánchez et al. 2012; Padial et al. 2014; Páez and Ron 2019; Ron et al. 2020; Sánchez-Nivicela et al. 2021; Arroyo et al. 2022). Among other results, many non-monophyletic species groups and genera have been redefined so as to represent monophyletic groups (Padial et al. 2014; Gonzáles-Durán et al. 2017; Páez and Ron 2019; Chávez et al. 2021; Zumel et al. 2021). Still, most nominal *Pristimantis* species remain unassigned to any species group, and the affinities of many assigned ones remain uncorroborated, both because of our limited understanding of phenotypic variation and a lack of genetic data (e.g., Padial et al. 2014).

Hedges et al. (2008) erected the *Pristimantis peruvianus* species group for some species formerly in the *P. conspicillatus* and *P. unistrigatus* groups. However, these authors did not study topotypic material of *P. peruvianus*. Instead, they relied on samples from populations in the Amazon lowlands of SW Peru, which at the time were considered *P. peruvianus* (Padial and De la Riva 2005) and which later were identified as belonging to a new species, *P. reichlei* (Padial and De la Riva 2009). Thus, the nominal species group could not be maintained as the species for which it was named belonged to a different clade. Padial et al. (2014) re-allocated *P. peruvianus* to the *P. conspicillatus* group and erected the *P. danae* species group for some species formerly considered part of the *P. peruvianus* group (sensu Hedges et al. 2008), which included *P. danae* and *P. reichlei* as well as others (*P. albertus*, *P. aniptopalmatus*, *P. cuneirostris*, *P. ornatus*, *P. pharangobates*, *P. rhabdolaemus*, *P. sagittulus*, *P. stictogaster*, *P. toftae*). They found this group to be monophyletic and sister to the *P. conspicillatus* group. Further research resulted in new species that were part of this species group (Lehr and von May 2017; Herrera-Alva et al. 2023; Lehr et al. 2017; Venegas et al. 2023), adding six species to it, all but two (*P. clarae*, *P. similaris*), from high elevations of the Pui Pui Protected Forest in central Peru (*P. attenboroughi*, *P. bounides*, *P. humboldti*, *P. puipui*). In addition, Herrera-Alva et al. (2023) revealed *P. scitulus* as part of the *P. danae* species group.

The systematics among several populations in southwestern Amazonia and along the eastern Andean slopes of Bolivia and Peru, today considered part of the *P. conspicillatus* and *P. danae* species groups, has been historically complex and partly chaotic, with certain populations misidentified at the species level and repeatedly allocated to the wrong groups (see Padial and De la Riva 2005, 2009). The reason for these long-lasting uncertainties was caused by superficial morphological similarities among species as well as by traits shared among species in both species groups. Padial and De la Riva (2009), with the descriptions of *P. koehleri* (*P. conspicillatus* group) and *P. reichlei* (*P. danae* group), cleared part of the existing chaos, as both species were previously confused with other taxa of both groups, respectively. They also demonstrated that among Amazonian frogs, there could be many sister and similar-looking unnamed species (i.e., sibling species; see Bickford et al. 2007) hidden under names that are applied to populations of more than one species.

In a recent study on the taxonomy and systematics of species in the *P. conspicillatus* group, Köhler et al. (2022) included species of the related *P. danae* group and provided a first indication of the presence of two divergent lineages among populations currently considered part of *P. reichlei*, as later also demonstrated by Herrera-Alva et al. (2023). In this new study, we follow clues provided by Köhler et al. (2022) and Herrera-Alva et al. (2023), and, based on genetic and bioacoustic evidence, we describe and name a new species of *Pristimantis* that is morphologically most similar and phylogenetically sister to *P. reichlei*, and with which it occurs in sympatry in some areas of the southwestern Amazon basin.

# Material and methods

## Fieldwork and voucher specimens

Fieldwork was conducted in different areas of the southwestern Amazon basin. Specimens were observed and collected during opportunistic searches during the day and night using torchlights and headlamps. Geographic position was recorded using handheld GPS receivers set to WGS84 datum. Collected specimens were euthanized with an overdose of 5% lidocaine or benzocaine gel applied to the ventral surfaces of individuals (McDiarmid 1994). Tissue samples were taken prior to fixation and stored in pure ethanol, while specimens were fixed using 96% ethanol or 4% formalin and subsequently stored in 70% ethanol. Newly collected specimens were deposited in the herpetological collections of the Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (**MUSM**), Lima, Peru; Zoologische Staatssammlung München (**ZSM**), Germany; Museo de Biodiversidad del Perú (**MUBI**), Cusco, Peru; and Carnegie Museum of Natural History (**CM**), Pittsburgh, USA. **KU** refers to the University of Kansas, Museum of Natural History, Lawrence, USA; **MNCN** refers to the Museo Nacional de Ciencias Naturales, Madrid, Spain; **MCZ** refers to the Museum of Comparative Zoology, Harvard University, Cambridge, USA; **MNK-A** refers to the amphibian collection of the Museo Noel Kempff Mercado, Santa Cruz, Bolivia; **NMP6V** refers to the Department of Zoology, National Museum, Prague, Czech Republic; **SMNS** refers to Staatliches Museum für Naturkunde Stuttgart, Germany; **ZFMK** refers to Zoologisches Forschungsmuseum A. Koenig, Bonn, Germany; **FGZC** refers to Frank Glaw field numbers.

## External morphology

Morphometric measurements (in millimeters) were taken by CAP, JCC, JK, and JMP with calipers to the nearest 0.1 mm. Measurements taken and used throughout the text are those used by Köhler et al. (2022): **SVL**, snout–vent length (distance from tip of snout to posterior end of the body); **TL**, tibia length (distance from knee to distal end of tibia); **HW**, head width (at level of angle of jaws); **HL**, head length (from posterior margin of lower jaw to tip of snout); **IOD**, interorbital distance (distance between upper eyelids); **ED**, horizontal eye diameter (length of the visible eye); **E–N**, eye–nostril distance (straight line distance between anterior corner of orbit and posterior margin of external nares); **TD**, tympanum diameter (horizontal distance between the peripheral borders of the tympanic annulus); **HandL**, hand length (from proximal border of outer metacarpal tubercle to tip of third finger); **FootL**, foot length (from proximal border of inner metatarsal tubercle to tip of fourth toe). Fingers and toes are numbered preaxially to postaxially from I–IV and I–V, respectively. The lengths of fingers I and II were determined by adpressing the fingers against each other. For character state definitions, we followed Duellman and Lehr (2009). Descriptions and diagnostic schemes follow Padial et al. (2016). Coloration in life was described based on digital photographs.

## Bioacoustics

Vocalizations in the field were recorded using an Edirol R-09 digital recorder with built-in microphones (at 44.1 KHz and 16-bit resolution, saved as uncompressed wave format) or a Sony WM-D6C tape recorder with an attached directional microphone Sennheiser Me-80 (for calls of *P. reichlei*). Recordings were sampled or re-sampled at 22.05 kHz and 32-bit resolution and analyzed using the software Cool Edit Pro 2.0. Frequency information was obtained through Fast Fourier Transformation (FFT; width 1024 points) at the Hanning window function. Spectrograms were obtained with the Blackman window function with 256 bands resolution. Sensitive filtering was applied to remove background sounds, but only to frequencies outside the prevalent bandwidths of calls. Temporal measurements are summarized as a range, with the mean ± standard deviation in parentheses. Description, terminology, and methods follow those recommended by Köhler et al. (2017), using the call-centered terminological scheme. Representative sections of the call recordings analyzed were archived in the Zenodo repository under DOI https://doi.org/10.5281/zenodo.10852627.

## Molecular data

Monophyly and sister relationships between the focal lineage and putative new species were tested through phylogenetic analyses of a fragment of the mitochondrial 16S rRNA gene. As revealed by preliminary data presented by Köhler et al. (2022), the focal lineage (*P.* cf. *reichlei* sensu Köhler et al. 2022; hereafter referred to as *P.* aff. *reichlei*) is part of the *P. danae* species group. For taxon sampling, we therefore selected representative samples of this group as well as of its sister clade, the *P. conspicillatus* group (Padial et al. 2014). As the focal lineage is morphologically most similar to *P. reichlei* and *P. danae*, which are sister species (Padial and De la Riva 2009; Padial et al. 2014), we included all homologous DNA sequences of these species available from GenBank. We rooted our tree with *Yunganastes*, which is sister to *Pristimantis* (Padial et al. 2009). We extracted DNA using a standard salt extraction protocol and performed polymerase chain reactions (PCR) with primers 16SAr-L (5’-CGCCTGTTTATCAAAAACAT-3’) and 16SBr-H (5’-CCGGTCTGAACTCAGATCACGT-3’) (Palumbi et al. 1991). PCR products were then directly sequenced on automated DNA sequencers by LGC Genomics (Berlin, Germany). All new DNA sequences were submitted to GenBank (accession numbers PP621188–PP621210). We used MAFFT (Katoh and Standley 2013) with the G-INS-i algorithm as implemented in Concatenator (Vences et al. 2022) to align sequences to reference sequences of other *Pristimantis* downloaded from GenBank. After removing differences in length resulting from variations in priming regions, we obtained an alignment of 496 base pairs and 75 terminals. We used these data to infer the best model (TIM2+F+I+R3) under Model Finder (Kalyaanamoorthy et al. 2017), as well as the maximum likelihood tree (log-likelihood: -4805.1412) (Nguyen et al. 2015), using IQ-TREE 2.2.0 (Minh et al. 2020). Branch support was assessed through 500 replicates of standard non-parametric bootstrapping as well as 1000 iterations of the Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT) (Shimodaira and Hasegawa 1999), both implemented in IQ-TREE. Genetic divergences were quantified as uncorrected pairwise distances (p-distances) on pairwise alignments, not considering gaps using TaxI2 (Vences et al. 2021). A table with all used sequences, GenBank accession numbers, voucher numbers, and locality information is archived in the Zenodo repository under DOI https://doi.org/10.5281/zenodo.10958854.

# Results

## Molecular data

The inferred maximum likelihood tree (log-likelihood: -4805.1412; Fig. 1), based on an alignment of 496 nucleotides of the 16S rRNA gene, recovered the *Pristimantis conspicillatus* and *P. danae* species groups as monophyletic, with higher support for the latter. Our samples of *P. reichlei* are grouped into two well-supported and highly divergent clades, although the support for their sister relationship is low (bootstrap/SH-aLRT = 45/78). One clade includes all topotypical samples, and we considered it *P. reichlei* sensu stricto. Within this clade of nominal *P. reichlei*, considerable differentiation is evident among samples. The other clade includes samples from Panguana, central Peru, south-eastern Peru, and northernmost Bolivia. We labeled this clade *P.* aff. *reichlei*. The clade containing *P.* aff. *reichlei* and *P. reichlei* is sister to *P.* aff. *danae* samples from Bolivia, and the clade containing *P.* aff. *reichlei*, *P. reichlei*, and *P.* aff. *danae* is sister to nominal *P. danae* samples from Peru. These four mentioned subclades together are sister to all samples in the *P. danae* group from high elevations, and these all together are sister to samples of *P. cosnipatae* + *P. toftae* and *Pristimantis* sp. (GenBank accession number KY652655; a sample formerly identified as *P. pharangobates*; see Herrera-Alva et al. 2023), although some of the basal nodes received no significant bootstrap support.

Genetic divergences between the target lineages were rather high compared to other known sister lineage divergences in *Pristimantis* (e.g., Köhler et al. 2022; Herrera-Alva et al. 2023). *Pristimantis* aff. *reichlei* differed from *P. reichlei* in uncorrected pairwise distances by 9.5–13.5% (mean 11.4%), and *P.* aff. *danae* from Bolivia differed from *P. danae* from Peru invariably by 9.4% for all available sequences. *Pristimantis* aff. *danae* differed from *P. reichlei* by 11.9–15.2% (mean 12.7%) and from *P.* aff. *reichlei* by 7.4–9.1% (mean 8.3%). The respective values for nominal *P. danae* were 12.3–14.8% (mean 13.2%) and 7.4–9.1% (mean 8.3%).

Genetic distances were also high within two of these four main lineages. While no sequence divergence was found among the *P. danae* samples from Peru (all from the same or nearby localities) and only up to 0.2% among sequences from Bolivian *P.* aff. *danae*, uncorrected pairwise distances within *P.* aff. *reichlei* amounted up to 5.0% and within *P. reichlei* up to 9.3% (between samples from the Bolivian type locality Los Guácharos and Santo Domingo de Carabaya, Peru, but <6% between numerous other populations). See the Zenodo archive (DOI https://doi.org/10.5281/zenodo.10958854) for a table with all pairwise distances.

## Morphology

Our external morphological examination of specimens of the focal lineage *P.* aff. *reichlei* and comparisons with paratype specimens of *P. reichlei* from close to its type locality (Provincia Chapare, Departamento Cochabamba, Bolivia; ZFMK 66975, 72564–72565, 72587–72589) revealed no diagnostic differences in measurements (Table 1), proportions, or color pattern. The only qualitative morphological differences observed are comparatively faint and were: (1) specimens of *P.* aff. *reichlei* exhibit a barely recognizable small, flat, round outer metatarsal tubercle in life, which is virtually absent in preservative, whereas in *P. reichlei* it is distinct, prominent, ovate, and subconical, both in life and preservative (Fig. 4); (2) a difference in the outline of the canthus rostralis when viewed in straight angle from above. In *P.* aff. *reichlei*, the canthus rostralis runs almost straight from the anterior corner of the eye to the tip of snout, whereas in *P. reichlei*, the canthus rostralis is concavely curved around the nostrils, resulting in an indentation that allows to see a larger portion of the nostrils in straight dorsal view (Fig. 5). However, given the limited number of individuals available for direct examination (Table 1), we are not completely confident that the latter is a constant diagnostic character to distinguish among both major lineages across their entire ranges.

## Bioacoustics

The advertisement call of *P.* aff. *reichlei* from Panguana is characterized by being a single pulsatile note, simple in structure, and repeated at irregular intervals (see species account below for a detailed call description). Compared to Bolivian calls of *P. reichlei* from close to its type locality (Köhler 2000; Márquez et al. 2002 [as *Eleutherodactylus danae*]), the sister taxon of the focal lineage, the following differences are evident: Advertisement calls of *P. reichlei* are always composed of two pulsed calls (= notes) emitted in conjunction (a two-note call when following the note-centered terminology of Köhler et al. 2017) that differ from each other in duration and dominant frequency, with the second call being slightly longer in duration and higher in dominant frequency compared to the first call. Call duration (= note duration) in calls of *P. reichlei* is longer (49–68 ms) compared to calls of *P.* aff. *reichlei* at Panguana (23–47 ms), without overlap, and calls are emitted at a different temporal pattern (see Köhler 2000). Moreover, calls of *P. reichlei* are distinctly pulsed, with pulses being clearly separated and countable (pulse rate 155–194 pulses/second), versus pulses being largely fused and barely separated from each other but emitted at a distinctly higher rate in *P.* aff. *reichlei* (pulse rate ca. 300–375 pulses/second). Despite similar male body sizes (Table 1), the dominant frequency in calls of *P. reichlei* is significantly lower (1685–2384 Hz) when compared to those of *P.* aff. *reichlei* (3289–3628 Hz). Calls of both species are immediately distinguishable in the field, as they sound quite different to the human ear.

Although only very few call recordings are available for analysis and respective comparisons among the focal lineages in consideration (calls of two individuals of *P.* aff *reichlei* and three individuals of *P. reichlei*), the qualitative and quantitative differences in advertisement call structure revealed are far beyond those that could

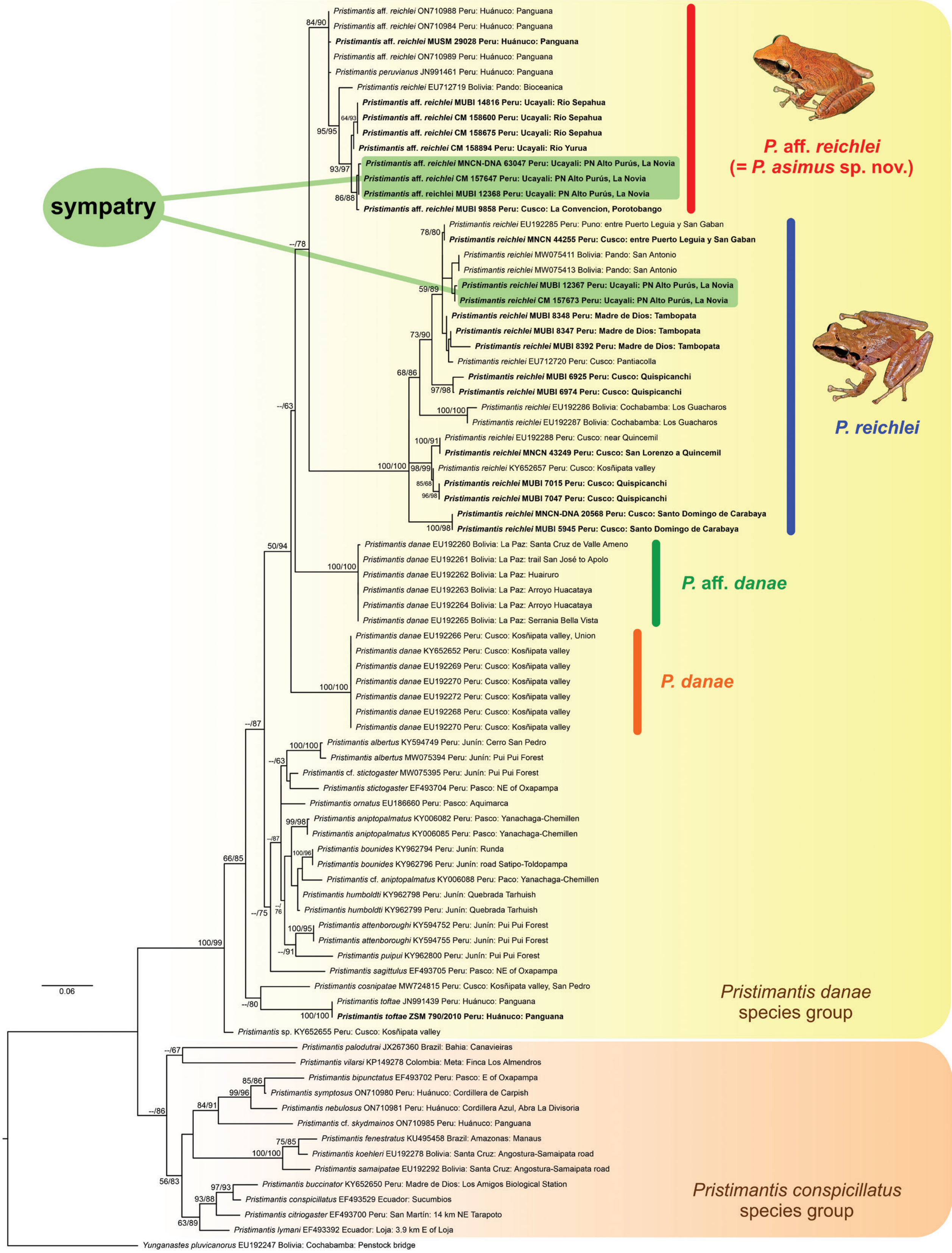

**Figure 1.** Maximum likelihood phylogenetic tree of selected samples of *Pristimantis*, representing the *P. conspicillatus* and *P. danae* species groups, inferred from an alignment of 496 nucleotides of the mitochondrial 16S rRNA gene. *Yunganastes pluvicanorus* was used to root the tree. Numbers at nodes are bootstrap values in percent (500 replicates; not shown if <50%) followed by SH-aLRT values (1000 iterations; not shown if <50) as calculated with IQ-Tree. The taxon name is followed by the GenBank accession number, or voucher number for newly produced sequences (bold terminals), and sample locality. Samples highlighted in green indicate the sympatric locality of *P. asimus* and *P. reichlei*. Inset photos depict paratopotypes of *P. asimus* sp. nov. (MUSM 29074) and *P. reichlei* (ZFMK 66975) in life, respectively.

**Table 1.** Morphometric measurements (in mm) of specimens of *Pristimantis asimus* sp. nov., *P. reichlei*, and *P. danae*, including holotypes and paratypes. BO = Bolivia; PE = Peru; m = male; f = female; subad. = subadult (all other adult specimens); * = paratype; ** = holotype; — = not available. For other abbreviations used, see Material and methods.

| | sex | SVL | TL | HL | HW | IOD | ED | TD | E-N | HandL | FootL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ***P. asimus*** | | | | | | | | | | | |
| MUSM 41241** PE: Panguana | m | 30.6 | 17.9 | 12.1 | 11.2 | 3.1 | 4.3 | 1.9 | 4.1 | 7.8 | 14.3 |
| MUSM 29028* PE: Panguana | m | 27.7 | 17.1 | 10.6 | 9.8 | 3.1 | 4.3 | 1.7 | 3.3 | 7.6 | 13.6 |
| MUSM 29073* PE: Panguana | m | 27.7 | 17.1 | 11.2 | 9.9 | 3.6 | 4.0 | 2.0 | 3.3 | 7.7 | 13.3 |
| MUSM 29074* PE: Panguana | m | 28.3 | 17.0 | 11.3 | 10.1 | 3.5 | 4.0 | 2.1 | 3.6 | 8.1 | 14.2 |
| ZSM 1986/2008* PE: Panguana | m | 28.4 | 17.3 | 12.7 | 10.4 | 3.2 | 4.1 | 1.9 | 3.5 | 7.8 | 14.2 |
| ZSM 1985/2008* PE: Panguana | m | 30.6 | 17.2 | 12.8 | 10.9 | 3.4 | 3.9 | 2.0 | 4.2 | 7.6 | 13.9 |
| SMNS 8856* PE: Panguana | m | 29.8 | 17.7 | 11.9 | 10.6 | 3.7 | 3.9 | 2.1 | 3.9 | 10.0 | 14.1 |
| MUBI 12368* PE: La Novia | m | 30.6 | 18.5 | 11.9 | 11.1 | 3.0 | 4.0 | 1.9 | 4.0 | 8.6 | 16.9 |
| MUBI 14816* PE: Rio Sepahua | f subad. | 26.0 | 15.4 | 9.6 | 9.5 | 2.7 | 3.0 | 1.5 | 3.3 | 7.2 | 11.4 |
| SMNS 6386* PE: Panguana | f | 37.9 | 22.4 | 14.7 | 13.7 | 4.7 | 4.8 | 2.5 | 5.1 | 10.8 | 17.7 |
| ***P. reichlei*** | | | | | | | | | | | |
| ZFMK 66975* BO: Chapare | m | 30.5 | 19.8 | 12.7 | 11.1 | 3.2 | 4.0 | 2.0 | 3.9 | 9.0 | 16.3 |
| ZFMK 72588* BO: Chapare | m | 27.7 | 15.6 | 10.7 | 9.8 | 3.1 | 4.0 | 1.7 | 3.8 | 8.3 | 14.0 |
| ZFMK 72589* BO: Chapare | m | 28.2 | 18.3 | 11.3 | 10.8 | 3.5 | 4.6 | 2.2 | 3.6 | 9.3 | 15.4 |
| ZFMK 72564* BO: Chapare | m | 28.8 | 16.9 | 13.1 | 10.5 | 3.6 | 3.9 | 2.0 | 4.0 | 8.6 | 14.4 |
| ZFMK 72565* BO: Chapare | m | 27.9 | 16.9 | 11.4 | 9.6 | 2.8 | 3.9 | 2.3 | 3.2 | 8.5 | 14.5 |
| MNCN 43028* BO: Chapare | m | 25.3 | 15.4 | 9.6 | 9.1 | — | 3.6 | 1.6 | 3.2 | — | 12.6 |
| MNK-A 6620** BO: Chapare | f | 32.3 | 20.5 | 12.6 | 11.8 | — | 3.8 | 1.7 | 4.0 | — | 17.0 |
| MNCN 43024* BO: Chapare | f | 35.0 | 21.4 | 12.7 | 12.1 | — | 4.6 | 2.0 | 4.4 | — | 17.4 |
| ZFMK 72587* BO: Chapare | f | 39.1 | 24.4 | 15.2 | 15.0 | 4.3 | 5.4 | 2.6 | 4.8 | 11.8 | 21.5 |
| MUBI 8347 PE: Tambopata | f | 33.4 | 19.8 | 12.1 | 11.4 | 4.0 | 4.2 | 1.8 | 4.2 | 8.7 | 14.8 |
| MUBI 8347 PE: Tambopata | f | 31.5 | 17.3 | 12.0 | 11.3 | 3.8 | 3.4 | 1.8 | 3.7 | 8.5 | 13.5 |
| MUBI 12367 PE: La Novia | f | 30.9 | 19.2 | 12.3 | 11.4 | 3.4 | 4.0 | 1.9 | 4.0 | 8.5 | 14.8 |
| ***P. danae*** | | | | | | | | | | | |
| KU 162307** PE: Kosñipata | m | 34.3 | 16.8 | 13.6 | 12.9 | — | 5.6 | 2.1 | 4.3 | — | 16.6 |
| MCZ 93305* PE: Kosñipata | m | 30.7 | 17.6 | 12.0 | 11.4 | — | 4.9 | 1.9 | 4.1 | — | 15.1 |
| MCZ 93306* PE: Kosñipata | m | 26.0 | 15.9 | 10.6 | 9.6 | — | 4.4 | 1.7 | 3.7 | — | 13.4 |
| MUSM 13957* PE: Santa Isabel | m | 24.3 | 15.7 | 10.9 | 9.9 | 3.5 | 4.2 | 1.8 | 3.6 | 7.9 | 12.5 |

be attributed to intra-specific call variation (see Köhler et al. 2017). Even if more recordings become available in the future, it is more than unlikely that an increased sample size would alter the conclusions derived from our bioacoustic analyses by interpreting observed differences in calls as intra-specific variation. On the contrary, call parameters and spectrograms available in the published literature (e.g., Schlüter 1980; Rodríguez 1994; Duellman 2005) indicate a misidentification of respective populations and corroboration of the call characteristics described for both lineages compared herein (see below).

Calls of *P. danae* from Manu National Park in Peru (https://soundcloud.com/user-416416746/pristimantis-danae?in=user-416416746/sets/cantos-de-nfibios-del-pn-manu) are trill-like calls of approximately 80–120 ms duration, composed of 3–4 widely spaced pulses (pulse rate approximately 30 pulses/second). Calls of *P.* aff. *danae* from Bolivia are characterized by being composed of two notes, each note containing two distinctly separated pulses only (Padial and De la Riva 2009).

In summary, the bioacoustic differences observed among the clades here allocated to *P. reichlei*, *P. danae*, *P.* aff. *danae*, and the focal lineage *P.* aff. *reichlei* provide further evidence for the specific distinctness of respective lineages, particularly in view of the partly sympatric occurrence of *P. reichlei* and *P.* aff. *reichlei* (see below). Consequently, we describe the lineage hitherto referred to as *P.* aff. *reichlei* as a new species.

## Taxonomy

### ***Pristimantis asimus* sp. nov.**

https://zoobank.org/298F59D4-1918-4833-8C64-B935FA7E826E

**Remarks.** This species has been previously referred to as *Eleutherodactylus peruvianus* by Schlüter (1980) and Lehr (2002 [partim]); *Eleutherodactylus fenestratus* by Rodríguez (1994); *Eleutherodactylus* aff. *conspicillatus* by Schlüter (2005); *Pristimantis* cf. *danae* by Moravec and Aparicio (2005); *Pristimantis reichlei* by Padial and De la Riva (2009 [partim]), Moravec et al. (2020 [partim]), and Herrera-Alva et al. (2023 [partim]); *Pristimantis peruvianus* by Pinto-Sánchez et al. (2012), de Oliveira et al. (2020), and Fouquet et al. (2022); and *Pristimantis* cf. *reichlei* by Köhler et al. (2022).

**Type material.** ***Holotype*.** MUSM 41241 (FGZC 5342, formerly ZSM 177/2017), adult male (Figs 2, 3), from the Área de Conservación Privada Panguana (9.6166°S, 74.9333°W, 260 m above sea level), lower Río Yuyapichis, Provincia Puerto Inca, Departamento Huánuco, Peru, collected on 29 September 2017 by F. Glaw. GenBank accession number for 16S: ON710989.

***Paratypes*.** A total of 16 specimens: ZSM 1985–1986/2008 (FGZC 3388–3389), two adult males, same locality as holotype, collected on 10 December 2008 by F. Glaw; MUSM 29073–29074 (FGZC 3300, 3273), two adult males, MUSM 29028 (FGZC 3274), an adult male (call voucher), same locality as holotype, collected between 26 November and 2 December 2008 by F. Glaw; FGZC 6334 (to be deposited in ZSM), an adult male, same locality as holotype, collected on 17 November 2019 by E. Castillo-Urbina, F. Glaw and J. Köhler; SMNS 6386, an adult female, same locality as holotype, collected on 11 November 1985 by A. Schlüter; SMNS 8856, an adult male, same locality as holotype, collected in 1972 by R. Aussem; MUBI 14816, a subadult female, CM 158675, an adult male, from Campamento 4, between Quebrada Sungaro and Quebrada Esther, Río Sepahua (11.0801°S, 72.1258°W, 395 m a.s.l.), Distrito

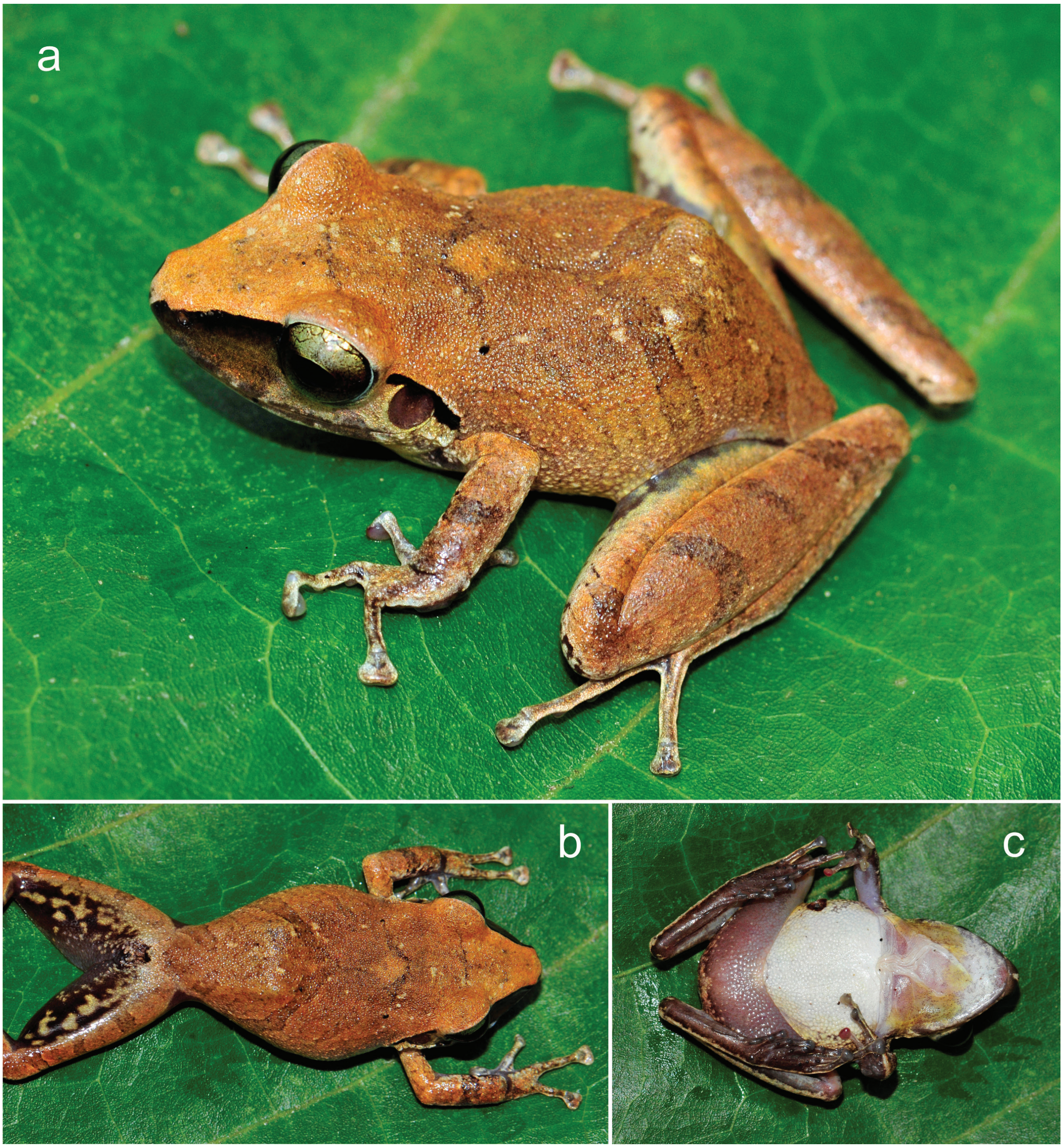

**Figure 2.** Male holotype of *Pristimantis asimus* sp. nov. (MUSM 41241, FGZC 5342) from Panguana, Departamento Huánuco, Peru, in life (SVL 30.6 mm): **a.** Dorsolateral view; **b.** Dorsal view (showing posterior thighs coloration); **c.** Ventral view.

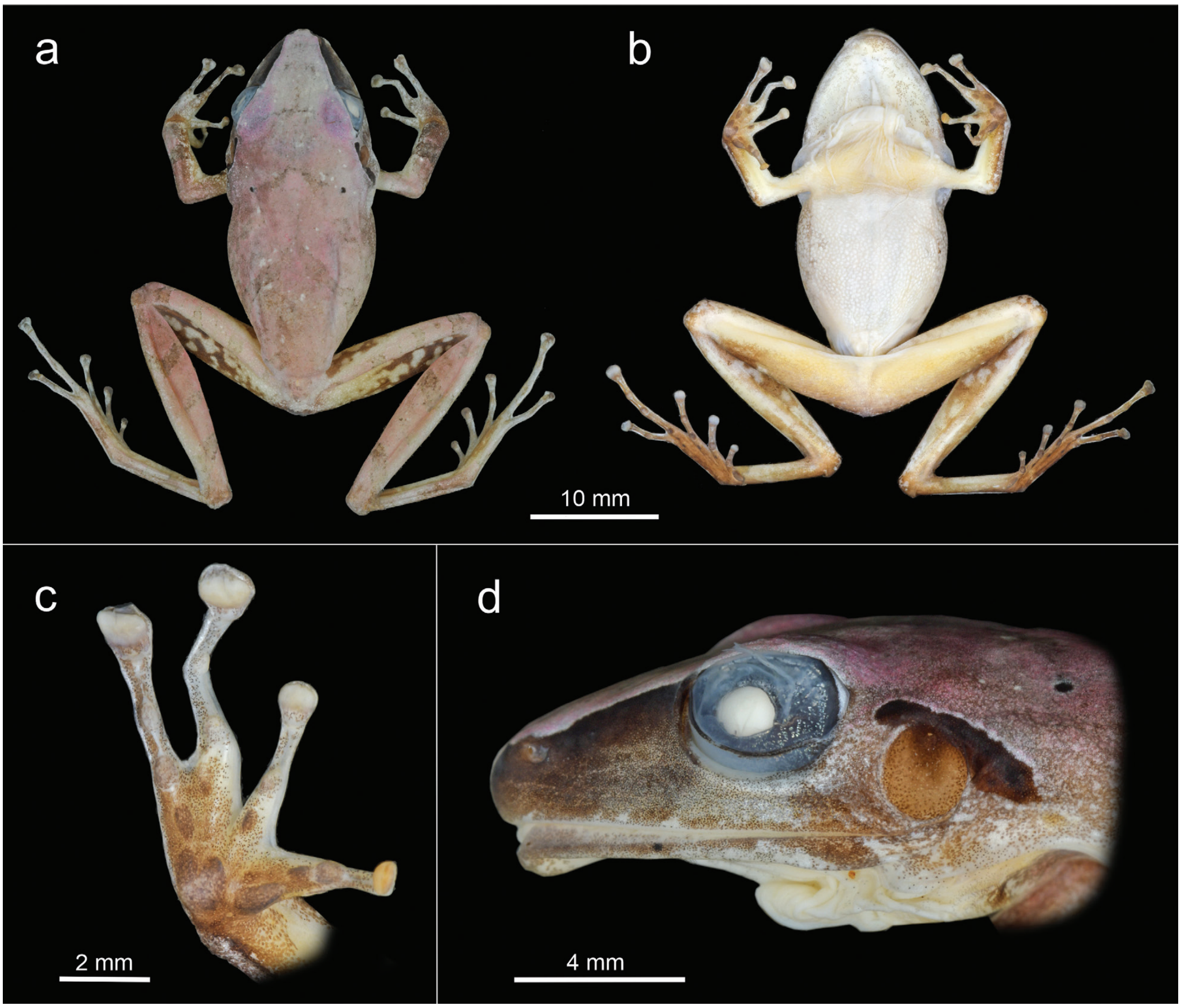

**Figure 3.** Preserved male holotype of *Pristimantis asimus* sp. nov. (MUSM 41241, FGZC 5342) from Panguana, Departamento Huánuco, Peru: **a.** Dorsal view; **b.** Ventral view; **c.** Palmar surface of the right hand; **d.** Lateral view of the head.

Sepahua, Provincia Atayala, Departamento Ucayali, Peru, collected on 3 and 6 March 2014, respectively, by J. M. Padial, L. A. G. Gagliardi, J. C. Chaparro and R. Gutiérrez; CM 158600, an unsexed adult, from a track across the river from Campamento 2, Río Sepahua (11.0491°S, 72.4489°W, 408 m a.s.l.), Distrito Sepahua, Provincia Atayala, Departamento Ucayali, Peru, collected on 24 February 2014 by J. M. Padial, L. A. G. Gagliardi, J. C. Chaparro and R. Gutiérrez; CM 158894, an adult male from a track ca. 4 km west of Breu, on the road to Victoria (9.5451°S, 72.7933°W, 223 m a.s.l.), Distrito Yurúa, Departamento Ucayali, Peru, collected on 12 February 2015, by J. M. Padial, L. A. G. Gagliardi, R. Gutiérrez, O. Rojas and S. Castroviejo-Fisher; MUBI 12368, an adult male, from Puesto de Control y Vigilancia La Novia, Río La Novia (9.9883°S, 70.7084°W, 262 m a.s.l.), Distrito Purús, Departamento Ucayali, Peru, collected on 25 January 2013 by J. M. Padial, L. A. G. Gagliardi, R. Gutiérrez and S. Castroviejo-Fisher; MUBI 9858, an unsexed adult from Porotobango (11.4311°S, 73.3471°W, 469 m a.s.l.) Provincia La Convención, Departamento Cusco, Peru, collected on 25 January 2010 by L. Tejada; NMP6V 72578/1–2, two adult males (also paratypes of *P. reichlei*), from Bioceanica (11.1333°S, 69.3666°W, 290 m a.s.l.), Departamento Pando, Bolivia, collected on 25 January 2005 by J. Moravec.

**Definition.** A medium-sized species of the *Pristimantis danae* species group (based on molecular relationships and morphological similarity), with 27.7–30.6 mm SVL in adult males (n = 7), and 37.9 mm SVL in adult females (n = 1), characterized by: (1) skin on dorsum finely shagreened, lacking enlarged tubercles or warts; throat smooth, venter areolate; discoidal fold inconspicuous; dorsolateral folds absent; upper eyelid lacking tubercles and granules; posterior surfaces of thighs smooth; (2) tympanic membrane and annulus distinct, slightly higher than long, their length less than half of eye diameter; supratympanic fold prominent, curved, slightly covering upper edge of tympanic annulus; (3) head slightly longer than wide; snout subacuminate in dorsal view, bluntly rounded in lateral profile; canthus rostralis straight in dorsal view, slightly rounded in profile; (4) cranial crests absent; (5) dentigerous process of vomers elongate, oblique, situated posteromedial to choanae; (6) males with vocal slits, single subgular vocal sac; indistinct nuptial asperities on dorsal surface of thenar tubercle; (7) hands with slender fingers, first finger slightly shorter or about equal in length to second; subarticular tubercles subconical, prominent; supernumerary tubercles absent; palmar tubercle cordiform; thenar tubercle prominent, elongated; terminal discs of inner two fingers enlarged and round, those of external fingers enlarged, truncate, about twice the width of digit proximal to disc; circumferential grooves conspicuous, ungual flap not indented; narrow lateral fringes on fingers weakly developed; basal webbing between fingers absent; (8) ulnar tubercles absent; (9) tubercles on heel and tarsus absent, tarsal fold present; (10) inner metatarsal tubercle prominent, ovate; outer metatarsal tubercle small, round, flat, barely recognizable in life, virtually absent in preservative; supernumerary tubercles absent; (11) toes long and slender; lateral fringes narrow, weak; basal toe webbing present; toe V reaching beyond distal level of penultimate subarticular tubercle of toe IV; tips of toes rounded to slightly ovate, enlarged; circumferential grooves conspicuous; (12) in life, dorsal coloration light brown, reddish-brown, or tan, usually with dark brown chevrons and flecks on dorsum; dark brown bars on dorsal surfaces of arms and legs; a pair of black spots dorsolaterally in scapular region; black supratympanic stripe; black canthal stripe; belly creamy white; throat with fine dark mottling in males; posterior surfaces of thighs blackish with yellowish-cream spots and flecking; iris bronze, with black reticulation in life; posterior iris periphery cream to turquoise; bones white; (13) advertisement call consisting of a single pulsatile note of 23–47 ms duration and with a dominant frequency of 3289–3628 Hz, repeated at irregular intervals, containing groups of 2–4 calls repeated in faster succession.

**Diagnosis.** *Pristimantis asimus* differs from other species in the *Pristimantis danae* species group as follows: The new species differs from *P. albertus*, *P. attenboroughi*, *P. bounides*, *P. clarae*, *P. cosnipatae*, *P. humboldti*, *P. ornatus*, *P. pharangobates*, *P. puipui*, *P. rhabdolaemus*, *P. sagittulus*, *P. similaris*, *P. stictogaster*, and *P. toftae*, at least by the lack of dorsolateral folds (versus presence). Furthermore, *P. attenboroughi*, *P. bounides*, *P. humboldti*, and *P. puipui* have stout bodies with relatively shorter legs when compared to *P. asimus*. *Pristimantis attenboroughi* and *P. puipui* lack a tympanum (prominent in the new species), and *P. attenboroughi* lacks vocal slits in males (present in the new species). *Pristimantis clarae* additionally differs from the new species by dorsal and ventral color pattern, advertisement call, and smaller adult male size (12.9–15.6 versus 27.7–30.6 mm). As the new species, *P. aniptopalmatus* lacks dorsolateral folds (Duellman and Hedges 2005) but differs by its smaller adult male size (16.5–23.2 versus 27.7–30.6 mm), presence of a tubercle on the upper eyelid, and color pattern (Duellman and Lehr 2009). *Pristimantis cuneirostris* lacks dorsolateral folds but differs from the new species by a long wedge-shaped snout (unique in the *P. danae* species group; Duellman and Pramuk 1999; Duellman and Lehr 2009), lack of toe webbing (versus basal webbing present), and posterior surfaces of thighs uniformly brown (versus blackish with yellowish-cream blotches and flecks). *Pristimantis scitulus* mainly differs from the new species by the presence of a single conical tubercle on upper eyelid (absent), dentigerous processes of vomers absent (present), presence of a conical tubercle on heel (absent), and webbing on toes absent (basal webbing present) (Duellman 1978).

Morphologically, *P. asimus* is most similar or even cryptic to *P. danae* and *P. reichlei*. However, as a tendency, nominal *P. danae* (from Kosñipata valley, Peru) exhibit a more contrasting dorsal color pattern in life (see, e.g., Duellman and Lehr 2009: fig. 150) when compared to *P. asimus*. Moreover, in dorsal view, the snout in *P. danae* is rounded (versus subacuminate in *P. asimus*). From *P. reichlei*, the new species seems to differ by a very small, round, flat outer metatarsal tubercle, barely recognizable in life and virtually indistinguishable in preservative (versus distinct, ovate, subconical, recognizable in life and in preservative; Fig. 4). Moreover, there are slight differences in the dorsal outline of the canthus rostralis, with a relatively smaller portion of the nostrils being visible when viewed from straight above in *P. asimus* compared to *P. reichlei*, where nostrils are almost completely visible from above as the canthus rostralis shows a curved indentation around the nostrils (Fig. 5). The new species differs from both *P. danae* and *P. reichlei* by substantial differentiation in the 16S gene and differences in the advertisement call (see above).

The new species occurs in sympatry with some species of the *P. conspicillatus* group, which superficially may have a similar appearance. However, these are distinguishable from *P. asimus* by molecular phylogenetic relationships, differences in advertisement calls, and most of them by different relative finger lengths, i.e., the first finger being longer than the second. However, the sympatric *P. iiap* has the first and second fingers equal in length but differs from the new species at least by exhibiting distinct dorsolateral folds (Padial et al. 2016).

**Description of the holotype.** An adult male, in good state of preservation (Fig. 3), with subgular vocal sac and vocal slits. Head slightly longer than wide (HL/HW = 1.08); snout subacuminate in dorsal view, bluntly rounded in profile; nostrils oriented posterolaterally; canthus rostralis straight in dorsal view, slightly rounded in profile; loreal region slightly concave; lips not flared; upper eyelid without tubercles; cranial crests absent. Supratympanic fold prominent, long, slightly curved, covering uppermost tympanic annulus; tympanic membrane and annulus distinct; tympanic membrane slightly higher than long, its length slightly less than half the eye diameter; one flat round postrictal tubercle. Choanae not concealed by palatal shelf of the maxillary arch when roof of mouth is viewed from below; choanae large, oval, separated by distance equal to five times diameter of a choana; dentigerous process of vomers present, but barely evident, flat,

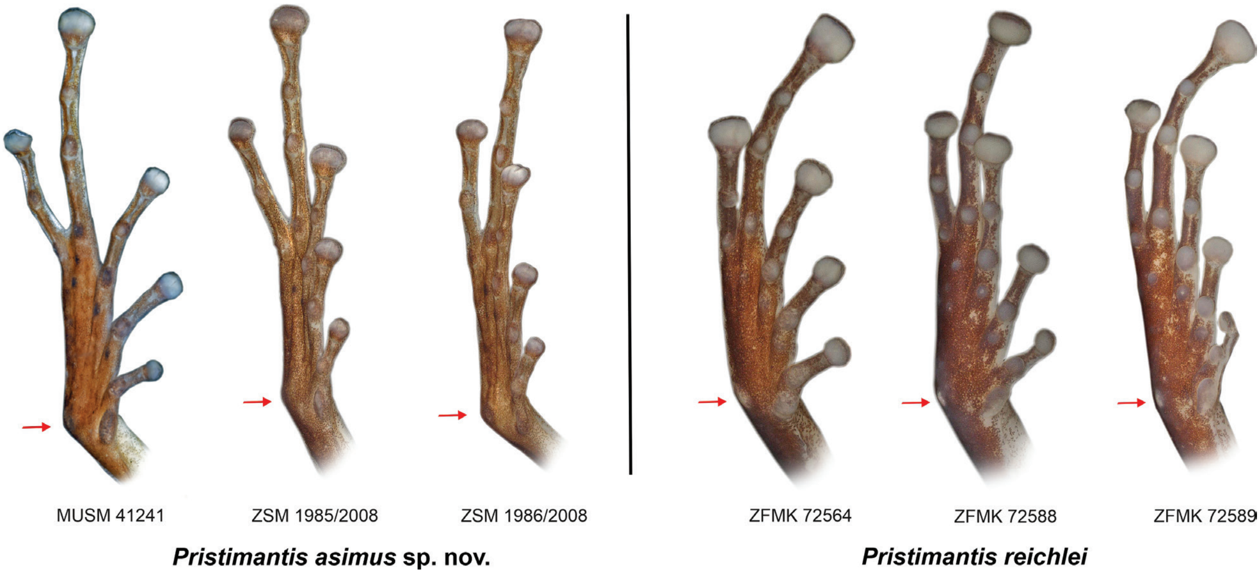

**Figure 4.** Comparative plate showing plantar surfaces of the right feet of preserved type specimens of *Pristimantis asimus* sp. nov. (all from Panguana, Huánuco, Peru) and *P. reichlei* (all from Chapare, Cochabamba, Bolivia). Red arrows point to the outer metatarsus, showing the virtual absence versus the presence of an outer metatarsal tubercle. Not to scale.

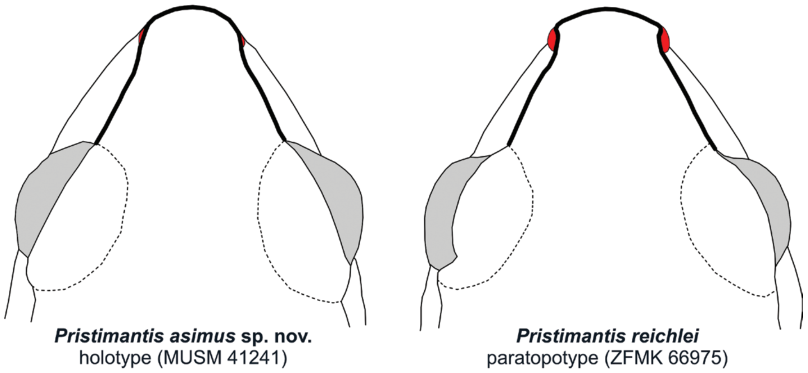

**Figure 5.** Schematic line drawings of dorsal views of the heads of male *Pristimantis asimus* sp. nov. and *P. reichlei*, exemplifying the differing outlines of the canthus rostralis (bold line), resulting in the visibility of different portions of the nostrils (red) in straight dorsal view. Not to scale.

elongate, not in contact, oblique, situated posteromedial to choanae, bearing vomerine teeth; tongue removed for tissue sample; vocal sac subgular, vocal slits placed posterolaterally. Skin on dorsum finely shagreened, lacking enlarged tubercles or warts; dorsal surfaces of hind limbs finely shagreened, dorsal surfaces of forearms and flanks finely shagreened; skin on throat and chest smooth, that on belly areolate; occipital folds absent; dorsolateral folds absent; discoidal fold indistinct. Arm without ulnar tubercles; palmar tubercle cordiform, about double in size to elongate thenar tubercle; supernumerary tubercles absent; subarticular tubercles prominent, subconical; fingers long and slender; finger tips enlarged, rounded on inner two fingers, on two outer fingers truncate, their width about twice the width of digit proximal to disc; circumferential grooves conspicuous, ungual flap not indented on outer fingers; lateral fringes and keels on fingers weak, barely recognizable; basal webbing between fingers absent; relative length of fingers III>IV>II≥I; nuptial asperities on dorsal surface of thenar tubercle indistinct. Toes long and slender (FootL 47% of SVL); heel and tarsus lacking tubercles; tarsal fold present; inner metatarsal tubercle ovate, prominent; outer metatarsal tubercle not recognizable; supernumerary tubercles absent; subarticular tubercles prominent, subconical; narrow lateral fringes on toes present, weakly developed; basal toe webbing present; toe tips enlarged, rounded, their width about 1.5 times the width of toe proximal to disc; circumferential grooves conspicuous; relative length of toes IV>V>III>II>I; toe V reaching slightly beyond distal level of penultimate subarticular tubercle of toe IV. Tibiotarsal articulation reaching distinctly beyond tip of snout when hindlimb flexed parallel to axis of body; heels broadly overlapping when hind limbs flexed perpendicular to axis of body. For morphological measurements, see Table 1.

In life (Fig. 2), dorsal ground color light brown, with darker brown chevron-shaped marking slightly anterior to sacral region; irregular dark brown U-shaped line in scapular region, bordered posteriorly by orange-brown blotch; irregular indistinct dark brown markings and lines

**Figure 6.** Paratypes of *Pristimantis asimus* sp. nov. in life (dorsolateral and ventral views): **a.** ZSM 1985/2008 (FGZC 3388; SVL 30.6 mm); **b.** ZSM 1986/2008 (FGZC 3389; SVL 28.4 mm); **c.** MUSM 29074 (FGZC 3273; SVL 28.3 mm), all from the type locality Panguana, Huánuco, Peru; **d.** CM 158600, from Río Sepahua, Ucayali, Peru.

on dorsum; minute cream flecks, irregular in outline, scattered on dorsum; triangular cream fleck on snout tip; dark brown bars on dorsal surfaces of arms and legs; dark brown interorbital line, partly interrupted, not extending to upper eyelids; a pair of small black spots dorsolaterally in scapular region; black supratympanic stripe; broad blackish canthal stripe; lips dark brown to black, irregularly barred with cream; flanks light brown with irregular dark brown markings; belly creamy-white; anterior throat grayish-white with scattered fine gray mottling, posterior throat yellowish with irregular fine brown mottling; chest pinkish-white; ventral surfaces of thighs and shanks pinkish-gray; posterior surface of thighs blackish with irregular yellow-cream blotches and flecks; tarsus, plantar, and palmar surfaces dark brown; iris bronze, with black reticulation, with a dark reddish-brown median streak; posterior iris periphery cream with a turquoise tint; bones white. After six years in preservative (Fig. 3), the general color pattern remains the same as in life. Brown ground coloration turned to grayish-tan, with some pinkish tint, particularly on upper eyelids; brown flecks, bars, and markings on dorsum slightly faded; chest and ventral surfaces of thighs yellowish-cream; belly creamy-white; throat creamy-white with gray mottling.

**Variation.** For variation in morphological measurements among type specimens, see Table 1. Females are significantly larger than males, approximately reaching 25% greater SVL. We observed some limited variation in color and color pattern among the specimens studied. In some individuals, the dorsal ground coloration is yellowish-tan to yellowish-brown in life (e.g., ZSM 1985/2008; Fig. 6a), whereas in most specimens, the dorsal ground color was reddish-brown in life (e.g., ZSM 1986/2008, MUSM 29074, CM 158600; Fig. 6b, c, d), as it was in the holotype. Darker dorsal markings might be more or less distinctly outlined with fine cream lines, with some individuals seemingly lacking these fine cream lines (Fig. 6a, b). A dark interorbital stripe is present in all specimens but narrow, less conspicuous, and barely extending on upper eyelids in the holotype and some paratypes (Figs 2, 6a, b), whereas a broader, distinct stripe outlined with fine cream lines extends to the upper eyelids in other specimens (Figs 6c, d, 7). Scattered dorsal cream spots and flecking might be present to different extent or completely absent. The contrasting color pattern on the posterior surfaces of thighs (dark brown with yellow-orange flecking) is present in all studied specimens, with CM 158600 having some orange color extending to the groin (Fig. 6d). Some males exhibit shades of

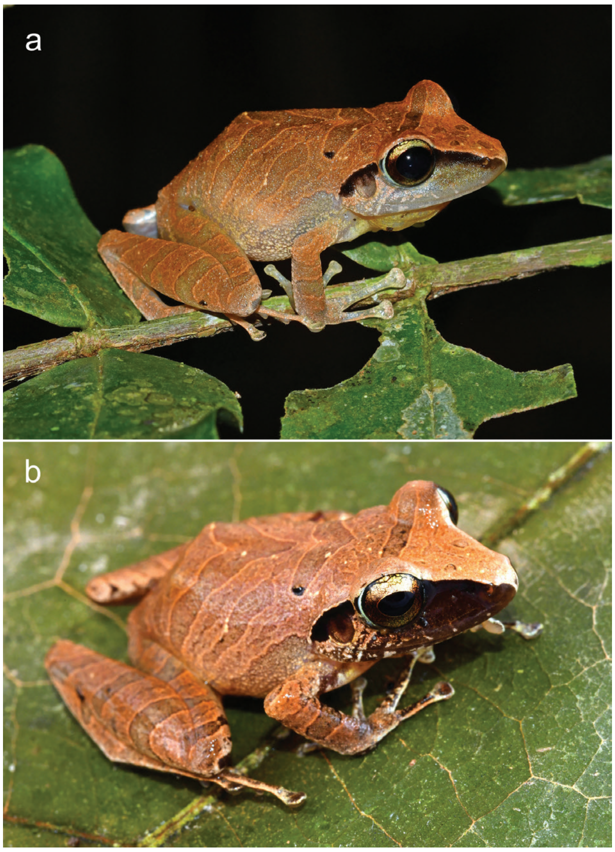

**Figure 7.** Male paratopotype (FGZC 6334) of *Pristimantis asimus* sp. nov. in life at: **a.** Night coloration in situ; **b.** Day coloration the day after collection, demonstrating a limited ability of color change. Note the dark brown, almost black, loreal region and upper lip in the day coloration, resulting in a dark facemask.

yellow on the throat in life (Fig. 6). Remarkably, the male paratype MUBI 14816 exhibits a well-marked, W-shaped occipital fold that is lacking in other specimens.

**Natural history.** At Panguana, individuals of this species have been observed being active at night, perching on small trees and bushes within disturbed primary forest. Schlüter (1980) reported males calling from vegetation mainly at 1–2 m height, with calling activity being most intense at dusk and at dawn, which is confirmed by our own observations. Schlüter (1980) described a vertical calling position with the head down as typical, but we observed calling males in a horizontal position on the upper side of leaves. Individuals of this species have some limited ability to change color, with nocturnal color being characterized by a light brown loreal region, whereas diurnal color is characterized by a dark loreal region and upper lip, turning dark brown to almost black (see Fig. 7). The habitat close to the type locality has recently been impacted by illegal gold mining activities, which constitute a potential threat to the anuran fauna of Panguana.

**Vocalization.** Advertisement calls of *Pristimantis asimus*, emitted by the male MUSM 29028, were recorded on 29 November 2008, at dusk (18:15 h) at the type locality (ambient temperature not recorded). The calling male was sitting on top of a horizontally oriented leaf at approximately 2.1 m height within the forest. The call consists of a single short pulsatile note, repeated at somewhat irregular intervals (see below). In our recording, 4 to 14 calls were emitted in succession and then interrupted by few seconds of silence. Each call (= note) has a clearly pulsatile structure, although 'pulses' are largely fused and, in most cases, barely countable. However, in some cases, distinct and thus countable energy peaks (4–10/call) are recognizable in the oscillograms of calls. In these calls, the pulse rate varies between 300 and 375 pulses/second. There is further amplitude modulation recognizable within each call, with maximum call energy being present around the center of each call, rapidly decreasing to a lower level, and further fading towards the call's end. Calls were usually emitted in slow succession but regularly contained sections of more rapidly repeated calls, usually 2–4 calls emitted at shorter intervals, altering the relatively regular pattern of call repetition (see Fig. 8a). In other words, call repetition rate may change temporarily from ca. 140 calls/min to ca. 900 calls/min. The character of calls in these rapidly repeated sections did not differ from other calls. It remains unknown whether this increased speed in call succession has a different function (e.g., territorial) or is just part of the ordinary advertisement call. Numerical parameters for 65 analyzed calls of the mentioned male are as follows: call duration (= note duration) 23–47 ms (36.0 ± 7.1 ms); inter-call interval in slow calling sections 305–597 ms (394.6 ± 73.6 ms); inter-call interval in rapid calling sections 26–37 ms (31.8 ± 4.6 ms); dominant frequency 3289–3628 Hz (3518 ± 97 Hz); second frequency peak at around 2000 Hz; prevalent bandwidth 1600–5400 Hz.

Schlüter (1980) described the call from the same locality (under the name *Eleutherodactylus peruvianus*). The spectrogram and numerical parameters (call duration ca. 40 ms) provided by him agree with our analysis. The call described by Rodríguez (1994) as that of *Eleutherodactylus fenestratus* from Cocha Cashu, Manu National Park, Madre de Dios, Peru, is also clearly referable to *P. asimus*. The parameters described by Duellman (2005) for calls of *P. fenestratus* from Cusco Amazónico clearly differ from those of *P. asimus*, but the corresponding audiospectrogram and oscillogram are in disagreement with the numerical parameters provided and may possibly correspond to *P. asimus*. Although difficult to compare according to another terminology used, the call described for *E. fenestratus* from Manaus, Brazil, by Zimmerman and Bogart (1984) seems to agree with that of *P. asimus*.

**Distribution.** As far as known and confirmed by bioacoustic and/or genetic data, *P. asimus* occurs in lowland rainforests from the southern Departamento Huánuco (Panguana, type locality) and eastern Departamento Ucayali (Breu, Río Yurua) southward across most of the lowlands of southeastern Peru, reaching the border of Machiguenga Communal Reserve (Departamento Cusco) and Manu National Park (Departamento Madre de Dios) in the south as well as northernmost Bolivia (Bioceanica, Departamento Pando) to the east (Fig. 9). All known localities are at elevations between 220 and 470 m a.s.l. The new species occurs

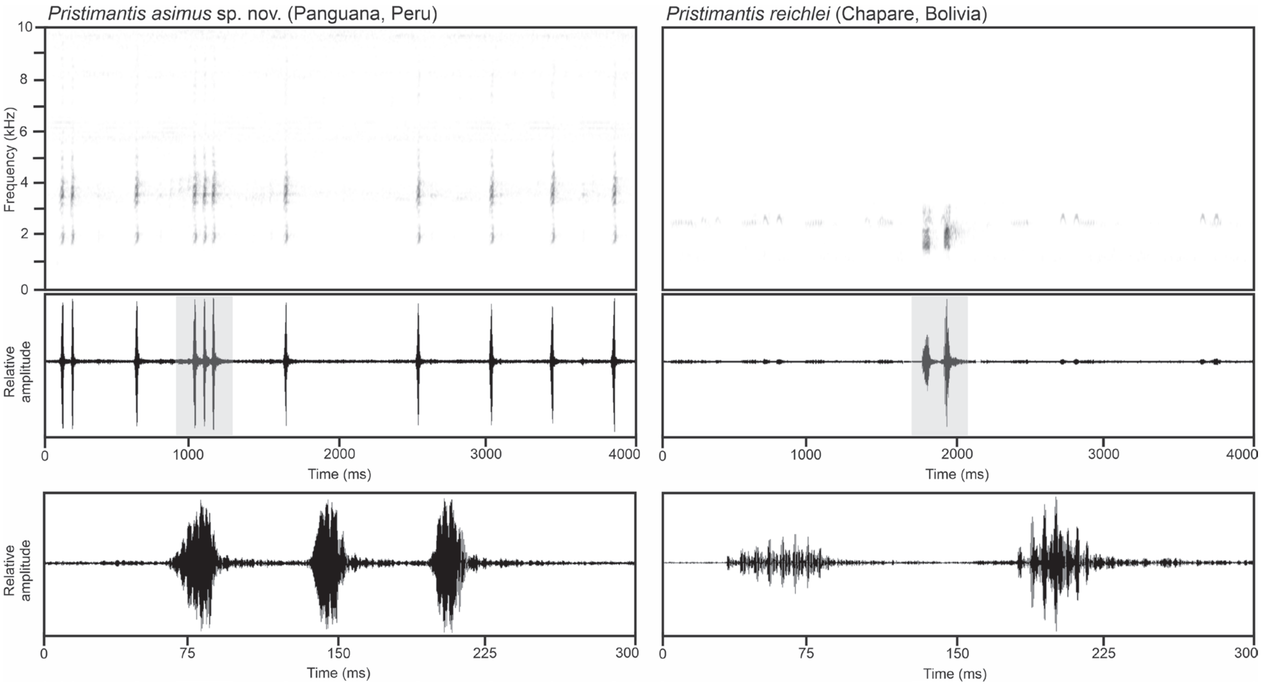

**Figure 8.** Audiospectrograms and corresponding oscillograms of the advertisement calls of *Pristimantis asimus* sp. nov. from the type locality Panguana, Peru (emitted by paratype MUSM 29028) and *Pristimantis reichlei* from close to its type locality in the Chapare region, Bolivia, at the same temporal and spectral scale (4000 ms and 10 kHz, respectively). Oscillograms at the bottom show respective calls (those highlighted in gray in the oscillograms above) at an expanded time scale of 300 ms. Recording of *P. asimus* high-pass filtered at 700 Hz and that of *P. reichlei* band-pass filtered at 900–3600 Hz.

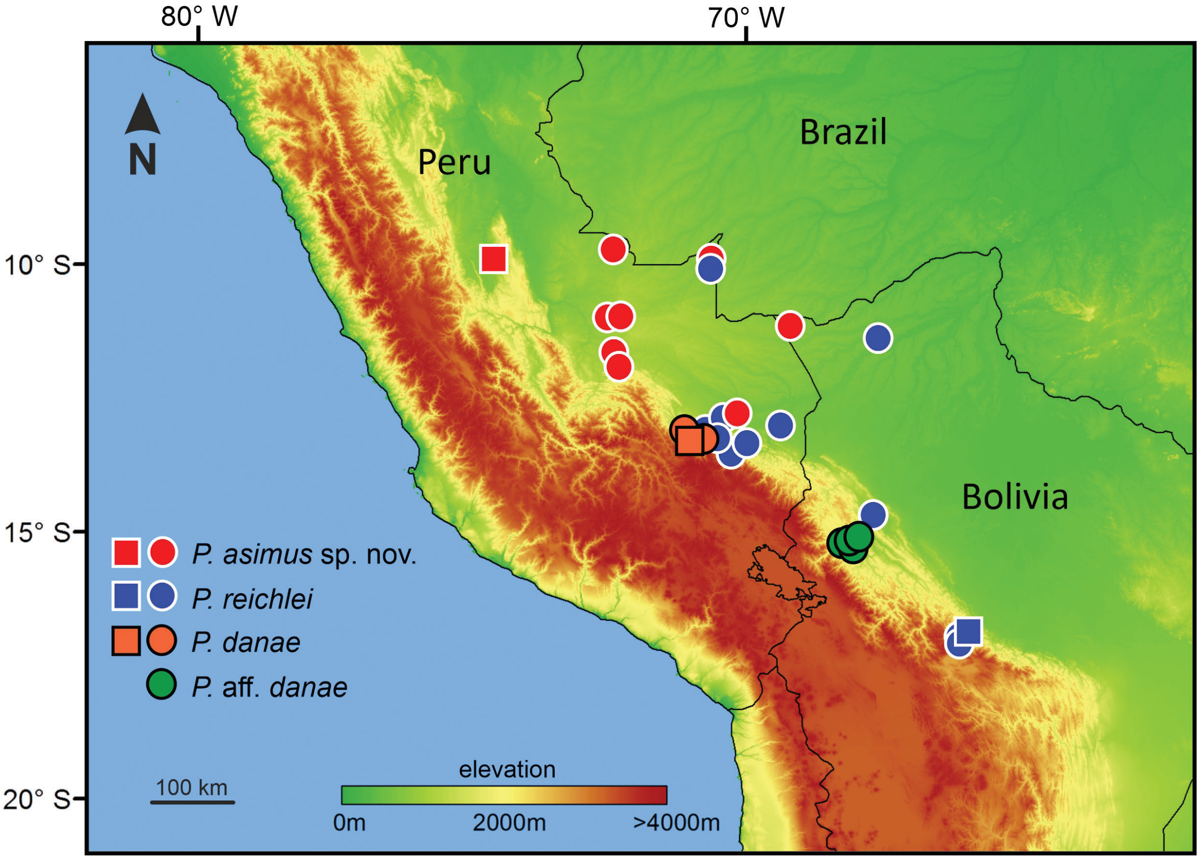

**Figure 9.** Schematic map of central-western South America showing the known distribution of species of the *Pristimantis danae* group referred to in the text, as inferred by molecular genetics and/or bioacoustic data. Broadly overlapping symbols may refer to the same locality. Square symbols refer to the type localities of the respective species.

in syntopy with *P. reichlei* at least in Alto Purús National Park (La Novia, Departamento Ucayali, Peru) and probably in other places in southeastern Peru and northern Bolivia. The new species most likely also occurs further east in the Brazilian Amazon, as indicated by the call description of Zimmerman and Bogart (1984) from Manaus, but respective records are in need of clarification (see Discussion).

**Etymology.** The specific epithet is a Latinized adjective derived from the Greek άσημος (ásimos), meaning 'inconspicuous, nameless'. It refers to the morphologically cryptic nature of the new species and the fact that it has been associated with different species names in the past, missing its status as a separate species to be named.

## Discussion

Morphological crypsis among genetically distant (i.e., non-sister) species seems to be uncommon among anuran species (e.g., Castroviejo-Fisher et al. 2017). Striking similarity between sister or closely related species (i.e., siblings; Mayr 1942) is nonetheless common and has been recognized for a long time (see Winker 2005). Similarity caused by recent shared descent poses difficulties distinguishing among species in complexes with little morphological variation, yet difficulties tend to fade once additional evidence becomes available to clarify the sophisticated issues of the complex (e.g., Scherz et al. 2022). Thus, for example, it is not uncommon that after a study reveals multiple closely related species hidden under a single name, the new species discovered are more readily distinguishable once the external morphology is examined anew with the results of genetics in mind, or when different dimensions of the phenotype and the geographic ranges of the species are known better (Saéz and Lozano 2005; Korshunova et al. 2019).

Like in the case of the species pair *Pristimantis asimus* and *P. reichlei*, it is increasingly common that studies of Neotropical amphibians reveal sister and similar-looking species showing large genetic divergences (Padial and De la Riva 2009; Padial et al. 2009; Hutter and Guayasamin 2015; Ortega-Andrade et al. 2015; Páez and Ron 2019; Trevisan et al. 2020). In many cases, these are allopatric species that, upon closer examination, are readily diagnosable morphologically, but cases of sympatry/parapatry are not rare either, as in the example reported herein. The challenge with such cases is to ascertain whether such morphologically similar but genetically divergent frogs are, in fact, different species. Vicariance with secondary contact after reproductive isolation is the usual evolutionary scenario invoked to explain the pattern (Mayr 1942; Wiley and Lieberman 2011). Thus, when mitochondrial DNA (mtDNA) analyses of populations of one putative species reveal the existence of two distinct genetic lineages in sympatry, and the mtDNA-divergent individuals are found to also differ at least faintly in other, unlinked characters, the results are interpreted as strong evidence for the existence of similar-looking sibling/cryptic species isolated reproductively. However, as shown by Chan et al. (2020) and others (e.g., Ruane et al. 2014), gene flow and past introgression with another species can lead traditional tree analysis of gene sequences and genetic distance analyses to results identical to those produced by speciation through vicariance. Therefore, populations of the same species, some of which may carry part of the mitochondrial genome of a closely related species, will be inferred as two divergent lineages, even in sympatry. As discussed by Chan et al. (2020), this has serious implications. Many barcoding and integrative analyses relying on 16S or COI and traditional ML or Bayesian inference tree analyses are reporting cases of divergent mtDNA gene lineages in sympatric populations. These are often considered full species that are readily described and named. In order to detect such cases, it is key to rely on additional sources of evidence that may allow us to critically evaluate whether the distinct genetic lineages are part of two distinct species ('integrative taxonomy'; see Padial et al. 2010), and in cases where they are undescribed, they can be classified as a confirmed candidate species (Vieites et al. 2009). On the other hand, mitochondrial divergences in situations of sympatry can be among the strongest possible lines of evidence for the species status of the lineages involved, as soon as they are fully concordant with differentiation in unlinked nuclear-encoded markers, bioacoustics, or morphology, and this concordance is confirmed with substantial sample sizes (Miralles et al. 2024). In our case, support for the existence of two species is provided by differences in the respective advertisement calls, which are most likely explained as mechanisms of reproductive isolation (Köhler et al. 2017), and possibly by a few morphological traits. However, it remains desirable to assess both the genealogies and differences in calls with increasing sampling to refine and corroborate the limits of these two species.

Within the *Pristimantis danae* species group, Padial and De la Riva (2009) were unable to identify any qualitative morphological character distinguishing *P. danae* from *P. reichlei*, and the statistical differences in morphometric measurements were at least ambiguous, thus emphasizing the cryptic nature of both species. With the description of *P. asimus*, we add another morphologically cryptic sibling species to this complex of frogs. Although we detected two minor morphological differences between topotypes of *P. asimus* and *P. reichlei* (virtual absence versus presence of the outer metatarsal tubercle in preservative; different outlines of the canthus rostralis in straight dorsal view of the head), variation in these character states has yet to be studied in additional specimens and across the entire species' ranges. However, *P. asimus*, *P. danae*, and *P. reichlei* clearly differ by pronounced molecular divergence (but see above) and advertisement call differences.

Given that morphological differences between *P. asimus* and *P. reichlei* are yet to be corroborated, identification of the two species can be considered reliable only with the aid of bioacoustic and/or molecular genetic analyses. Consequently, several published records referring to *P. reichlei* should be reviewed. These include lowland records from the Bolivian departments of Beni and La Paz (Padial et al.

2004; Padial and De la Riva 2009), records from Manu National Park in southeastern Peru (Villacampa-Ortega et al. 2017), from Serrania de Sira, central Peru (Whitworth et al. 2016), and Brazilian records from the state of Acre (Melo-Sampaio and de Souza 2010; Bernarde et al. 2011; Melo-Sampaio 2015). These and other populations (e.g., Manaus, Brazil; Zimmerman and Bogart 1984) should be studied genetically and/or bioacoustically for their correct allocation to one of the two species, and researchers should check for the respective morphological character states.

With the description of *P. asimus*, we accounted for a more adequate taxonomic resolution of frogs formerly subsumed under the name *P. reichlei* (sensu Padial and De la Riva 2009). However, the high genetic diversification within *P. reichlei* sensu this work, with several deeply differentiated mtDNA lineages (Fig. 1) and uncorrected 16S p-distances of up to 9.3%, argue for further investigation of these populations, particularly of their calls, as possibly the name *P. reichlei* as used herein still represents a complex of species.

As evidenced by our molecular phylogenetic analysis and the mentioned differences in advertisement calls, the situation of *P. asimus* and *P. reichlei* is somehow paralleled by a second species pair, namely *P. danae* from Peru and *P.* aff. *danae* from Bolivia (see Fig. 1). Currently, the respective populations from the montane rainforests of southern Peru and western Bolivia are all treated as *P. danae* (Padial and De la Riva 2009). However, the genetic differentiation, with uncorrected p-distances in 16S of 9.4% between both major lineages and apparently significant differences in advertisement calls (see above), makes it highly improbable that nominal *P. danae* from the Kosñipata valley in Peru are conspecific with *P.* aff. *danae* from the Yungas de La Paz, Bolivia, although data from the area in between these two regions are lacking (Fig. 9). Solving the taxonomic status of Bolivian *P.* aff. *danae* was beyond the scope of this study, and a detailed investigation of *P. danae* remains necessary.

Despite the still substantial limitations in geographic and character sampling outlined above, our understanding of species limits in *Pristimantis* is rapidly growing. It is now customary in phylogenetic studies of *Pristimantis* to discover the existence of unnamed species overlooked and confounded across the range with their similar-looking nominal sister species or closely related ones (e.g., Hutter and Guayasamin 2015; Ortega-Andrade et al. 2015; Páez and Ron 2019; Trevisan et al. 2020; Castillo-Urbina et al. 2023; Herrera-Alva et al. 2023). This increasing level of species resolution is a fundamental step toward more rigorous inferences of evolutionary scenarios as well as to attain taxonomic stability (Padial and De la Riva 2021).

In contrast to the growing taxonomic knowledge in the *P. danae* group, there is a disproportional lack of knowledge about the ecology and natural history of its species, which remains almost completely unknown. Martínez and Rodríguez (2007) reported on egg clutch size and egg numbers in *P. danae* from Peru. Quintero-Muñoz and Aguayo (2022) described observations on parental care (clutch guarding) and reproduction in *P. reichlei* from Bolivia, where a collected gravid female laid an egg clutch in captivity (without male individuals being present during capture or in the tank), from which half the eggs developed into froglets. This remarkable observation indicates either the presence of internal fertilization or parthenogenesis in this group of frogs. These two potential explanations deserve further study, as it either constitutes a rarely documented case in oviparous anurans (e.g., Townsend et al. 1981) or is so far undocumented in frogs (natural parthenogenesis). Such observations and potential phenomena argue for a more careful scientific consideration of these inconspicuous, brown-colored frogs.

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