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A new species of rain frog (Anura: Strabomantidae: *Pristimantis*) from the Guiana Shield and amended diagnosis of *P. ockendeni* (Boulenger, 1912)

Alexander Tamanini Mônico¹, Miquéias Ferrão², Juan Carlos Chaparro^{3,4}, Antoine Fouquet⁵, Albertina Pimentel Lima¹

¹ Programa de Pós-Graduação em Biologia (Ecologia), Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil

² Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, United States of America

³ Museo de Biodiversidad del Perú, Cusco, Peru

⁴ Museo de Historia Natural, Universidad Nacional de San Antonio Abad del Cusco, Cusco, Peru

⁵ Laboratoire Evolution et Diversité Biologique, Université Paul Sabatier, Toulouse, France

<http://zoobank.org/573953FE-8BE5-42FE-938E-C48170C045EA>

Corresponding author: Alexander Tamanini Mônico (alexandermonico@hotmail.com)

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Abstract

Pristimantis is already the most speciose genus among vertebrates, yet the current number of species remains largely underestimated. A member of the *P. unistrigatus* species group from the Guiana Shield has been historically misidentified as *P. ockendeni*, a species described from southern Peru. We combined mitochondrial (16S and COI) and nuclear (RAG1) loci, external morphology, skull osteology (μ -CT scan), vocalization (advertisement and courtship calls), geographic distribution and natural history data to differentiate the Guiana Shield populations from *P. ockendeni*, and describe them as a new species. The new species is crepuscular and nocturnal and inhabits the understory of unflooded (*terra firme*) forests in Brazil, Guyana and Suriname. It is phylogenetically related to *P. ardalonychus*, *P. martiae* and undescribed species from Brazilian Amazonia. The new species notably differs from *P. ockendeni* and its congeners in the *P. unistrigatus* species group occurring in the Guiana Shield by the combination of the following characters: absence of dentigerous processes of vomers, presence of vocal slits in males, body size (SVL 16.2–20.7 mm in males and 21.4–25.7 mm in females), advertisement call (call with 4–6 notes, call duration of 158–371 ms and dominant frequency of 3,466–4,521 Hz) and translucent groin coloration in life. To facilitate the recognition and description of cryptic species previously hidden under the name *P. ockendeni*, we provide an amended diagnosis of this taxon based on external morphology and advertisement call of specimens recently collected nearby the type locality and additional localities in southwestern Amazonia.

Keywords

Amazonia, Amphibia, Brachycephaloidea, integrative taxonomy, natural history, Terrarana

Introduction

With more than 590 currently valid nominal species (Frost 2022), *Pristimantis* Jiménez de la Espada, 1841 is the most speciose genus among vertebrates (Hedges et al. 2008; Padial and De la Riva 2009; Ortega-Andrade et al. 2015; Chavez and Catenazzi 2016). Moreover, this number is increasing at a fast pace suggesting that the number of extant species of *Pristimantis* remains largely underestimated (De Oliveira et al. 2019; Paéz and Ron 2019; Vacher et al. 2020; Zumel et al. 2022, Fouquet et al. 2022a).

Members of the *Pristimantis unistrigatus* species group occupy mostly the Andes and Amazonia and are generally cryptically colored and small-bodied. Although the monophyly of this speciose group remains highly contentious (Padial et al. 2014), our focal species belong to one of the well-supported clades supposedly forming the *P. unistrigatus* species group (Zapata-Brito et al. 2021; Frost 2022). Some populations of this species group from central Amazonia and the Guiana Shield have been historically identified as *P. ockendeni* (Boulenger, 1912)—a species described based on three syntypes from La Union, Huacamayo River, Carabaya, Departamento Puno, southeastern Peru, at about 800 m above sea level (a.s.l.). *Pristimantis ockendeni* has been subsequently reported from Brazil, Colombia, Ecuador, Guyana, Suriname (Zimmerman and Rodrigues 1990; Lima et al. 2006; Lima et al. 2012; Ocampo et al. 2016; Silva-e-Silva and Costa-Campos 2018; Azevedo et al. 2021; Torralvo et al. 2021; Fouquet et al. 2022b) and Bolivia (Padial et al. 2004; Elmer and Cannatella 2008; Ocampo et al. 2016; Frost 2022). However, the identification of these populations as *P. ockendeni* and, thus, the wide geographic distribution of the species, has been considered doubtful by Elmer et al. (2007a, 2007b), who suggested the existence of undescribed species erroneously associated with this taxon. Later, congruence between genetic and morphologic evidence supported the formal description of three new species historically hidden under the name *P. ockendeni* in Ecuador (Elmer and Cannatella 2008). Due to the unavailability of recently collected topotypical material, Elmer and Cannatella (2008) recognized the difficulties to clarify the identity of *P. ockendeni* sensu stricto. Similarly, populations from Brazil are unlikely conspecific to *P. ockendeni* (Fouquet et al. 2013; Ocampo et al. 2016). More recently, Fouquet et al. (2022b) highlighted specifically the case of these populations from central Amazonia and the Guiana Shield that have been identified as *P. cf. ockendeni* (also indicated by Vacher et al. 2020), showing that they form a species complex (“trans-Amazon complex”) which is distinct from all the other species of the Guiana Shield while possibly related, though also distinct, to western Amazonian species.

In the present study, we evaluate the taxonomic status of the populations from the Guiana Shield (Brazil, Guyana and Suriname) previously identified as *P. ockendeni* and confirm that they belong to a new species that

is described herein. We provide information regarding the ecology, phylogeny, call, and natural history of the new species. We also collected new morphological, bio-acoustic and genetic data of *P. ockendeni* that permitted clarifying its phylogenetic position, and amend its diagnosis.

Material and methods

Sampling

Fifty-six adults of the new species were manually collected in five localities in Brazil between November 2019 and December 2021 [eighteen individuals at Reserva Floresta Adolpho Ducke (RFAD), municipality of Manaus (2°55'53.6"S; 59°58'25.7"W); six individuals from Km 144 of the BR-174 Highway (1°45'26.7"S; 60°08'24.2"W) and eight individuals from Cachoeira da Suçuarana in Balbina (1°54'45.5"S; 59°24'30.8"W), municipality of Presidente Figueiredo, state of Amazonas; ten individuals from vicinity of the municipality of São João da Baliza (0°57'08.9"N; 59°53'29.7"W), state of Roraima], one locality in Suriname in April 2014 [five individuals from Sipaliwini (2°01'40.0"N; 56°07'32.2"W)] and the last one in Guyana between November 2002 to June 2004 and August 2010 [nine individuals from Mabura Hill Forest Reserve (5°09'20.6"N; 58°42'00.4"W)]. Eight adults of *Pristimantis ockendeni* sensu stricto (Appendix 1) were collected, being six individuals from municipalities of Manoel Urbano (Floresta Estadual do Afluente; 8°42'16.6"S; 69°32'02.6"W, to 522 Km from Type Locality – TL) and two individuals from Feijó (8°38'46.5"S; 69°43'29.7"W, to 522 Km from TL), state of Acre, Brazil. Specimens were anaesthetized and killed with 5% lidocaine. Muscle or liver tissue was preserved in 100% ethanol for genetic analysis, whereas the specimens were fixed in 10% formalin and preserved in 70% ethanol. Specimens were deposited in the Herpetological Collection of the Instituto Nacional de Pesquisas da Amazônia – **INPA-H** (Manaus, Brazil), Museu Paraense Emílio Goeldi – **MPEG** (Belém, Brazil), Museum National d'Histoire Naturelle – **MNHN-RA** (Paris, France), Staatliches Museum für Naturkunde – **SMNS** (Stuttgart, Germany), and Museum für Tierkunde in Dresden – **MTD** (Dresden, Germany).

Sequencing and phylogenetic analyses

Genomic DNA was extracted from tissues (muscle or liver) of 26 individuals of the new species, including three individuals from each locality previously mentioned, in addition to three (JJLR007, JJLR010 and JJLR016; Appendix 1) from Reserva Biológica do Uatumã, state of Amazonas, and one (MPEG 33750; Appendix 1) from Oriximiná municipality, Pará state, Brazil. We also extracted genomic DNA of five individuals of *Pristimantis*

ockendeni from Brazil (previously mentioned) and three from Peru, deposited in Museo de Biodiversidad del Perú (MUBI): two (MUBI 10538 and MUBI 14568; Appendix 1) from Manu Province (Reserva Comunal Amarakaeri, to 154 Km from TL) and one (MUBI 13049; Appendix 1) from Paucartambo Province (Kosñipata District, to 196 Km from TL) (Appendix 1). We extract genomic DNA using PureLink™ Genomic DNA (Invitrogen by Thermo Fisher Scientific, Carlsbad, CA, USA). Fragments of two mitochondrial (16S and Cytochrome C Oxidase sub-unit 1 – COI) and a nuclear gene (Recombination Activating 1 – RAG1) were amplified through polymerase chain reaction (PCR).

The 16S was amplified using primers 16Saf (5'-CG-CCTGTTTATCAAAAACAT-3') and 16Sbr (5'-CCG-GTCTGAACTCAGATCACGT-3') (Palumbi 1996) under the following conditions: 5 m at 94°C followed by 35 cycles of 94°C (30 s), 50°C (50 s) and 72°C (120 s), and final extension of 7 minutes at 72°C. The final volume of the PCR reaction was 15 µL and contained 1.5 µL of 25 mM MgCl₂, 1.5 µL of 10 mM dNTPs (2.5 mM each dNTP), 1.5 µL of tampon 10× (75 mM Tris HCl, 50 mM KCl, 20 mM (NH₄)₂SO₄), 1.5 µL of each primer (2 µM), 6.4 µL of ddH₂O and 0.1 µL of 1 U Taq DNA Polymerase and 1 µL of DNA (30–50 ng/µL). The COI was amplified using primers Chmf4f (5'-TYTCWACWAAY-CAYAAAGAYATCGG-3') and Chmr4r (5'-ACYTCRG-GRTGRCCRAARAATCA-3') (Che et al. 2012) under the following conditions: 60 s at 94°C followed by 35 cycles of 94°C (20 s), 50°C (50 s) and 72°C (90 s), and final extension of 10 minutes at 72°C. The final volume of the PCR reaction was 15 µL and contained 1.2 µL of 25 mM MgCl₂, 1.2 µL of 10 mM dNTPs (2.5 mM each dNTP), 1.5 µL of tampon 10× (75 mM Tris HCl, 50 mM KCl, 20 mM (NH₄)₂SO₄), 0.5 µL of each primer (10 µM), 8.95 µL of ddH₂O and 0.15 µL of 1 U Taq DNA Polymerase and 1 µL of DNA (30–50 ng/µL). Finally, RAG1 was amplified using primers R182 (5'-GCCATAACTGCTGGAG-CATYAT-3') and R270 (5'-AGYAGATGTTGCCTGG-GTCTTC-3') (Heinicke et al. 2007) under the following conditions: 5 m at 94°C followed by 40 cycles of 94°C (30 s), 59°C (30 s) and 72°C (60 s), and final extension of 7 minutes at 72°C. The composition of the PCR reaction was the same used for 16S. PCR products were visualized in 1 agarose with SYBRsafe (Life Inc.) and purified using PEG 8000 protocol (Sambrook and Russell 2001) and submitted to sequencing using standard protocols of the Big Dye™ Terminator Kit (Applied Biosystems, Inc., Grand Island, NY, USA). Amplicons were sequenced in an ABI PRISMI 3130XL (Thermo Fisher) using the forward and reverse primers of each gene. Sequences were subjected to BLAST searches (Altschul et al. 1997) in GenBank to verify if the target had been amplified. Newly generated sequences were deposited in GenBank, and accession numbers are available in Appendix 2.

To infer the phylogenetic relationships of the new species, the above newly generated sequences were inserted into a data set containing homologous sequences retrieved from GenBank. Sequences retrieved from Gen-

Bank are detailed in Appendix 2. We selected sequences used in the most recent phylogenetic analyses that involved *Pristimantis ockendeni* and related Andean species (Zapata-Britto et al. 2020) and species historically assigned to the *P. unistrigatus* species group of the Guiana Shield (Fouquet et al. 2022b). In total, 252 sequences (Appendix 2) of the three genes were selected (135 for 16S, 66 for COI and 51 for RAG1), which corresponded to 136 terminals. We aligned sequences of each gene using MAFFT online server with default parameters for each gene, except by the use of E-INS-i strategy for 16S gene and G-INS-i for protein coding genes (Katoh and Standley 2013). The final matrix was concatenated in Mesquite (Maddison and Maddison 2021) and composed of 136 terminals with 1,843 bp (569 pb for 16S, 638 pb for COI, and 636 pb for RAG1).

Best-fit evolutionary models and partition schemes were determined through ModelFinder (Kalyaanamoorthy et al. 2017) using nine partitions: one for the 16S and one for each codon of protein-coding genes. The best evolutionary models for partitions in the concatenated matrix were: TIM2 + F + R5 for 16S, COI 2nd and RAG 3rd codons, TIM3 + F + R3 for COI 1st position, and HKY + F + G4 for COI 3rd, RAG 1st and 2nd codons. Phylogenetic relationships were reconstructed under Maximum Likelihood inference (ML). The ML tree was inferred using IQTREE (Nguyen et al. 2015) as implemented in the webserver <http://iqtree.cibiv.univie.ac.at> (Trifinopoulos et al. 2016). Clade support was estimated with 10,000 ultrafast bootstrap replications (Hoang et al. 2018), 1,000 maximum iterations, and a minimum correlation coefficient of 0.99. We calculated pairwise genetic distances (*p* and Kimura-two-parameter distances; Kimura 1980) among populations of new species and *P. ockendeni* sensu stricto using MEGA 11 (Tamura et al. 2021). Interspecific distances were also calculated among the new species and other species of the *P. unistrigatus* species group from the Guiana and the Pantepui regions. Genetic distances were calculated using pairwise deletion.

Morphology

We measured 25 morphometric measurements from adults of the new species (males = 43 and females = 13) and *Pristimantis ockendeni* sensu stricto (males = 9 and females = 2, including three syntypes). Measurements follow Duellman and Lehr (2009) (eye diameter [ED], eye-nostril distance [EN], foot length [FL], interorbital distance [IOD], internarial distance [IND], head length [HL], head width [HW], snout-vent length [SVL], tibia length [TL] and tympanum diameter [TD]), Caldwell et al. (2002) (forearm length [FAL], hand length [HAND], snout length [SL], disc width of Finger III [WFD]), Heyer et al. (1990) (tarsus length [TAL], thigh length [THL], upper arm length [UAL]) and Lima et al. (2007) (hand length from proximal edge of palmar tubercle to tip of Finger I [HANDI], Finger II [HANDII] and Finger IV [HANDIV]). Additionally, we measured foot length from

proximal edge of outer metatarsal tubercle to tip of Toe I (FLI), Toe II (FLII), Toe III (FLIII), Toe V (FLV), and disc width of Toe IV (WTD). All measurements, including the ones of the eggs, were taken using a Leica stereo-microscope (model S8APO) coupled to a Leica DFC295 camera, with a precision of 0.001 mm, except for SVL, which was measured with digital calipers (precision of 0.01 mm). Diagnostic characters follow Duellman and Lehr (2009), and morphological description follows Kok and Kalamandeen (2008), Duellman and Lehr (2009), and Kok et al. (2011). Color in life was described based on photographs taken in the field.

We obtained μ -CT scans of the skull to confirm the presence/absence of dentigerous processes of vomers in one male (MNHN-RA-2020.0115) and one female (MNHN-RA-2020.0114). Specimens were scanned (kV = 40–70, resolution < 20 μ m) using an EasyTom 150 from the MRI platform of ISEM (Institute of Evolutionary Sciences of Montpellier, France). Segmentation of the full skeleton and of the cranium was done using Avizo (FEI Visualization Sciences Group, Burlington, MA, USA) and Biomedisa (Lösel et al. 2000).

Bioacoustics

We recorded advertisement calls of 34 males of the new species and seven males *Pristimantis ockendeni* sensu stricto with a digital recorder “Marantz PMD660” (Marantz, Japan) coupled to a Sennheiser K6/ME66 unidirectional microphone (Sennheiser, Germany). Air temperatures during calls ranged between 22.4 and 25.6°C. We recorded three minutes of calls per individual using frequency rate of 44 kHz and 16 bits of resolution in the mono pattern. Call recordings were deposited at Fonoteca Neotropical Jacques Viellard (FNJV) of the Universidade de Campinas (UNICAMP, Campinas, SP – Brazil) and Sonothèque du Museum National d’Histoire Naturelle – MNHN-SO, Paris, France.

Bioacoustic variables were analyzed with Raven Pro 1.6 software, 64-bit version (Bioacoustics Research Program 2014) set as follow: window = Blackman, Discrete Fourier Transform = 2,048 samples and 3dB filter bandwidth = 80.0 Hz. Dominant frequency was measured using the *Peak frequency* function; maximum and minimum frequencies were measured 20dB below the peak frequency to avoid background noise interference. Call centered terminology and categorization follows Köhler et al. (2017): call duration (CD), number of notes (NN), note duration (ND), inter-note interval (SBN), minimum (LF), maximum (HF), and dominant frequency (DF). Inter-call interval was not measured because it is affected by microclimatic conditions at the time of recording (i.e., on rainy days males call more often in a short period of time than on days without rain). Spectrogram and oscillogram were generated in R environment (R Core Team 2019) through the ‘seewave’ package 2.0.5 (Sueur et al. 2008) using a Hanning window, 256 points of resolution (Fast Fourier Transform) and an overlap of 85.

Statistical Analyses

We used a principal component analysis (PCA) via FactoMineR package in R v.3.2.4 (Lê et al. 2008; R Core Team 2019) associated with analysis of variance (ANOVA) to investigate the variation of morphometric and bioacoustic data between the new species and *P. ockendeni* sensu stricto.

We conducted the analysis with morphometric ratios (morphometric measurements divided by SVL) using only males since the *P. ockendeni* sensu stricto has insufficient data for females. We normalized data with the function ‘*data.Normalization*’ of the package ‘clusterSin’ (Walesiak and Dudek 2020) and tested for homoscedasticity with the Levene Test using the function ‘*leveneTest*’ of the package ‘car’ (Fox and Weisberg 2019). PCAs were run using the function ‘*prcomp*’ of the package *stat* with parameters ‘scale’ and ‘center’ set as ‘True’. We obtained the number of retained principal components (PCs) with the broken stick model using the function ‘*screeplot*’ of the package ‘vegan’ (Oksanen et al. 2022), which retained the first two PCs for both PCAs. Finally, we prepared the graphs using the function ‘*autoplot*’ of the package ‘*ggfortify*’ (Horikoshi and Tang 2016; Tang et al. 2016).

We conducted an ANOVA for PC1 and PC2 for morphometric and bioacoustic analyses to test the existence of statistical difference in the space occupied by males of the new specie and *P. ockendeni*. Morphological and bioacoustic analyses were conducted on the R platform (R Core Team 2021).

Results

Phylogenetic relationships and genetic distances

The two focal species are not closely related, even though they are both nested in a large strongly supported clade formed by species of the *Pristimantis unistrigatus* species group. Relationships inferred from the Maximum Likelihood analysis within that clade are well-supported (Bootsstraps > 70%; Fig. 1). The individuals of the new species from the Guiana Shield form a monophyletic group, and display low intraspecific genetic distances on 16S (*p*-distance = mean 0.4%, maximum 1.6%). The new species is nested within a strongly supported clade (100%) formed with four additional major lineages (mean *p*-distance between 5.8 and 7.3% to the new species) located south of the Amazon River that were historically confused with *P. ockendeni*. This clade corresponds to the “trans-Amazon complex” of Fouquet et al. (2022b) and is distantly related to two species from Peru: *P. ardalonychus* (Duellman and Pramuk, 1999), *p*-distance = 13.3%, and *P. martiae* (Lynch, 1974), *p*-distance = 10.4% (Table 1).

Pristimantis ockendeni from Peru (near to the type locality) clusters with specimens from Acre state (Brazil;

Table 1. Interspecific and intraspecific genetic distances between *Pristimantis guianensis* **sp. nov.** and closely related taxa. Uncorrected *p*-distances (%; lower diagonal) and Kimura-2-parameter (%; upper diagonal) for sequences in a matrix with 562 bp from 16S mtDNA gene and expressed as percentages. Bold numbers in the diagonal represent intraspecific *p*-distance.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1 <i>P. guianensis</i> sp. nov.	0.4	6.1	6.2	7.3	7.7	17.9	13.4	10.2	16.1	14.2	15.8	8.3	16.5	10.4	14.1	15.9	13.2	13.0	10.4
2 <i>P. sp.</i> South PA	5.8	1.4	5.3	4.5	4.9	16.8	13.7	11.0	16.8	15.1	16.6	5.9	16.4	10.5	12.4	16.7	11.6	13.0	10.2
3 <i>P. sp.</i> Abacaxis1	5.9	5.11	–	7.0	6.7	16.1	13.7	8.9	15.6	12.8	15.8	5.9	14.0	8.6	11.7	16.2	12.6	12.2	8.3
4 <i>P. sp.</i> South AM, RO	6.9	4.3	6.6	1.6	5.6	17.1	15.0	11.3	17.2	15.3	17.9	8.7	16.1	11.2	12.5	16.3	13.6	14.0	10.6
5 <i>P. sp.</i> Abacaxis2	7.3	4.7	6.4	5.3	–	17.6	14.4	11.4	18.2	14.9	17.7	6.5	15.8	12.5	14.1	16.7	14.2	14.4	10.9
6 <i>P. ockendeni</i> SS	15.7	14.8	14.1	15.1	15.6	0.2	14.8	8.1	14.1	12.4	12.9	5.4	13.6	8.0	7.0	14.7	10.5	10.6	8.0
7 <i>P. ardalonychus</i>	12.1	12.4	12.5	13.4	13.1	13.2	–	7.2	12.2	9.7	9.5	5.7	13.3	7.1	9.5	11.6	10.1	8.4	6.9
8 <i>P. bogotensis</i>	9.4	10.1	8.3	10.3	10.4	7.6	6.8	–	8.8	6.5	5.9	5.0	11.8	7.3	9.1	4.8	6.7	5.1	5.7
9 <i>P. buenaventura</i>	14.2	14.7	13.9	15.1	15.9	12.6	11.1	8.1	–	9.7	10.1	4.8	15.1	8.9	10.6	14.9	10.4	11.5	7.4
10 <i>P. cajamarcensis</i>	12.8	13.5	11.7	13.7	13.4	11.3	9.0	6.1	9.0	–	8.5	4.8	13.9	6.5	9.4	12.0	10.7	8.5	5.6
11 <i>P. ceuthospilus</i>	14.1	14.7	14.1	15.6	15.7	11.7	8.9	5.6	9.3	8.0	–	7.0	11.6	6.6	8.2	11.6	8.8	8.0	6.0
12 <i>P. daquilemai</i>	7.7	5.6	5.6	8.0	6.1	5.1	5.4	4.8	4.6	4.6	6.6	0.0	7.6	8.0	5.9	6.9	6.7	5.6	5.1
13 <i>P. delius</i>	14.6	14.6	12.7	14.3	14.2	12.3	12.0	10.7	13.4	12.6	10.6	7.4	–	12.4	13.5	13.9	15.8	8.9	10.7
14 <i>P. martiae</i>	9.6	9.6	8.1	10.3	11.4	7.5	6.7	6.8	8.2	6.1	6.3	7.5	11.3	–	9.6	7.1	10.0	5.4	6.2
15 <i>P. matidiktyo</i>	12.7	11.3	10.7	11.4	12.7	6.6	8.9	8.5	9.8	8.8	7.7	5.6	12.3	9.0	0.0	8.5	10.5	7.2	8.4
16 <i>P. miyatai</i>	14.2	14.8	14.3	14.4	14.9	13.1	10.6	4.6	13.1	10.9	10.6	6.6	12.5	6.7	8.0	–	8.0	10.2	6.6
17 <i>P. taeniatus</i>	11.9	10.6	11.5	12.2	12.8	9.7	9.3	6.4	9.5	9.8	8.1	6.4	14.0	9.2	9.7	7.5	3.8	8.1	8.7
18 <i>P. unistrigatus</i>	11.9	11.8	11.3	12.7	13.0	9.8	7.9	4.9	10.5	8.0	7.6	5.3	8.3	5.2	6.8	9.4	7.6	–	4.4
19 <i>P. zophus</i>	9.6	9.4	7.8	9.7	10.1	7.5	6.5	5.4	7.0	5.3	5.7	4.9	9.9	5.9	7.9	6.3	8.1	4.2	4.9

Table 2. Loadings of 23 morphometric ratios on the first two principal components. Values generated by a principal component analysis based on 43 males of *Pristimantis guianensis* **sp. nov.** and nine males of *Pristimantis ockendeni* sensu stricto.

Variables	PC1	PC2
HW	0.038	-0.332
HL	-0.017	-0.382
SL	-0.013	-0.223
IND	-0.024	-0.139
EN	-0.071	-0.078
IOD	0.068	-0.131
ED	-0.171	-0.141
TD	0.197	0.013
TL	0.064	-0.362
TAL	-0.214	-0.184
FL	0.311	-0.133
FLI	0.241	-0.197
FLII	0.293	-0.078
FLIII	0.339	-0.101
FLV	-0.167	-0.268
WTD	-0.079	-0.161
UAL	-0.302	-0.184
FAL	-0.136	-0.274
HAND	0.332	-0.137
HANDI	0.265	-0.031
HANDII	0.334	0.129
HANDIV	0.279	-0.117
WFD	-0.009	-0.014

Table 3. Loadings of seven bioacoustic measurements on the first two principal components. Values were generated by a principal component analysis based on the advertisement call of 34 males of *Pristimantis guianensis* **sp. nov.** and seven males of *Pristimantis ockendeni* sensu stricto.

Variables	PC1	PC2
CD	0.430	0.235
ND	0.345	-0.479
NN	0.320	0.675
INI	0.365	0.272
LF	-0.405	0.191
HF	-0.366	0.054
DF	-0.403	0.382

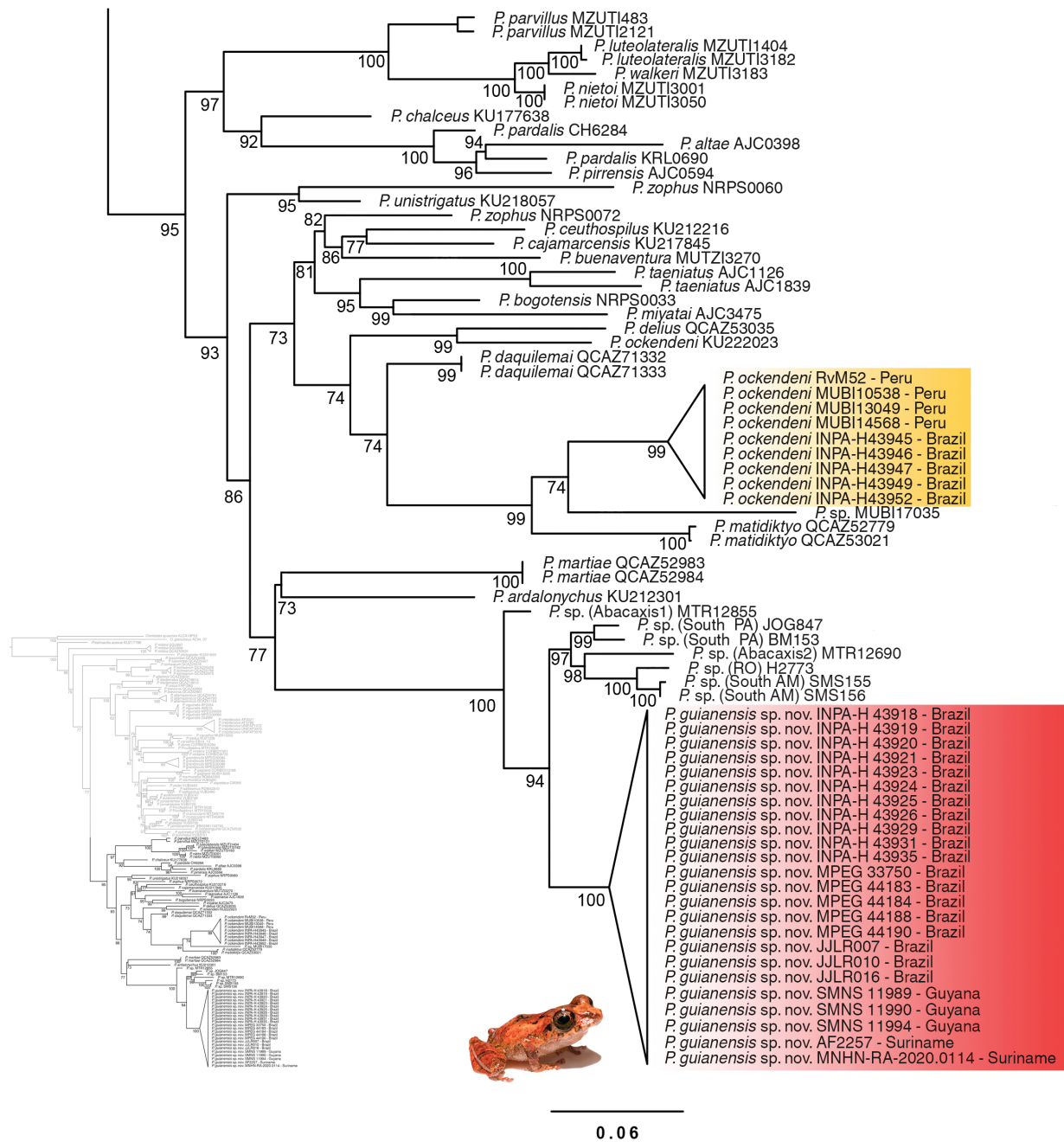


Figure 1. Part of the phylogenetic reconstruction showing the relationships of *Pristimantis guianensis* sp. nov. and *P. ockendeni*. Maximum likelihood tree inferred based on 16S, COI and RAG1. Non-parametric bootstrap support is shown above branches. The species name is preceded by the specimen voucher number (continuation of the tree in Appendix 3).

p -distance = 0.2%) and Peru and this clade is phylogenetically related to another undescribed species from Peru (voucher MUBI 17035 [Appendix 1], p -distance 10.9%) which altogether form a well-supported clade with *P. matidiktyo* Ortega-Andrade and Valencia, 2012, p -distance 7.5 (Fig. 1; Table 1).

Morphometric and acoustic analyses

The first two Principal Components (PCs) of morphometric and bioacoustic PCAs explained together ~47 and ~82% of data variance, respectively. Neither morphometric (Fig. 2A) nor bioacoustic (Fig. 2B) spaces occupied

by the new species and *Pristimantis ockendeni* overlap in graphic representations. ANOVAs showed that these species are significantly different along the first ($S^2 = 259.6$, $F = 142.3$, $DF = 47$, $p < 0.0001$) and second morphometric (ANOVA: $S^2 = 20.69$, $F = 5.5$, $DF = 47$, $p = .023$) and first bioacoustic PCs (first: $S^2 = 153.8$, $F = 207.6$, $DF = 35$, $p < 0.0001$). However, they do not significantly differ along bioacoustic PC2 (ANOVA: $S^2 = 1.0$, $F = 1.3$, $DF = 35$, $p = .258$). The three main variables contributing to the variation captured in morphometric PC1 are FLIII, HAN-DII and HAND, and in bioacoustics PC1 are DC, LF and DF. Contribution of other variables in PC1 and PC2 of morphometric and bioacoustic analyses are shown in Table 2 and Table 3, respectively.

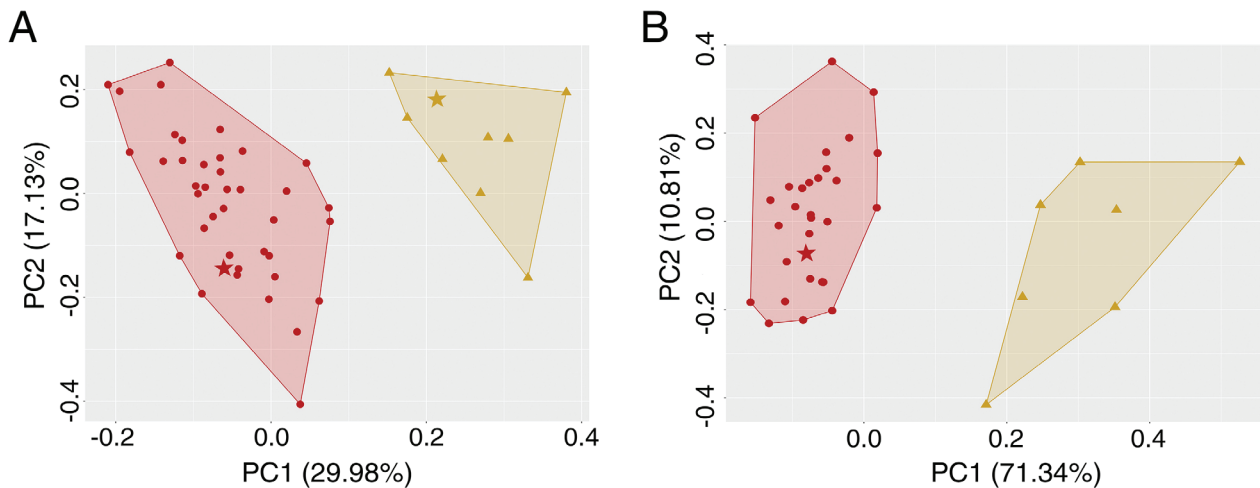


Figure 2. Principal Component Analysis (PCA) comparing *Pristimantis guianensis* sp. nov. (red circles) and *Pristimantis ockendeni* sensu stricto (yellow triangles). **A** morphometric data, **B** bioacoustic data. Red star indicates holotype of *P. guianensis* sp. nov. and yellow star indicates syntype of *Pristimantis ockendeni*.

Taxonomic account

Pristimantis ockendeni (Boulenger, 1912)

Figs. 3–5

Hylodes ockendeni – Boulenger (1912)

Eleutherodactylus ockendeni – Lynch (1996)

Pristimantis ockendeni – Heinicke et al. (2007), Hedges et al. (2008), Padiá et al. (2014), Ocampo et al. (2016), von May et al. (2017), Villacampa et al. (2017)

Syntypes. BMNH 1947.2.16.88, BMNH 1947.2.16.89 and BMNH 1947.2.16.90 (Fig. 3), collected on 07 May 1907 from La Union, Rio Huacamayo, Carabaya, south-eastern Peru (13°31'58.3"S, 69°45'06.7"W; 783 m elevation).

The specimens are in a good state of preservation, allowing for an easy view, for example, of the dark bar between the eyes, of oblique black streak in front of and behind the eye, oblique brown bars on the tibia, supratympanic stripe and melanophores in ventral surface.

We compared the newly collected individuals with the morphological diagnosis described by Boulenger (1912) and the syntypes of species (through high quality images made available by the Natural History Museum – London) to ensure that we are dealing with the same taxon. Moreover, the molecular analyses confirmed that our material is conspecific to a specimen (voucher RvM5_12) collected near the type locality (112 km linear distance) and made available by von May et al. (2017). Our samples are distributed at altitudes ranging from 223 m (in Brazil) to 924 m (in Peru), range that includes the altitude of the type locality (783 m).

The characters available in the species description are relatively vague. Boulenger (1912) defined the size of the species as 34 mm from snout to vent, based on the two female syntypes (NHM 1947.2.16.88 and NHM

1947.2.16.89). Boulenger might not have included the third syntype (NHM 1947.2.16.90) because he believed it was a juvenile individual, but our examination showed this specimen to be an adult male.

We acknowledge that >100 km separates the type locality from the closest newly collected population that could corresponds to *Pristimantis ockendeni*. Therefore, on the sole basis of geographical distance and considering the megadiversity and the cryptic morphology of this clade we cannot completely reject the hypothesis that our populations in fact belong to another species than *P. ockendeni*.

Nevertheless, four other nominal species of the *Pristimantis unistrigatus* species group are known to occur in the area [i.e. *P. altamazonicus* (Barbour & Dunn, 1921), *P. carvalhoi* (Lutz, 1952), *P. divnae* Lehr & von May, 2009, and *P. ventrimarmoratus* (Boulenger, 1912); Duellman 2005; Villacampa et al. 2017] and one undescribed ("*Pristimantis* sp. 3" sensu Villacampa et al. 2017). However, all of them have distinctive features on groin (yellow, orange or red marks) or belly (black marble) that readily distinguishes them from *P. ockendeni*. Also, the main lineages of the *P. unistrigatus* species group related to *P. ockendeni* [i.e., *P. matidiktyo*, *P. daquilemai* Brito-Zapata, Reyes-Puig, Cisneros-Heredia, Zumel & Ron, 2021, and *P. delius* (Duellman & Mendelson, 1995)], and possible any undescribed species related to them, can be easily distinguished from *P. ockendeni*. For example, *P. matidiktyo* differs by the dorsum without any marks and the presence of a small tubercle on the upper eyelid, *P. daquilemai* differs by its flanks densely tuberculated and the presence of a prominent rostral papilla at tip of snout, and *P. delius* is distinguished by the snout acutely rounded in dorsal view and the absence of dentigerous processes of vomers. Finally, the characters described by Boulenger (1912), although vague, but completed by the examination of the syntypes (see below), strongly suggests that the newly collected specimens are conspecific to *P. ockendeni*.

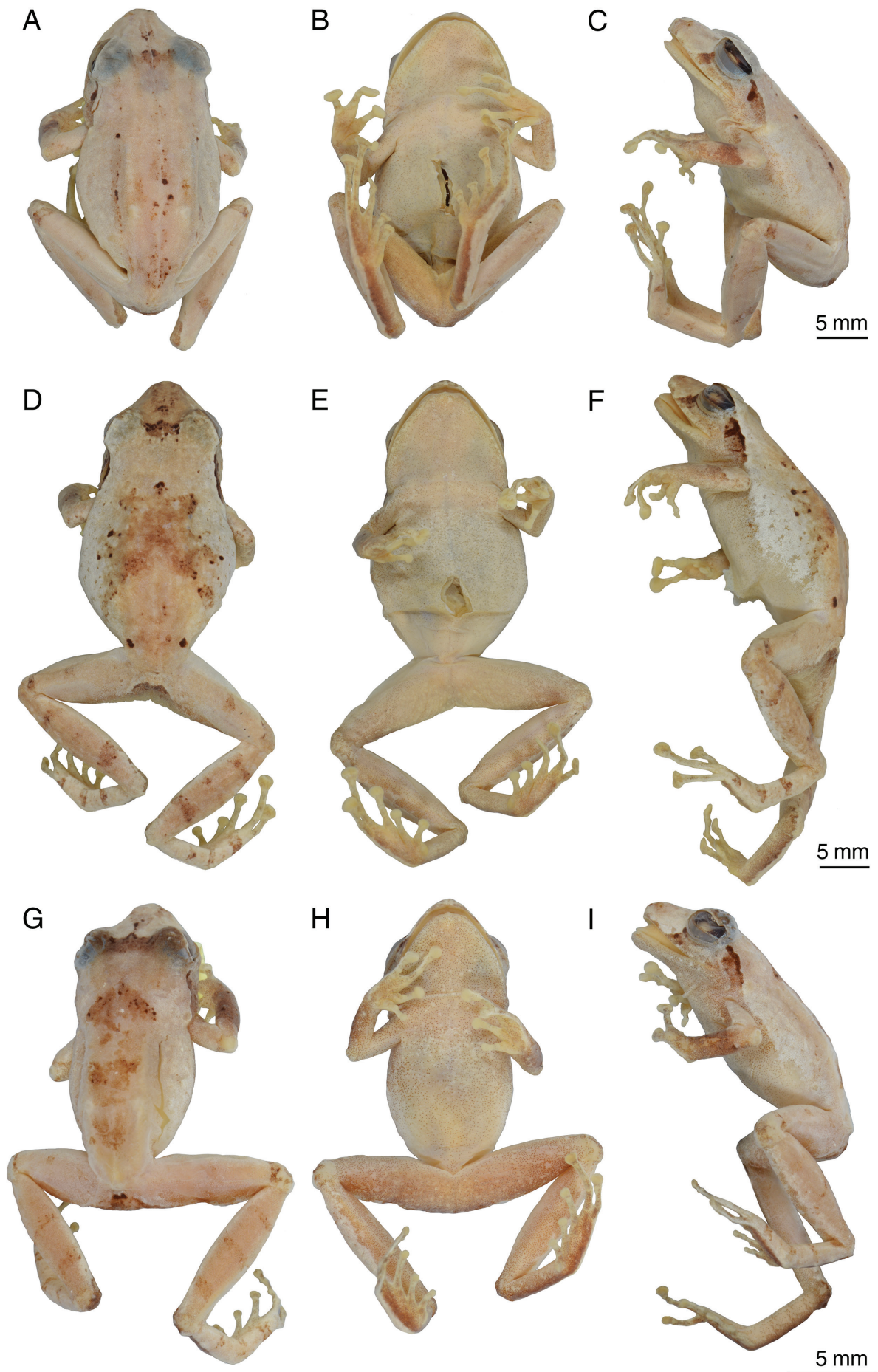


Figure 3. Dorsal, ventral and lateral views of syntypes of *Pristimantis ockendeni*. **A, B** and **C** female, NHM 1947.2.16.88; **D, E** and **F** female, NHM 1947.2.16.89; and **G, H** and **I** male, NHM 1947.2.16.90. Photographs: Natural History Museum of London.

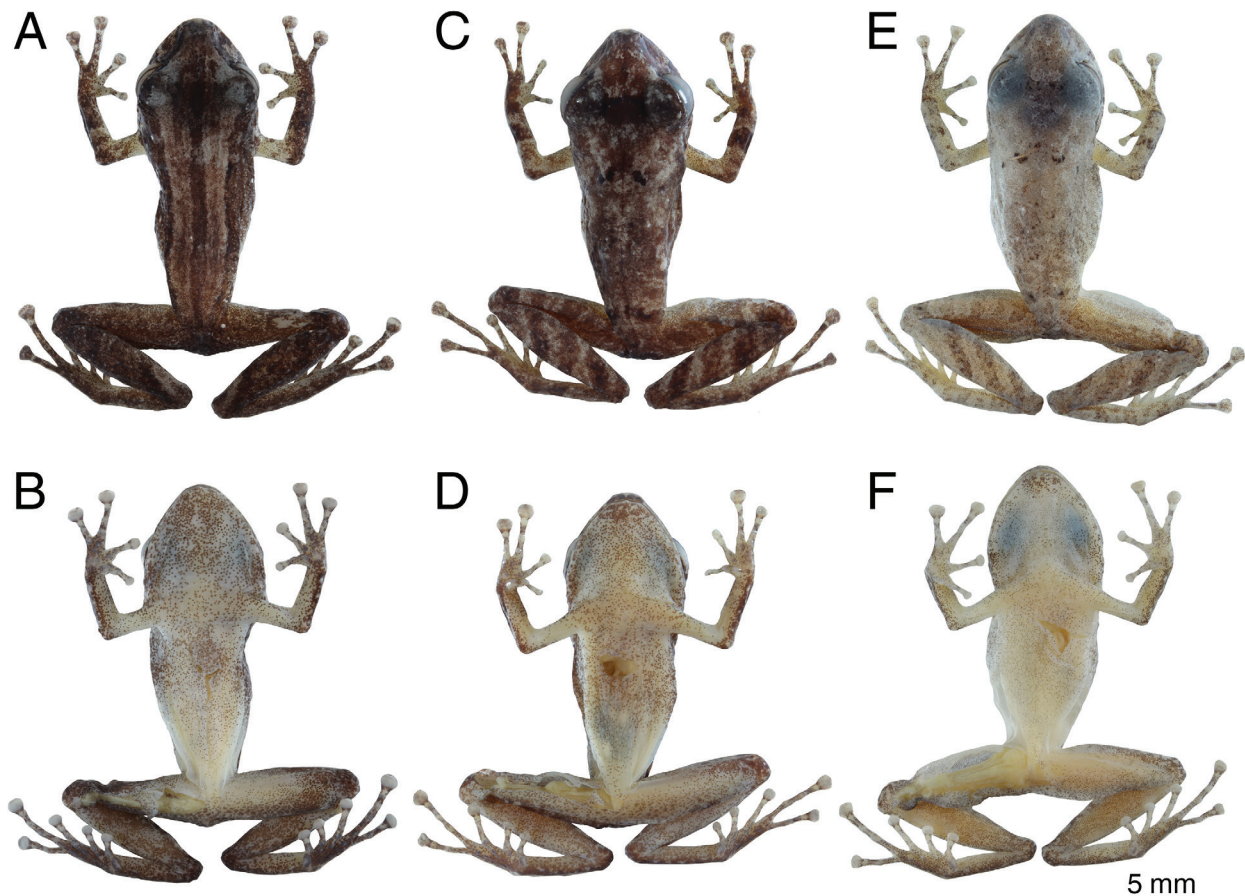


Figure 4. Newly collected specimens of *Pristimantis ockendeni* preserved, in dorsal and ventral view, respectively. **A** and **B** INPA-H 43946; **C** and **D** INPA-H 43949; and **E** and **F** INPA-H 43948. Photographs: A.T. Mônico.

Referred material. Available in Appendix 1.

Amended diagnosis. The species is characterized by the combination of the following characters, based on the description of Boulenger (1912) with additions to this study, obtained through examination of the syntypes (Fig. 3) and to the material recently collected (Fig. 4): (1) dorsal skin smooth to shagreen, frequently with distinctly enlarged tubercles, with or without W-shaped on scapular region, a narrow light vertebral line may be present; (2) tympanum very indistinct, its length 30 to 44% of eye length, tympanic membrane and tympanic annulus visible but sometimes inconspicuous; with or without supratympanic stripe; (3) snout short and subacuminate in dorsal view, with moderately strong, curved canthus and very oblique, concave loreal region; (4) upper eyelid usually bearing tubercles; dark bar between the eyes, and oblique black streak in front of and behind the eye; cranial crests absent; (5) nostril near the tip of the snout; interorbital space hardly as broad as the upper eyelid; (6) tongue oval, entire or indistinctly nicked behind; (7) dentigerous processes of vomers in two oblique oval groups just behind the level of the choanae, clearly visible; (8) vocal slits present, vocal sac median to subgular; nuptial pads absent; (9) Finger I shorter than Finger II; fingers moderate, discs ovoid to expanded (widest on fingers III and IV); (10) fingers without lateral fringes; (11) three to four ulnar tubercles, ill defined; (12) tibia length 49–56% of SVL; (13)

heel bearing one rounded tubercle; small tarsal tubercles, ovoid, poorly visible in fixed specimens; inner edge of tarsus with a short fold; (14) two feebly prominent metatarsal tubercles, being thenar tubercle ovoid to elliptical and small palmar tubercle ovoid; subarticular tubercles well developed but small; (15) toes without lateral fringes; scarcely a rudiment of web between them; (16) Toe I clearly smaller than Toe II, toes moderate, discs ovoid to expanded (widest on toes III to V); (17) dorsal coloration very variable; two or three oblique brown bars on the tibia; ventral surface smooth to slightly areolate, variable concentration of melanophores; and ventral region of the femur externally slightly areolate; (18) absence of spot on groin; (19) dichromatic iris in life, the lower part being coppery and the upper part cream; (20) SVL in adult males of 18.3–22.8 mm ($n = 9$; Table 4) and females of 30.4–30.6 mm ($n = 2$; Table 4); and (21) advertisement call with average call duration of 541 ± 151 ms, inter-note interval of 44 ± 5 ms (68 ± 11 ms), minimum frequency of 2,046–2,610 Hz, maximum frequency of 2,964–3,641 Hz and dominant frequency of 2,519–3,143 Hz.

Additional comments about *P. ockendeni* morphology. Dorsal pattern of males is highly variable, showing three main patterns ($n = 8$ specimens): (i) coloration strongly delimited and different from sideview, with or without dark transverse stripes on dorsum ($n = 4$ specimens; Fig. 4A; in life, Fig. 5A, 5B, 5D and 5F); (ii) irregular dark

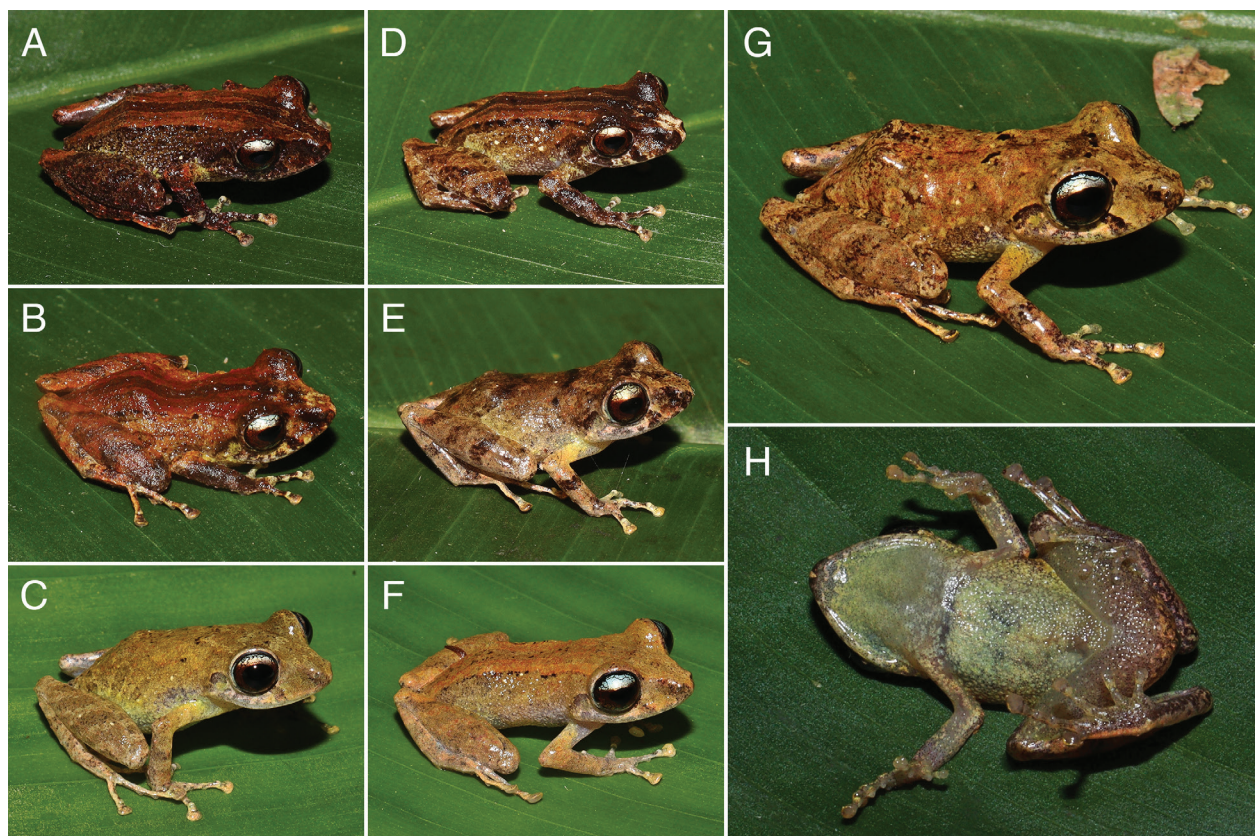


Figure 5. *Pristimantis ockendeni*. **A** INPA-H 43946 (SVL 20.1 mm); **B** INPA-H 43952 (SVL 18.9 mm); **C** INPA-H 43948 (SVL 19.4 mm); **D** INPA-H 43945 (SVL 19.0 mm); **E** INPA-H 43950 (SVL 18.4 mm); **F** INPA-H 43947 (SVL 18.8 mm); **G** INPA-H 43949 (SVL 19.6 mm) in dorsal view; and **H** INPA-H 43949 in ventral view. Photographs: A.T. Mônico.

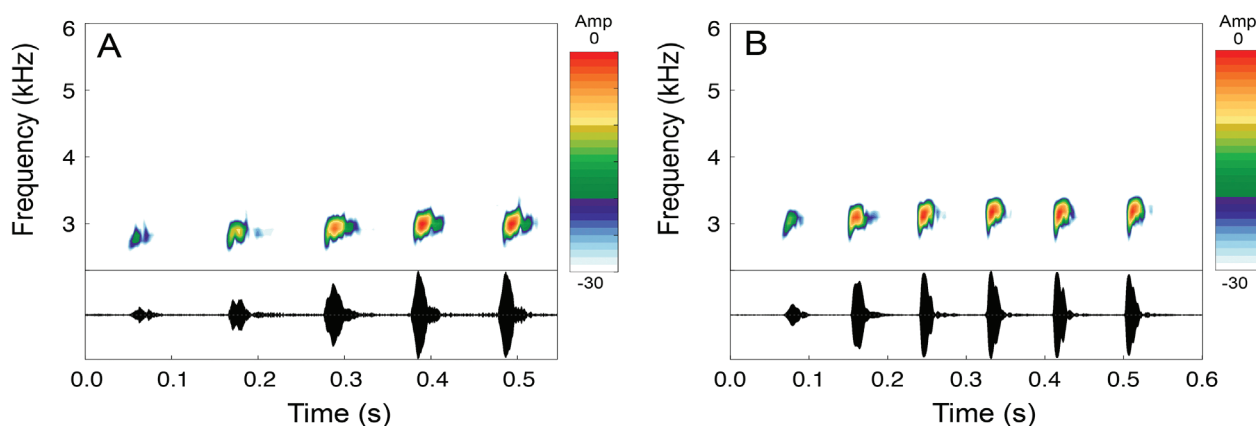


Figure 6. *Pristimantis ockendeni* advertisement calls spectrograms and oscillograms with five and six notes, respectively. **A** INPA-H 43952 (FNJV 58769) and **B** INPA-H 43946 (FNJV 58764), air temperature 24.1°C.

markings present on dorsum (Fig. 4C); and (iii) similar to the previous pattern, but lighter (Fig. E); mostly, interorbital region is covered by a dark band ($n = 5$ specimens). In addition, in some individual, W-shaped mark on scapular region present, even if not very evident (Fig. 4C; Fig. 5E and 5G). Ventral surface can have different shades, varying according to the concentration of melanophores: darker (Fig. 4B), intermediary (Fig. 4D) or lighter (Fig. 4F). Ventral skin texture is smooth (arms and chest region) to areolate (especially in belly and on femur region).

Dorsal coloration of males in life is highly variable, from brown reddish to yellow greenish (Fig. 5A–5G)

with or without interorbital bars. We do not have information on females. Arm is light yellowish. The iris is dichromatic (dark copper metallic down part and cream to silver upper part). Ventral surface is cream translucent (in the belly) and gray translucent (in the pelvic region) with dark melanophores (Fig. 5H).

Advertisement call of *Pristimantis ockendeni*. We recorded 40 calls of seven males from the underwood vegetation of about 2 m above the ground (air temperatures 23–25°C; relative humidity 82–99). Descriptive statistics of call parameters are presented in Table 5 and voucher in

Appendix 4. The advertisement call of *Pristimantis ockendeni* has a call duration of 541 ± 151 ms (334–939 ms, $n = 40$ calls) and 4–8 notes (6.1 ± 0.9 , $n = 243$ notes) with note duration of 24–158 ms (41 ± 31 ms). Calls with 6 and 7 notes were the most common arrangement (37.5% of recorded calls, $n = 15$, and 32.5% $n = 13$, respectively), followed by calls with 5 notes (25%, $n = 10$ calls), and rarely with 4 and 8 notes (2.5% each, $n = 1$). Inter-note intervals within calls average 62 ± 11 ms (38–87 ms). The spectral structure of the note has average minimum frequency of $2,389 \pm 190$ Hz (2,046–2,610 Hz), while average maximum frequency is $3,350 \pm 201$ Hz (2,964–3,641 Hz), and the dominant frequency is $2,864 \pm 202$ Hz (2,519–3,143 Hz) (Fig. 6A and 6B).

Pristimantis guianensis sp. nov.

<https://zoobank.org/2CD9F0BF-7BB8-4D12-B901-D56F5F489E2B>

Figs 7–11, 15 and 16

Eleutherodactylus ockendeni – Zimmerman and Rodrigues (1990), Heyer and Hardy (1991), Lima et al. (2006), Menin et al. (2007)

Pristimantis ockendeni – Rojas-Ahumada and Menin (2010); Lima et al. (2012), Fouquet et al. (2013)

Pristimantis cf. *ockendeni* – Vacher et al. (2020), Azevedo et al. (2021), Fouquet et al. (2022b)

Holotype. INPA-H 43918 (field number APL22783), adult male, collected at Reserva Florestal Adolpho Ducke, municipality of Manaus, state of Amazonas, Brazil ($2^{\circ}55'52.0''S$; $59^{\circ}58'25.8''W$, 74 m elevation), on 19 November 2019 by A.T. Mônico and I.Y. Fernandes.

Paratopotypes. Seventeen adult specimens (fourteen males and three females), same locality as the holotype: one male INPA-H 43917 (field number APL22782) collected in November 2019; nine males INPA-H 43919, INPA-H 439120, INPA-H 439121, INPA-H 43923, INPA-H 43924, INPA-H 43925, INPA-H 44248, INPA-H 44249, and INPA-H 44250 (field numbers APL22784–86, 22794–99, respectively) and one female INPA-H 43922 (field number APL22793) collected in December 2019 by A.T. Mônico, I.Y. Fernandes, E.D. Koch and A.P. Lima; two males MPEG 44181 and MPEG 44182 (field numbers APL22804 and 22805, respectively) collected January 2020 by A.T. Mônico and I.Y. Fernandes; two males INPA-H 43942 and INPA-H 43944 (field numbers APL23187 and 23189, respectively) and two females INPA-H 43941 and INPA-H 43943 (field numbers APL23187 and 23189, respectively) collected in 28–29 December 2020 by A.T. Mônico, U.F. Souza and I.Y. Fernandes.

Paratypes. Thirty-eight adult specimens (28 males and 10 females). BRAZIL: AMAZONAS: municipality of Presidente Figueiredo: Km 144 of the BR-174 Highway [six males MPEG 44183, MPEG 44184, INPA-H 43926,

INPA-H 44251, INPA-H 43927 and INPA-H 43928 (field numbers APL22806–11, respectively) collected in January 2020 by A.T. Mônico and E.D. Koch], Suçuarana's Fall at Vila de Balbina [seven males INPA-H 43929, INPA-H 43930, INPA-H 43931, INPA-H 43932, INPA-H 43933, MPEG 44185, MPEG 44186 (field numbers APL22812–18, respectively) and one female MPEG 44187 (field number APL22819) collected in January 2020 by A.T. Mônico and E.D. Koch]; RORAIMA: municipality of São João da Baliza [seven males INPA-H 43935, MPEG 44188, MPEG 44190, INPA-H 43937, INPA-H 43938, INPA-H 43939 and INPA-H 439340 (field numbers APL 22821, 22823, 22825 to 22829, respectively) and three females INPA-H 43934, INPA-H 43936 and MPEG 44189 (field numbers APL22820, 22822, and 22824, respectively) collected in June 2020 by A.T. Mônico, I.Y. Fernandes, M. Ferrão and A.P. Lima]. SURINAME: Sipaliwini [three males MNHN-RA-2020.0115–0117 (field numbers AF2255, 2256 and 2289, respectively) and two females MNHN-RA-2020.0113 and 2020.0114 (field numbers AF2155 and 2258, respectively) collected in April 2014 by A. Fouquet and J.P. Vacher]. GUYANA: Mabura Hill Forest Reserve [four males SMNS11987, 11989, 11993, 11994 and three females SMNS 11990, 11991 and 11995 collected between April 2003 and June 2004; and one male MTD 47769 and one female MTD 47770 collected in August 2010 by R. Ernst].

Referred material. Available in Appendix 1.

Diagnosis. The new species is characterized by the following unique combination of characters: (1) dorsal skin shagreened, frequently with distinctly tubercles, with or without W-shaped on scapular region; (2) tympanum visible, tympanic membrane poorly differentiated, tympanum diameter 24–34% of eye diameter and annulus partially visible externally; supratympanic black band; (3) snout subacuminate to sub-rounded in dorsal view and rounded in lateral profile, loreal region concave; (4) upper eyelid tubercles present; dark bar between the eyes, and three oblique black streaks below the eye; cranial crests absent; (5) nostril ovoid, slightly protuberant, directed laterally; interorbital distance 29–37% of head width; (6) tongue cordiform to ovoid; (7) absence of dentigerous processes of vomers (Fig. 7); (8) males with vocal slits, vocal sac median to subgular; nuptial pads absent; (9) Finger I slightly more shorter than II; finger discs ovoid to expanded (finger disc I less expanded compared to finger disc II, III and IV); (10) fingers without lateral fringes; (11) three to five enlarged ulnar tubercles, barely visible in fixed specimens (easily visible in individuals when in life); (12) tibia length 48–56% of SVL; (13) heel tubercle absent; tarsal tubercles aligned, ovoid to elliptical; tarsal fold short, barely visible; (14) thenar tubercle ovoid to elliptical; small palmar tubercle ill-defined, less than 30% of thenar tubercle; (15) toes without lateral fringes; rudiment of web absent; (16) toes I and II almost of the same size; toes discs ovoid (toe disc I and II, especially) to expanded (III, IV and V); (17) belly skin smooth to areolate, and ventral region of the femur externally are-

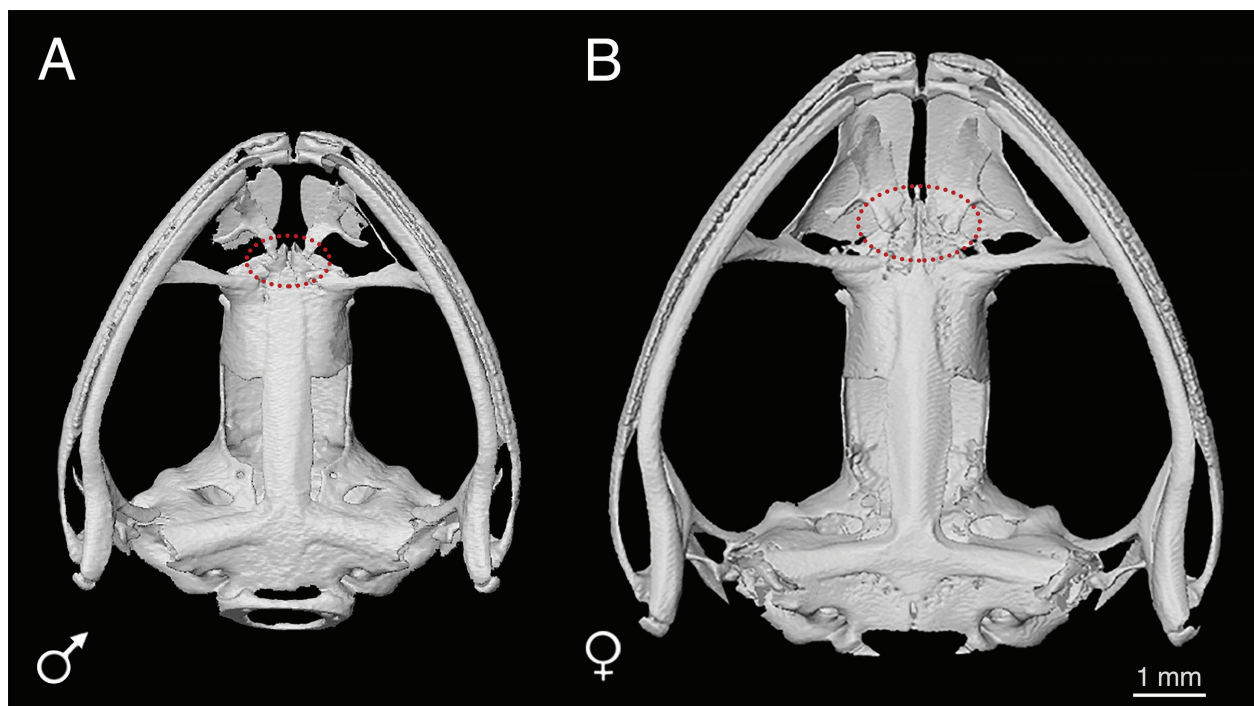


Figure 7. *Pristimantis guianensis* sp. nov. volumetric renderings of μ -CT scans of the skull in ventral views. **A** Male (Paratype, MNHN-RA-2020.0115) and **B** Female (Paratype, MNHN-RA-2020.0114). Absence of dentigerous processes of vomers can be noted in the dotted red circle.

olate; (18) in life, translucent groin with small, scattered dark melanophores and absence of bright colored blotches or marks; (19) in life, iris is dichromatic, brown lower part and cream upper part; (20) SVL in adult males of 16.2–20.7 mm ($n = 43$) and in females of 21.4–25.7 mm ($n = 13$); and (21) advertisement call with average call duration of 232 ± 42 ms, inter-note interval of 44 ± 5 ms (68 ± 11 ms), minimum frequency of 2,827–3,695 Hz, maximum frequency of 4,333–6,688 Hz and dominant frequency of 3,466–4,521 Hz.

Comparisons with other species. The new species is compared to other *Pristimantis* of the *P. unistrigatus* species group occurring in the Guiana Shield: *P. abakapa* Rojas-Runjaic, Salerno, Señaris & Pauly, 2013; *P. aureoventris* Kok, Means & Bossuyt, 2011; *P. crepitaculus* Fouquet, Peloso, Jairam, Lima, Mônico, Ernst & Kok, 2022; *P. espedeus* Fouquet, Martinez, Courtois, Dewynter, Pineau, Gaucher, Blanc, Marty & Kok, 2013; *P. grandoculis* (van Lidth de Jeude, 1904); *P. guaiquinimensis* (Schlüter & Rödder, 2007); *P. imthurni* Kok, 2013; *P. inguinalis* (Parker, 1940); *P. jamescameroni* Kok, 2013; *P. jester* Means & Savage, 2007; *P. marmoratus* (Boulenger, 1900), *P. memorans* (Myers & Donnelly, 1997), *P. pulvinatus* (Rivero, 1968); *P. saltissimus* Means & Savage, 2007; and *P. sarisarinama* Barrio-Amorós & Brewer-Carias, 2008 and to *P. ockendeni*, which occurs in southwestern Amazonia. Diagnostic characters of compared species are shown in parentheses unless stated otherwise.

Pristimantis guianensis sp. nov. differs from *P. ockendeni* by the absence of dentigerous processes of vomers (present) and smaller SVL: male 16.2–20.7 mm ($n = 43$

specimens) in *P. guianensis* sp. nov. (18.4–22.8 mm, $n = 09$ in *P. ockendeni*) and female 21.4–25.7 mm ($n = 13$ specimens) in *P. guianensis* sp. nov. (30.4–30.6 mm, $n = 02$ in *P. ockendeni*). Additionally, the advertisement call of *P. guianensis* sp. nov. has average call duration of 232 ± 42 ms (540 ± 150 ms), inter-note interval of 44 ± 5 ms (68 ± 11 ms) and is emitted at minimum frequency of 2,827–3,695 Hz (2,047–2,610 Hz), maximum frequency of 4,333–6,688 Hz (2,965–3,504 Hz) and dominant frequency of 3,466–4,521 Hz (2,519–3,144 Hz).

The absence of dentigerous processes of vomers easily distinguishes *Pristimantis guianensis* sp. nov. from *P. aureoventris*, *P. crepitaculus*, *P. espedeus*, *P. grandoculis*, *P. imthurni*, *P. jamescameroni*, *P. jester*, *P. marmoratus* and *P. saltissimus* (present in all species); and the presence of vocal slits in males (absent in *P. abakapa*, *P. aureoventris*, *P. grandoculis*, *P. imthurni*, *P. guaiquinimensis*, *P. jamescameroni*, *P. jester* and *P. saltissimus*).

Pristimantis guianensis sp. nov. differs from *P. espedeus*, *P. guaiquinimensis*, *P. imthurni*, *P. jamescameroni* and *P. pulvinatus* by having smaller male SVL of 16.2–20.7 mm (SVL 20.7–24.8 mm in *P. espedeus*; 33.4–34.7 mm in *P. guaiquinimensis*; 22.9 mm in *P. imthurni*; 22.9 mm in *P. jamescameroni*; 23.0–26.1 mm in *P. pulvinatus*; 22.6–25.8 mm in *P. sarisarinama*); from *P. espedeus*, *P. jamescameroni*, *P. guaiquinimensis* and *P. memorans* by smaller female SVL of 21.4–25.7 mm (SVL 29.4 mm in *P. espedeus*; 26.3–27.5 mm in *P. jamescameroni*; 32.4–33.6 mm in *P. guaiquinimensis*; 31–32 mm in *P. memorans*); by having a translucent groin with small, scattered dark melanophores and absence of bright colored blotches or marks in life, *P. guianensis* sp. nov. can be distinguished from *P. abakapa* (chocolate brown groin

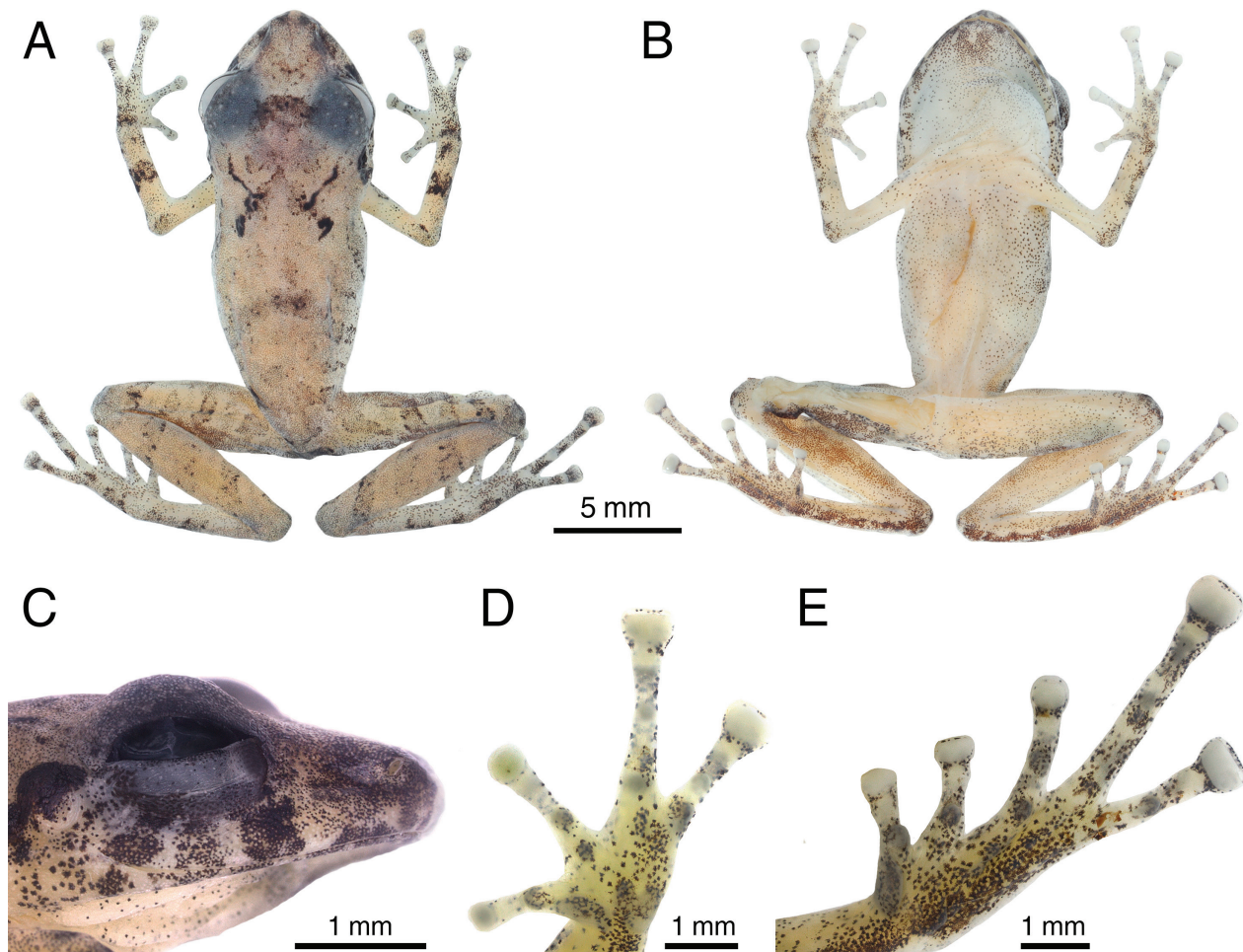


Figure 8. Preserved holotype of *Pristimantis guianensis* sp. nov. (INPA-H 43918) from Reserva Florestal Adolpho Ducke, Manaus municipality, Amazonas state, Brazil. **A** dorsal and **B** views of body; **C** lateral view of head; and ventral view of **D** hand and **E** foot. Photographs: A.T. Mônico.

with small white dots), *P. aureoventris* (black or brown, sometimes with some small golden spots), *P. crepitaculus* (dark grey groin), *P. espedeus* (reddish orange groin), *P. grandoculis* (dark grey groin), *P. imthurni* (brown groin), *P. inguinalis* (bright yellow spots on groin), *P. jamescameroni* (bright orange groin) and *P. marmoratus* (yellow or pale green wash on groin).

Description of holotype. INPA-H 43918 (field number APL 22783), an adult male (Fig. 8), SVL 17.7 mm; head slightly longer than wide (HL 105% of HW); head width 36.0% of SVL; head length 37.9% of SVL; cranial crest absent; snout subacuminated in dorsal (Fig. 8A) and rounded in lateral (Fig. 8C) views; snout short, END 77.3% of ED; nostril ovoid, slightly protuberant, directed laterally; internarial region slightly concave; canthus rostralis almost straight in dorsal view, rounded in profile; loreal region concave; lips rounded; small tubercles on upper eyelid; interorbital region straight, IOD 32.2% of HW; large eye (ED/TD = 3.4); supratympanic fold distinct, extending from posterior margin of eyelid angling posteroventrally behind tympanic annulus; tympanum visible and rounded, 28.5% of ED; tympanic membrane poorly prominent, directed laterally; tympanic annulus distinct, obscured anteriorly, dorsally, and posteriorly by

the supratympanic fold; two small postrictal tubercles, poorly visible; choanae of moderate sized, round, not concealed by palatal sheath of maxilla; dentigerous processes of vomers absent; tongue cordiform, longer than wide; vocal slits present; vocal sac median, simple and subgular.

Forearm slightly longer than hand (FAL 102.6% of HAND); four ulnar tubercles enlarged and aligned, poorly defined; size Finger I<II<IV<III (Fig. 8D); finger discs rounded (Finger I and II) to expanded (Finger III and IV); thenar tubercle ovoid; palmar tubercle bifid and poorly defined, almost twice the width of elongate thenar tubercle; subarticular tubercles well defined, most prominent on fingers I and IV, rounded in dorsal and lateral view; all fingers with ventral pads well defined by circumferential grooves.

Hindlimbs slender, with transversal bars complementary, being four on thigh, three on tibia and two on tarsus; tibia length 54% of SVL; heel without tubercles; tarsus with row of small, low tubercles, poorly visible; tarsal folds absent; foot length 41% of SVL; subarticular tubercles well defined (except in Toe IV), round in dorsal and lateral view; toes lacking lateral fringes and webbed; toes lengths I < II < III < V < IV (Fig. 8E); Toe I only slightly shorter than II; Toe V slightly longer than Toe III (Toe III

Table 4. Morphometric measurements in millimeters of adults of *Pristimantis guianensis* sp. nov. and *P. ockendeni*. The measurement of the two *P. ockendeni* females correspond to the syntypes. Values express mean \pm standard deviation (range). Measurement abbreviations are listed in the Material and Methods.

Variables	<i>Pristimantis guianensis</i> sp. nov.			<i>Pristimantis ockendeni</i>		
	Holotype	Males (n = 43)	Females (n = 13)	Males (n = 9)		Females (n = 2)
				Syntype	Newly collected (n = 8)	
SVL	17.7	17.9 \pm 0.8 (16.2–20.7)	23.8 \pm 1.3 (21.4–25.7)	18.6	19.6 \pm 1.3 (18.4–22.8)	30.4–30.6
HW	6.4	6.5 \pm 0.4 (5.7–7.6)	8.6 \pm 0.5 (7.5–9.6)	6.8	7.0 \pm 0.5 (6.7–8.2)	10.4–10.7
HL	6.7	6.8 \pm 0.4 (5.9–7.9)	8.7 \pm 0.7 (7.6–10.0)	6.9	7.2 \pm 0.4 (6.7–8.3)	10.8–10.8
SL	2.9	3.0 \pm 0.2 (2.6–3.5)	4.1 \pm 0.2 (3.8–4.5)	2.9	3.2 \pm 0.3 (2.7–3.6)	4.4–4.6
IND	1.9	1.8 \pm 0.2 (1.5–2.2)	2.3 \pm 0.2 (1.9–2.5)	1.8	2.0 \pm 0.1 (1.8–2.2)	2.6–2.7
EN	2.1	2.1 \pm 0.1 (1.7–2.3)	2.9 \pm 0.1 (2.8–3.2)	1.8	2.2 \pm 0.3 (1.8–2.6)	3.3
IOD	3.6	2.1 \pm 0.1 (1.8–2.5)	2.8 \pm 0.2 (2.4–3.1)	2.3	2.4 \pm 0.2 (2.0–2.7)	3.8–3.9
ED	2.7	2.7 \pm 0.2 (2.3–3.0)	3.2 \pm 0.1 (3.1–3.4)	2.7	2.7 \pm 0.1 (2.6–3.0)	4.1–4.3
TD	0.8	0.8 \pm 0.1 (0.6–1.0)	1.0 \pm 0.1 (0.8–1.2)	0.9	1.0 \pm 0.1 (0.9–1.2)	1.9
UAL	4.7	4.7 \pm 0.3 (4.0–5.2)	6.4 \pm 0.3 (5.8–6.7)	4.9	5.2 \pm 0.4 (4.8–6.2)	6.5–7.7
FAL	4.6	4.4 \pm 0.2 (3.8–4.9)	5.5 \pm 0.8 (3.8–6.3)	4.2	4.4 \pm 0.4 (3.8–5.1)	5.2–5.3
HAND	4.5	4.3 \pm 0.3 (3.6–5.1)	5.4 \pm 0.4 (4.7–6.1)	5.0	5.3 \pm 0.5 (4.9–6.6)	6.3–4.5
HANDI	2.2	2.2 \pm 0.2 (1.8–2.9)	2.8 \pm 0.3 (2.2–3.2)	2.6	2.7 \pm 0.3 (2.4–3.4)	3.9–4.0
HANDII	3.0	2.8 \pm 0.3 (2.0–3.4)	3.5 \pm 0.5 (2.6–4.1)	3.2	3.5 \pm 0.3 (3.2–4.2)	5.6–5.8
HANDIV	3.5	3.7 \pm 0.2 (3.2–4.3)	4.7 \pm 0.3 (4.3–5.1)	4.1	4.3 \pm 0.4 (3.9–5.3)	7.0–7.3
WFD	0.8	0.8 \pm 0.1 (0.5–1.0)	1.0 \pm 0.1 (1.0–1.2)	0.8	0.8 \pm 0.1 (0.8–0.9)	1.9
THL	9.2	8.7 \pm 0.6 (7.1–10.4)	10.7 \pm 0.7 (10.0–12.0)	9.2	9.3 \pm 0.5 (8.7–10.5)	14.9–15.6
TL	9.6	9.3 \pm 0.5 (8.2–10.7)	11.5 \pm 0.6 (10.7–12.5)	10.1	10.2 \pm 0.6 (9.5–11.4)	16.1–16.2
TAL	5.6	5.2 \pm 0.3 (4.4–5.9)	6.6 \pm 0.5 (5.7–7.3)	5.0	5.2 \pm 0.4 (4.9–5.7)	6.8–7.8
FL	7.2	6.9 \pm 0.4 (5.8–7.8)	8.6 \pm 0.5 (7.9–9.5)	7.9	8.3 \pm 0.8 (7.5–10.1)	12.3–12.5
FLI	2.4	2.3 \pm 0.2 (2.0–2.6)	3.0 \pm 0.2 (2.7–3.3)	2.4	2.6 \pm 0.3 (2.4–3.2)	4.1–4.5
FLII	2.9	3.0 \pm 0.2 (2.7–3.5)	3.8 \pm 0.2 (3.6–4.2)	3.4	3.5 \pm 0.2 (3.3–4.0)	5.5–8.4
FLIII	4.5	4.5 \pm 0.2 (4.1–5.1)	5.7 \pm 0.3 (5.4–6.3)	5.1	5.4 \pm 0.5 (4.9–6.6)	8.4–8.9
FLV	5.7	5.6 \pm 0.4 (4.3–6.4)	7.4 \pm 0.3 (6.9–7.8)	6.3	6.6 \pm 0.7 (5.8–8.2)	9.9–10.8
WTD	0.8	0.8 \pm 0.1 (0.5–1.0)	1.1 \pm 0.1 (0.9–1.3)	0.8	0.9 \pm 0.1 (0.7–1.0)	1.7–1.9

extending to border of toe discs V); discs well defined by circumferential grooves; toe discs ovoid (toes I, II and III) to expanded (toes IV and V); thenar tubercle ovoid and palmar tubercle small and ill-defined; all toes with ventral pads well defined, most prominent in toes IV and V.

Dorsal skin shagreened, with a W-shaped mark on scapular region, a dark blotch between the eyes and other ill-defined near the groin (Fig. 8A); upper eyelid shagreened, with several distinctly enlarged tubercles on each eyelid; upper and posterior surfaces of hindlimbs smooth; skin on flanks and chest smooth; slightly areolate on belly; and ventral region of the femur externally areolate; dorsolateral folds absent; pectoral and discoidal folds not visible; cloaca not protuberant, cloacal region without tubercles.

Morphometric measurements are presented in Table 4.

Coloration of holotype in preservative: After 30 months in 70% ethanol, colours of the holotype faded, notably glandular supracarpal pad (Fig. 8). The snout is dorsally dark brown and well-delimited by a transversal light gray interorbital band extending on half of the eyelids; dark bands below the eyes; supratympanic black band; black blotches in the tympanic region (Fig. 8C); a dark brown mark is well-defined on the scapular region posteriorly followed by two oblique transversal dark brown bands. Similar, transversal dark brown bands are present

on the arms, hands and legs and feet. Arms, belly and ventral surfaces of thighs, tibia and tarsus are cream with brown melanophores, arm and belly skin are the lightest, with melanophores sparsest, while ventral surfaces of hindlimbs have a greater amount of melanophores (Fig. 8B); upper arm without bands (only few melanophores) and lower arm with one dark transversal band; black blotch in the cloaca region; more colors details are shown in Fig. 8.

Coloration of holotype in life: In life, yellow dorsal surface with orange blotches and tubercles all over the dorsal surface; three horizontal dark bands on dorsum, the anterior one on interorbital region, the medial one bordering the W-shaped mark on the scapular region, and posterior one ill-defined near the groin (Fig. 10A). Black blotches located in the tympanic region and dark bands below the eyes. Upper surface of legs and arms with light brown transversal band. Ventral surface is cream covered with minute gray melanophores, while the throat region is slightly yellow. The arm and belly skin are the lightest and the melanophores are the sparsest. Ventral surfaces of thighs, tibia and tarsus are gray, due to the high amount of melanophores. During the day the coloration turns darker. The iris is dichromatic: lower part brown and upper part golden color with dark brown reticulation. More colors details are shown in Fig. 8A.

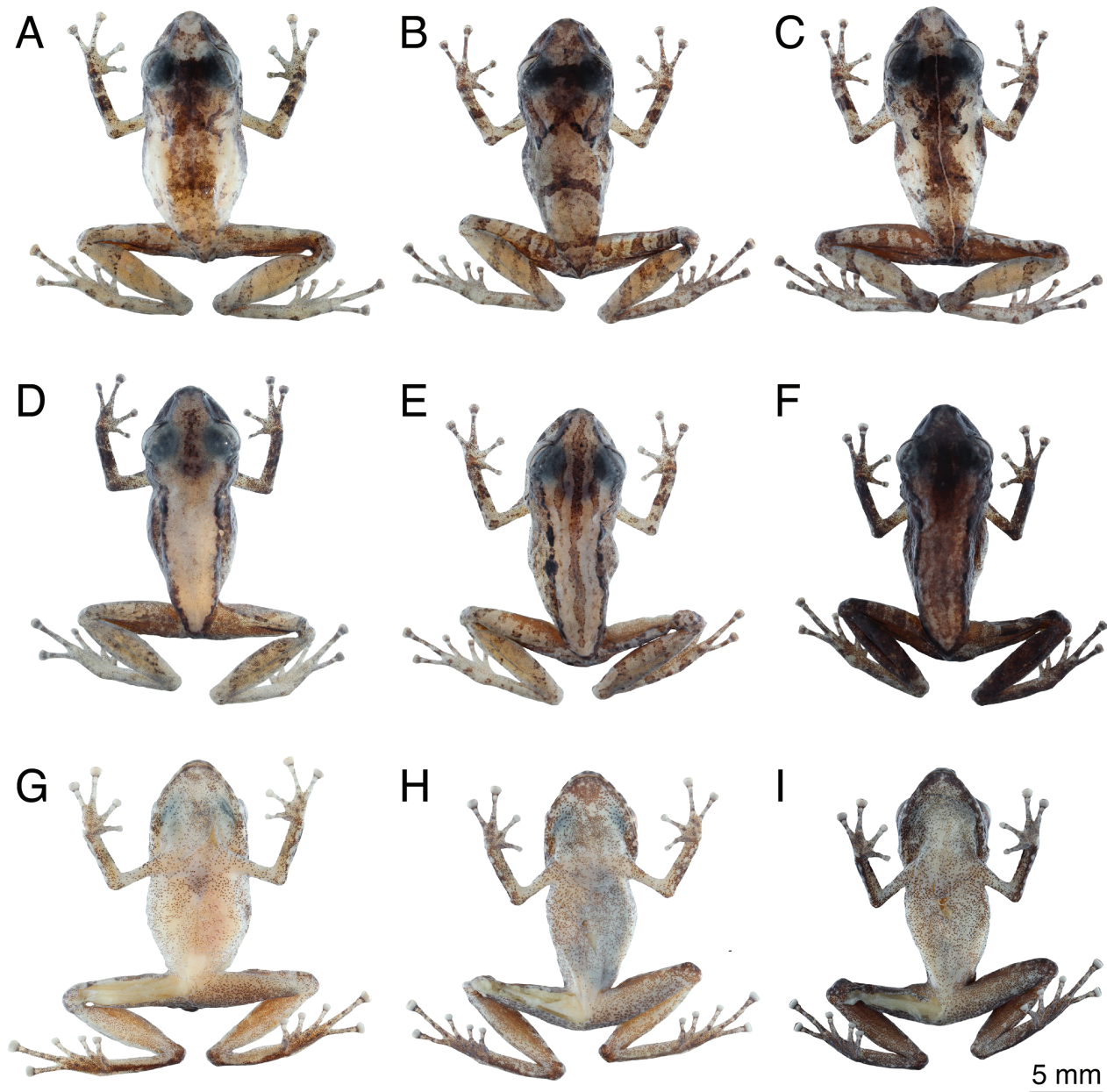


Figure 9. Preserved *Pristimantis guianensis* sp. nov. dorsal and ventral surface pattern variation. **A** MPEG 44181; **B** INPA-H 43929; **C** INPA-H 43928; **D** INPA-H 43939; **E** MPEG 44185; **F** INPA-H 43940; **G** MPEG 44181; **H** INPA-H 43929; and **I** INPA-H 43940. Photographs: A.T. Mónico.

Intraspecific variation. *Pristimantis guianensis* sp. nov. is small, SVL in adult males of 16.2–20.7 mm ($n = 43$; Table 4) and in females of 21.4–25.7 mm ($n = 13$; Table 4). Dorsal pattern of males is variable: most specimens of the type series (73% of type specimens) show irregular dark markings present on dorsum, similar to holotype (Fig. 9A), slightly dark middorsal and other ill-defined near the groin (12% of types; Fig. 9B), with snout-vent stripe (only one specimen; Fig. 9C), dorsal view coloration strongly delimited and different from sideview (13% of types; Fig. 9D, 9E and 9F), lighter (Fig. 9D) or darker (Fig. 9F), with or without dark transverse stripes on dorsum (Fig. 9E). Specimens of dorsal pattern with irregular dark markings (Fig. 9A and 9C) and slightly dark middorsal (Fig. 9B) has interorbital region is covered by a dark band (85.7% of specimens). In addition, these two patterns are closely

linked to the presence of a W-shaped on scapular region (66% of types), even if not very evident (16% of types). Dorsal skin texture is fairly variable too (probably depending on activity, as observed in many species of the genus (e.g., Guayasamin et al. 2015; Kok et al. 2018), from shagreened to granular, with or without tubercles.

Dark bands and blotches located below the eyes are present in the most specimens of type series, not very evident or absent (91%, 4%, and 5% of types, respectively). Transversal dark brown bands are present on the arms and hands, intermediary, or absent (89%, 4%, and 7% of types, respectively). In legs and feet, transversal dark bands are present in most specimens of the type series, intermediary, or absent (80%, 9%, and 11% of types, respectively). The three to five ulnar tubercles are small in 9% of types. Supratympanic black band in all species.

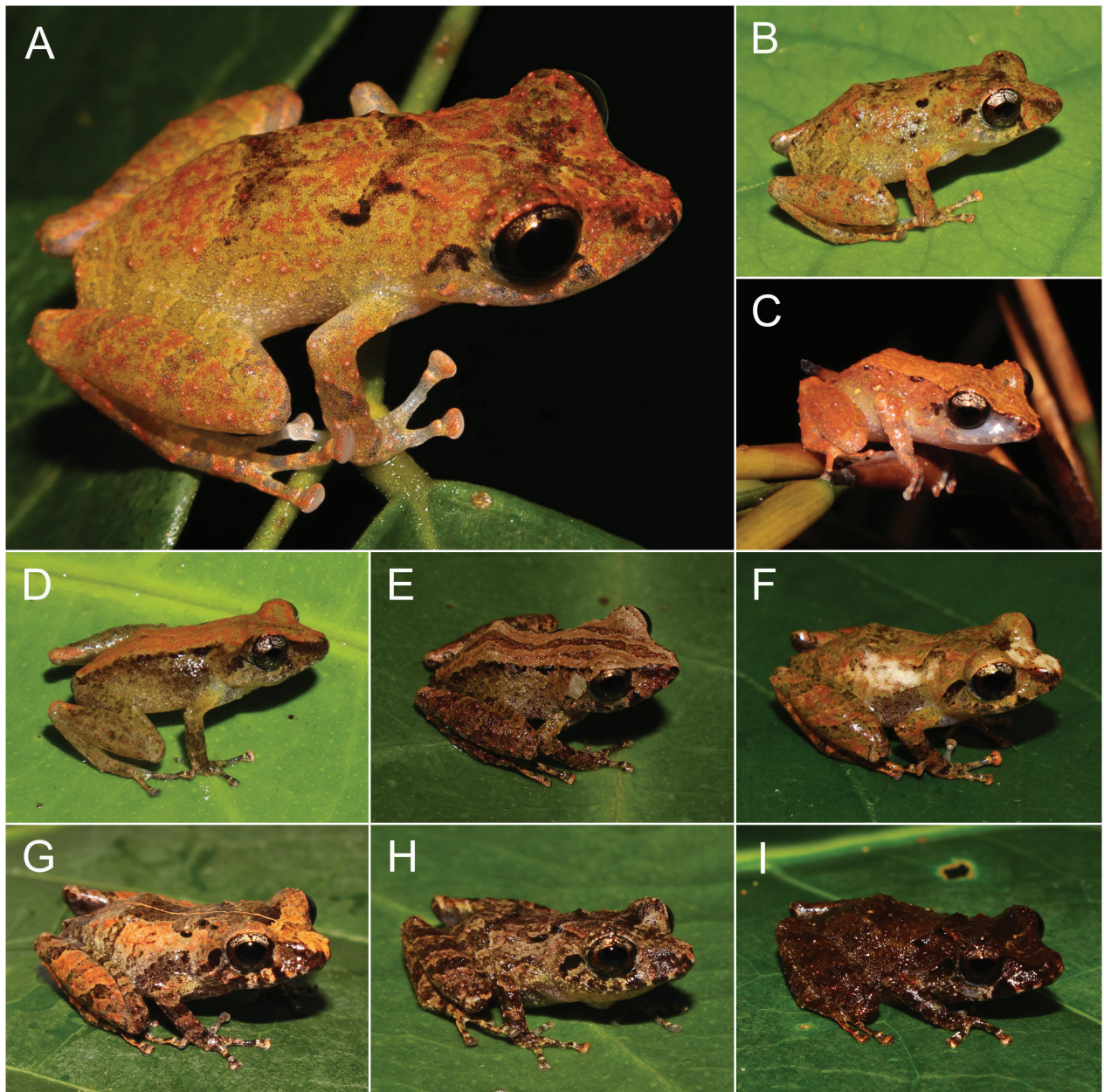


Figure 10. Males of *Pristimantis guianensis* sp. nov. in life. **A** Holotype INPA-H 43918 (SVL 17.7 mm); **B** Paratype MPEG 44188 (SVL 18.4 mm); **C** Paratopotype INPA-H 44250 (SVL 17.6 mm); **D** Paratype INPA-H 43939 (SVL 17.6 mm); **E** Paratype MPEG 44185 (SVL 17.9 mm); **F** Paratopotype MPEG 44181 (SVL 17.8 mm); **G** Paratype INPA-H 43928 (SVL 17.9 mm); **H** Paratype INPA-H 43931 (SVL 17.7 mm); and **I** Paratype INPA-H 43926 (SVL 16.9 mm). Photographs: A.T. Mônico, except (C) E.D. Koch.

Ventral surface can have different shades, varying according to the concentration of melanophores: light (Fig. 9G), intermediary (Fig. 9H) or dark (Fig. 9I). Most type specimens (57%) had the intermediate concentration, while light and dark were less frequent (29% and 14% of types).

Dorsal background coloration in life is widely variable, from greenish yellow (Fig. 10A, 10B and 10F) and light orange (Fig. 10C, 10F and 10G) to brown (Fig. 10E, 10H, 10I), while females do not vary as much. Snout-vent stripe can be present (Fig. 9G). The iris is dichromatic: brown to dark copper metallic lower part and cream to silver upper part with dark brown reticulation (Fig. 10). When active, the ventral surface is with cream background, cover by white granules and brown

melanophores (nocturnal, Fig. 11A), and gray at the day, which is the result of melanophores expansion (diurnal, Fig. 11B).

Advertisement call. We recorded 111 calls of 34 males from 2 m above the ground underwood vegetation at temperatures between 23–25°C and 82–99% relative air humidity. The list of call recordings is disponible in Appendix 4, and descriptive statistics of call parameters are in Table 5. The advertisement call of *Pristimantis guianensis* sp. nov. is short (mean 232 ms \pm standard deviation 42, range 158–371 ms) and composed of 4–6 notes (4.6 ± 0.7). Calls with four notes were the most common arrangement, corresponding to 51% of all analysed calls ($n = 57$ calls), followed by calls with five notes (38%; $n =$

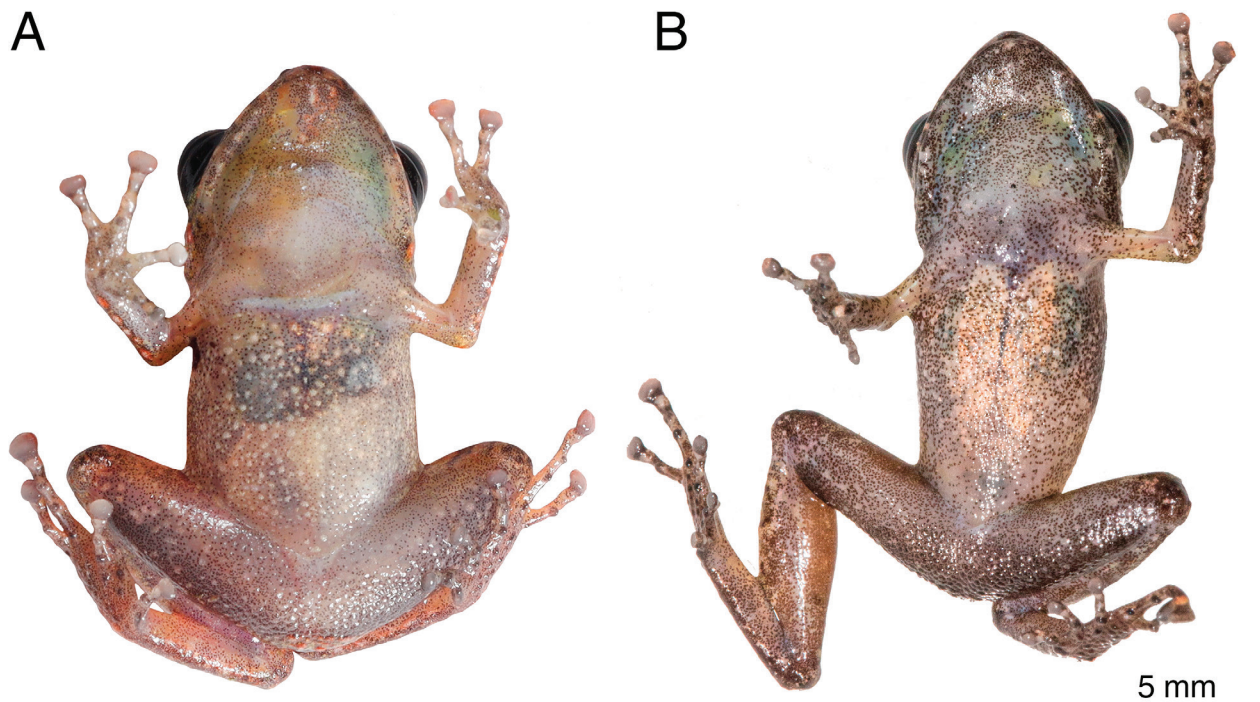


Figure 11. Ventral coloration of *Pristimantis guianensis* sp. nov. in life. **A** Paratopotype INPA-H 43944 (nocturnal); and **B** Paratype MNHN-RA-2020.0115 (diurnal). Photographs: (A) A.T. Mónico and (B) A. Fouquet.

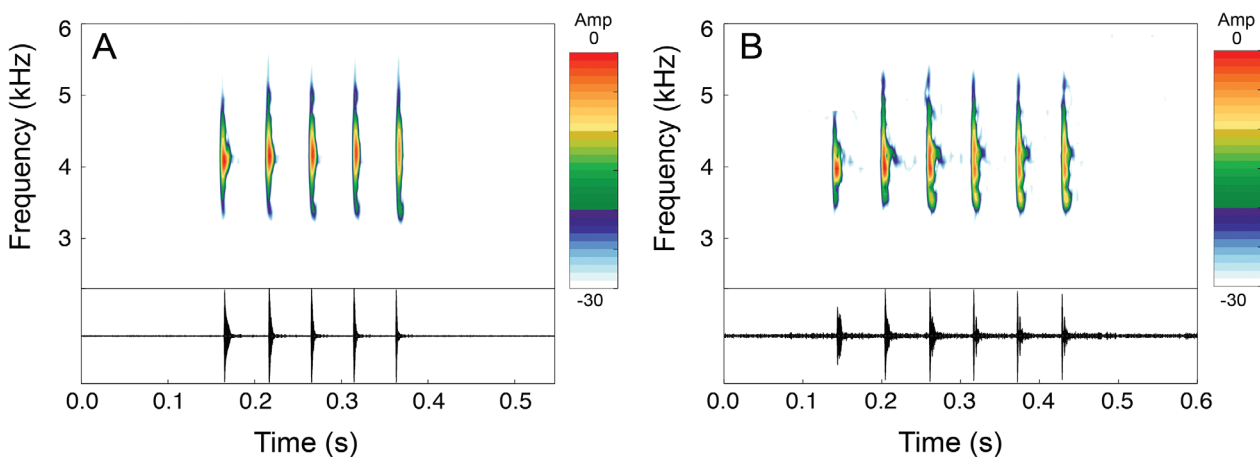


Figure 12. *Pristimantis guianensis* sp. nov. advertisement calls spectrograms and oscillograms with five and six notes, respectively. **A** Holotype INPA-H 43918 (FNJV 58730); **B** Paratopotype INPA-H 43919 (FNJV 58731), air temperature 23.2°C.

42 calls) and six notes (11%; $n = 12$ calls). Note duration averaged 16 ± 5 ms (5–30 ms; $n = 510$ notes). Inter-note intervals are homogeneous within calls (average = 44 ± 5 ms; range 14–56 ms; $n = 399$ inter-note intervals), i.e., inter-note the same temporal structure. Notes lack true harmonics. The average minimum frequency is $3,210 \pm 152$ Hz (2,827–3,695 Hz), while average maximum frequency is $5,264 \pm 639$ Hz (4,333–6,688 Hz), and the dominant frequency is $3,970 \pm 179$ Hz (3,466–4,521 Hz) ($n = 111$ calls) (Fig. 12A and 12B, Table 5).

We provide additional acoustic variables in Appendix 5.

Courtship call repertoire. We observed that when a female is present, males calls consist of 7–8 notes (7.75 ± 0.5 ; $n = 4$ calls of two males), with call duration of

359 ± 31 ms (321–391 ms), note duration of 13 ± 2 ms (10–14 ms) with inter-note intervals of 39 ± 1 ms (38–40 ms). The average minimum frequency is $3,097 \pm 55$ Hz (3,025–3,158 Hz), while average maximum frequency is $5,080 \pm 678$ Hz (4,134–5,861 Hz), and the dominant frequency is $3,914 \pm 191$ Hz (3,790–4,199 Hz).

We recorded the vocal repertoire of a male (INPA-H 43944, FNJV 58762) until the amplexus with a female (INPA-H 43943) for ~80 s (Fig. 13). The male jumped and positioned close to the female (approximately 20 cm) and then emitted one call with 8 notes (Fig. 13A), two-harmonic structure and more high-pitched than advertisements calls (maximum frequency reaching 8,182 Hz). The call duration was 386 s, where notes lasted 15 ± 1 ms (13–17 ms) with inter-note intervals of 38 ± 2 ms (35–41 ms). The call was emitted with a

Table 5. Acoustic variables of the advertisement call of *Pristimantis guianensis* **sp. nov.** (n = 111 calls, 34 males) and *Pristimantis ockendeni* (n = 40 calls, 7 males). Values express mean \pm standard deviation (range).

Acoustic variables	<i>P. guianensis</i> sp. nov.	<i>P. ockendeni</i>
Call duration (ms)	232 \pm 42 (158–371)	540 \pm 150 (334–940)
Number of notes per call	4.5 \pm 0.7 (4–6)	6.1 \pm 0.9 (4–8)
Note duration (ms)	18 \pm 11 (5–89)	41 \pm 31 (27–71)
Inter-note interval (ms)	44 \pm 5 (14–56)	68 \pm 11 (38–87)
Minimum frequency (Hz)	3,210 \pm 152 (2,827–3,695)	2,389 \pm 190 (2,047–2,610)
Maximum frequency (Hz)	5,264 \pm 639 (4,333–6,688)	3,350 \pm 201 (2,965–3,504)
Dominant frequency (Hz)	3,970 \pm 179 (3,466–4,521)	2,864 \pm 203 (2,519–3,144)

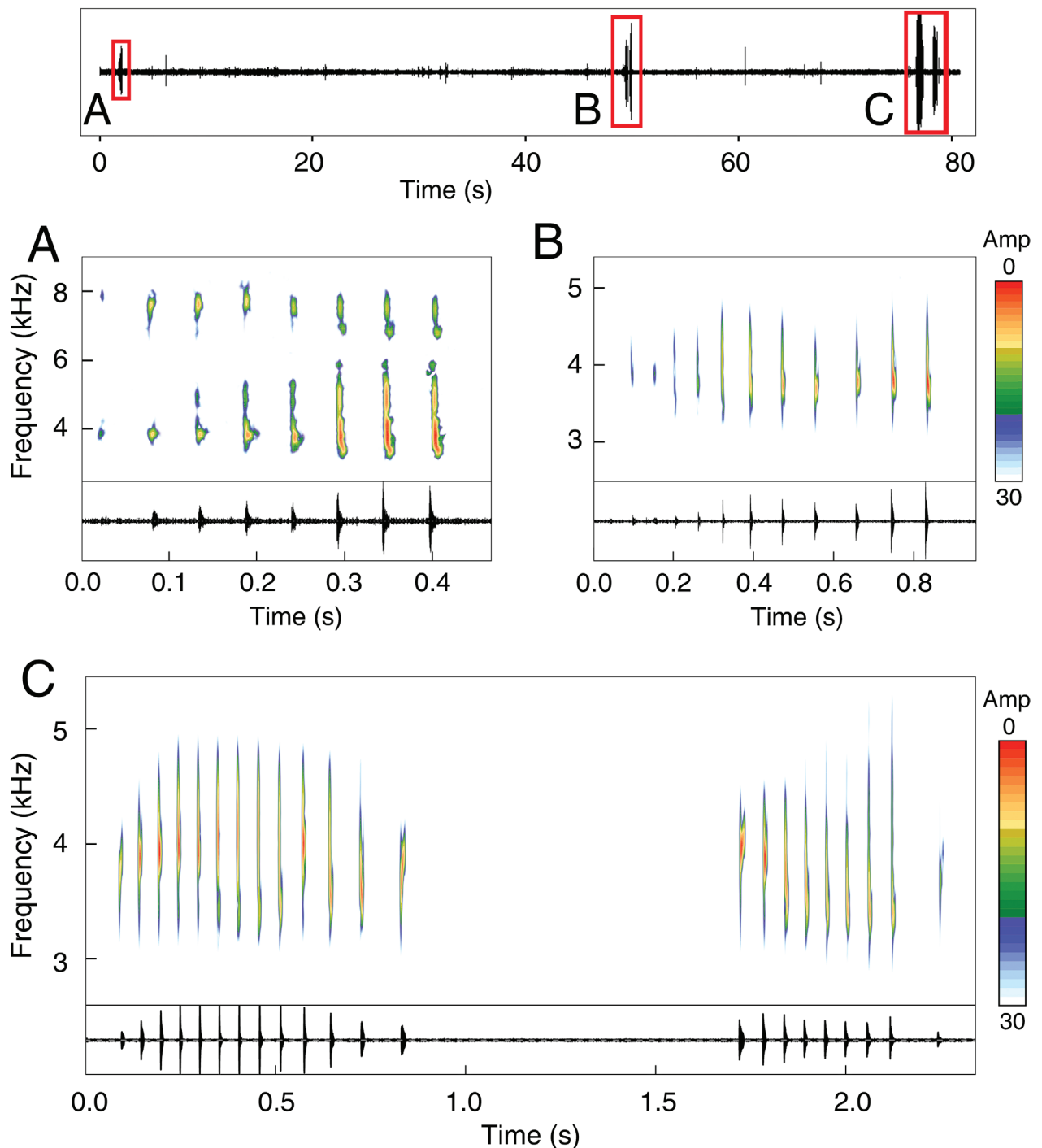


Figure 13. *Pristimantis guianensis* **sp. nov.** courtship call repertoire spectrograms for 80 seconds before the amplexus. Male INPA-H 43944, FNVJ 58762; air temperature 24.8°C and humidity 97%. **A** First call after approaching the female; **B** second call, issued after 47 s of previous call; **C** last two calls before forming the amplexus.

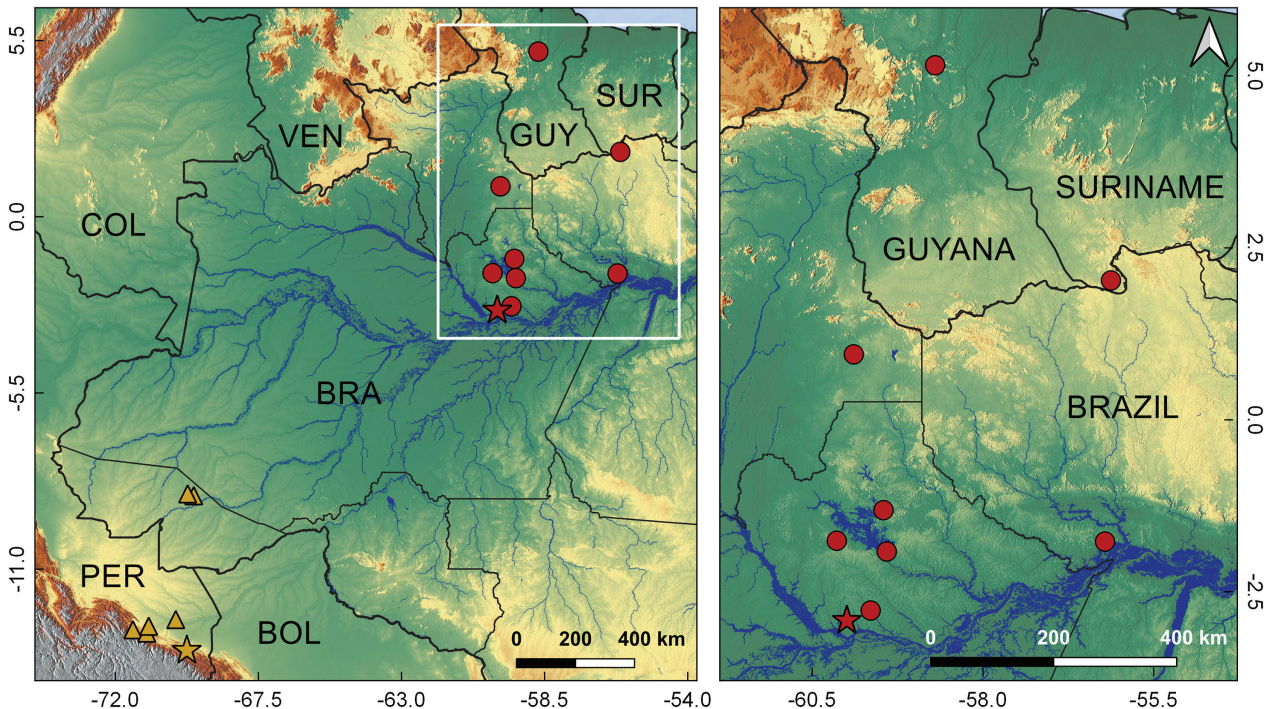


Figure 14. Geographic distribution of individuals of *Pristimantis guianensis* sp. nov. (red circles) and *Pristimantis ockendeni* sensu stricto (yellow triangles) used in our analyses. Stars indicate type localities of each species.

dominant frequency of $3,790 \pm 172$ Hz, minimum frequency of $3,231 \pm 168$ Hz and maximum frequency of $5,489 \pm 602$ Hz. The male came even closer to female and, after 47 s emitting the previous call, emitted a second call (Fig. 13B), less high-pitched and longer than the previous one (Fig. 9A): 11 notes, call duration 757 ms, note duration 20 ± 5 ms (17–33 ms), inter-note intervals 54 ± 19 ms (28–85 ms), minimum, maximum and dominant frequency of $3,144 \pm 111$ Hz, $4,780 \pm 158$ Hz and $3,704 \pm 151$ Hz, respectively. Finally, the male emitted two calls with inter-call interval of 860 ms between them (Fig. 13C): the former call has call duration of 761 ms and consisted of 13 notes with note duration of 19 ± 3 ms (14–22 ms), inter-note interval of 45 ± 16 ms (31–87 ms) s, and frequencies were similar to previous call (minimum $3,032 \pm 104$ Hz, maximum $4,845 \pm 245$ Hz and dominant of $3,876 \pm 213$ Hz); while the latter call lasted 547 ms and was composed of 9 notes, with note duration of 18 ± 3 ms (15–25 ms), inter-note interval of 39 ± 4 ms (32–45), except for last note (107 ms), dominant frequency was $3,962 \pm 219$ Hz, minimum frequency $2,995 \pm 76$ Hz and maximum frequency $4,705 \pm 214$ Hz. The female then bent down and formed the amplexus with the male.

Etymology. The specific epithet “*guianensis*” refers to the region of occurrence of the new species, widely distributed in the western Guiana region (the lowlands of the eastern part of the Guiana Shield).

Distribution, Natural history and Conservation. *Pristimantis guianensis* sp. nov. is likely endemic to the western portion of the Guiana region (Fig. 14) which is bounded by the Amazon and the Negro Rivers on the South,

the Branco River on the southwest. Similarly distributed species are *Allobates sumtuosus* (Morales, 2002) (Simões et al. 2013), *Synapturanus mirandaribeiroi* Nelson & Lescure, 1975 (Fouquet et al. 2021a), *Amazophrynella manaos* Rojas, Carvalho, Ávila, Farias & Hrbek, 2014, and *Anomaloglossus stepheni* (Martins, 1989) (Vacher et al. 2017). In Brazil, it is known from Manaus and Presidente Figueiredo (Amazonas), São João da Baliza (Roraima) and Oriximiná (Pará); in Guyana from the Mabura Hill Reserve, and in Suriname from Sipaliwini.

Pristimantis guianensis sp. nov. is crepuscular/nocturnal and inhabits the understory of unflooded (*terra firme*) forests. Its breeding activity takes place in the rainy season, between November and February in the state of Amazonas, and between May and August in the state of Roraima, both in Brazil. Calling males of *P. guianensis* sp. nov. aggregate in groups of about eight, separated by 2–3 m distance. At Reserva Ducke (Manaus, Brazil), understorey areas rich in Marantaceae are preferentially used (Fig. 15A). In other localities (e.g., São João da Baliza, Brazil), the species is found mainly on the edge of forest fragments. The new species occurs in sympatry with congeneric *P. koehleri* Padial & De la Riva, 2009 and *P. zimmermanae* (Heyer & Hardy, 1991) in Manaus and Presidente Figueiredo, *P. zeuctotylus* (Lynch & Hoogmoed, 1977) in São João da Baliza, *P. chiastonotus* (Lynch & Hoogmoed, 1977), *P. zeuctotylus* and *P. inguinalis* in Sipaliwini (Fouquet et al. 2015).

Males call perched on vegetation (Fig. 15B) about 2 m above the ground. They start calling at dusk (~18:00h) and remain active until ~19:00h, their activity then remains sporadic until around midnight. When the weather is rainy, the activity is sustained throughout the night. The amplexus ($n = 2$) is axillary (Fig. 15C). Juveniles and

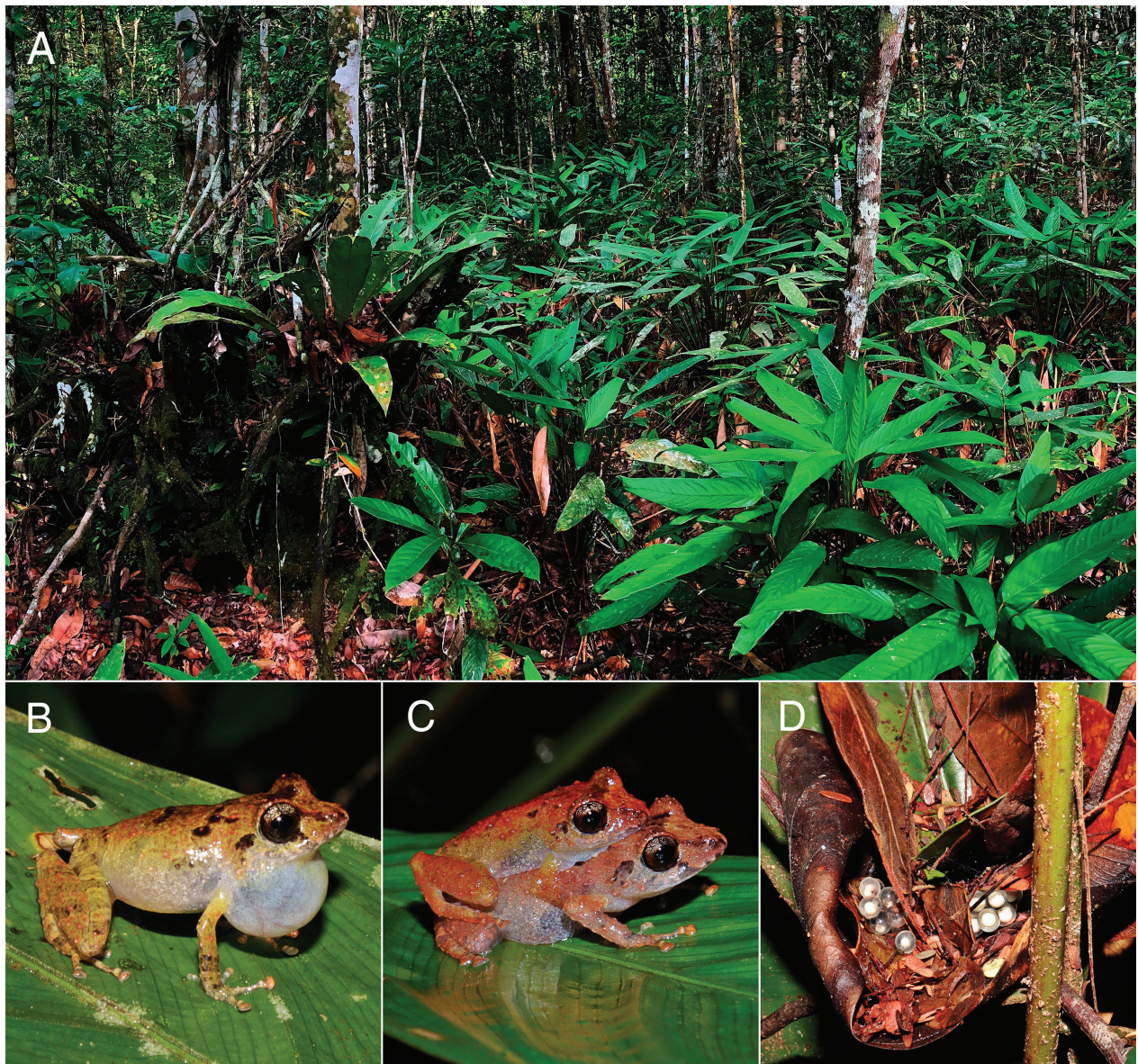


Figure 15. *Pristimantis guianensis* sp. nov. natural history and breeding aspects. **A** Example of habitat used by the new species; **B** calling male (INPA-H 43942); **C** amplexant pair (female INPA-H 43943, male INPA-H 43944), from RFAD, Manaus, Amazonas; **D** suspended litter spawning about 1 m above the ground, the two clutches are at different stages (left, more developed; right, recent oviposition), from São João da Baliza municipality, Roraima, Brazil. Photographs: A.T. Mônico, except (B) I.Y. Fernandes.

females are rarely found. We registered three clutches *in situ* in São João da Baliza (Roraima, Brazil), all within dead leaves hung in the vegetation above the ground. Two clutches were laid in the same nest (Fig. 14D) about 1 m above the ground, one was recent and the other in a more advanced developmental stage; the third clutch (about 40 cm above the ground) contained dead eggs, apparently due to fungal infestation. The first two clutches contained 11 and 13 large eggs. In the surroundings of the nests (up to 5 m), we observed adult males ($n = 6$ individuals) and females ($n = 3$).

On 29 December 2020 we found an amplexing pair (Fig. 14C) from Reserva Florestal Adolpho Ducke (Tinga basin), Manaus municipality. Male (INPA-H 43944) and female (INPA-H 43943) remained amplexed overnight in the same collection container. Oviposition of 13 eggs took place at ~9:30 on the next day. In laboratory, we

observed egg development until hatching (Fig. 16) in a sterile and moist container (at room temperature, between 24.8 and 28.3°C). Egg diameter increased from ~4.1 mm at oviposition to 5.8 mm before froglet hatching. The first froglet has hatched on the 25th day, and it lasted seven days (33rd day) to all the remnant froglets hatch. The SVL of froglets at hatching was ~4.3 mm.

As previously mentioned, *Pristimantis guianensis* sp. nov. has a wide geographic distribution, from Manaus, Brazil in the South to Mabura Hill Reserve, Guyana in the North (i.e., 800 km linear distance), and potentially 256 thousand km². The species inhabits primary and secondary forests, as well as edge areas. Furthermore, their populations are locally abundant. Therefore, we believe that the species fits into the category “Least Concern” of the International Union for Conservation of Nature (IUCN).

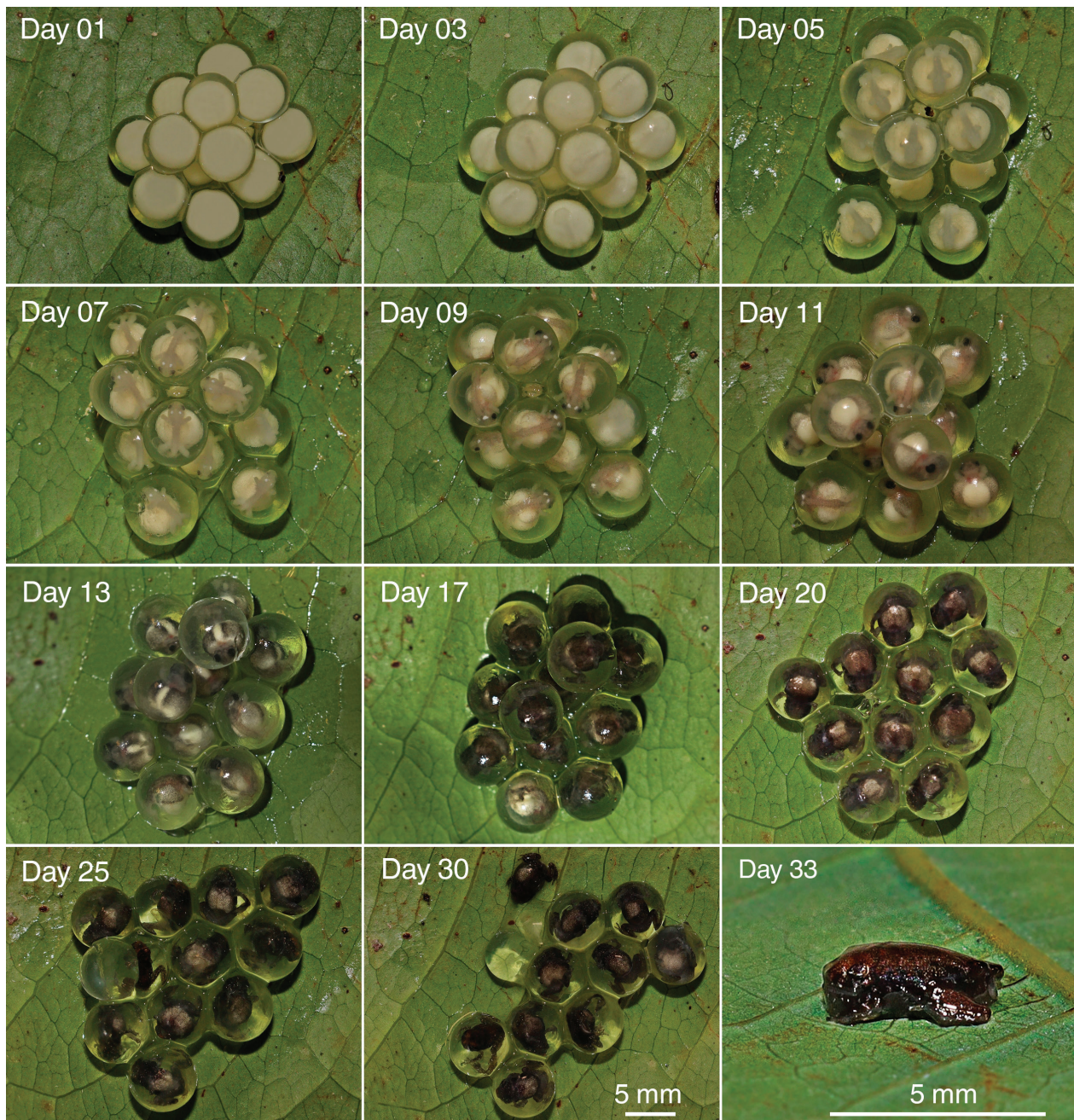


Figure 16. *Pristimantis guianensis* sp. nov. eggs and embryos from Reserva Florestal Adolpho Ducke (Manaus, Brazil). Photographs: A.T. Mônico.

Discussion

The integration of molecular, acoustic and morphological data can help decipher the large proportion of still hidden amazonian biodiversity (e.g., Páez and Ron 2019; Fouquet et al. 2021b), and lead to the discovery and description of new taxa (e.g., Lima et al. 2020; Carvalho et al. 2021; Fouquet et al. 2021a; Ferrão et al. 2022). However, taxonomic acts are not trivial since many Amazonian species have been historically misidentified, often lumped within putatively widespread taxa (e.g., *Amazophrynella minuta* Rojas-Zamora et al. 2014; *Dendropsophus brevifrons* Fouquet et al. 2015; *Boana geographica* Fouquet

et al. 2016). Therefore, tackling these taxonomic issues also requires examining types, and topotypical material and often redescribing or redefining these supposedly widespread species (e.g., *Pristimantis marmoratus* Kok et al. 2018; *Anomaloglossus baeobatrachus* Fouquet et al. 2019; *Pristimantis grandoculis* Fouquet et al. 2022b).

The case of *Pristimantis guianensis* exemplifies these taxonomic challenges. Populations of *P. guianensis* were misidentified as *P. ockendeni* (in Brazil) or *P. marmoratus* (in Guyana) for over 30 years (Zimmerman and Rodrigues 1990; Heyer and Hardy 1991), although Fouquet et al. (2013) previously questioned the identity of individuals from Manaus. The existence of many morphologically superficially similar species of the *P. unistrigatus*

species group remaining undescribed in Amazonia, associated with the difficulties to clarify the phylogenetic positions and the acoustic characteristics of *P. ockendeni* (as well as of *P. marmoratus*, recently resolved by Kok et al. 2018, and *P. grandoculis*, recently resolved by Fouquet et al. 2022b), represented challenges that needed to be overcome before being able to describe *P. guianensis*. We demonstrate that the new species is easily diagnosable based on characters that are widely used for *Pristimantis*.

The distribution of *Pristimantis guianensis* **sp. nov.** is restricted to the southwestern portion of the Guiana region sensu Vacher et al. (2020), where the range boundaries of many species overlap (e.g., *Allobates sumtuosus*, *Anomaloglossus stepheni* and *Amazophrynella manaos*), geographically bounded southward by the Amazonas River and the Negro River and westward by the Rupununi savanna and the Branco River. We do not expect that *P. guianensis* to occur on the opposite margins of these rivers where other related species occur, precisely as our ongoing investigations have detected morphological, acoustic and genetic differences between them. We do not expect *P. guianensis* to reach the Branco River, because it lacks suitable habitat in the open environments of savannas/lavrados in the Guyana and Roraima state, Brazil. Northwestward, the new species is bounded by the Pantepui region in Guyana. However, the landscape features that bound the distribution of *P. guianensis* and of this bioregion eastward and northward in Para and Suriname are much less clear.

Fouquet et al. (2022b) suggested that *Pristimantis guianensis* (treated as “*P. cf. ockendeni*”) might belong to a lineage apart from the other species of the Guiana Shield that evolved from western Amazonian ancestors and posteriorly dispersed to the Guiana Shield across the Amazonas River. Other species hidden under the name “*Pristimantis ockendeni*” likely occur in the Brazilian Amazon, since at least five lineages forming a “trans-Amazon complex”, proposed by Vacher et al. (2020) and Fouquet et al. (2022b). The taxonomic status of these candidate species and their historical relationships deserve to be clarified.

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Appendix 1

Referred material

Pristimantis ockendeni

BMNH 1947.2.16.88, Syntype, Female, PERU: Departamento Puno: Carabaya: Rio Huacamayo: La Union [13°31'58.3"S; 69°45'06.7"W], 07-May-1907.

BMNH 1947.2.16.89, Syntype, Female, PERU: Departamento Puno: Carabaya: Rio Huacamayo: La Union [13°31'58.3"S; 69°45'06.7"W], 07-May-1907.

BMNH 1947.2.16.90, Syntype, Male, PERU: Departamento Puno: Carabaya: Rio Huacamayo: La Union [13°31'58.3"S; 69°45'06.7"W], 07-May-1907.

INPA-H 43945, Male, BRAZIL: Acre: Manoel Urbano: Floresta Estadual do Afluente [8°42'16.6"S; 69°32'02.6"W], 23-Nov-2020 by A.T. Mônico, I.Y. Fernandes, U.F. Souza and C.H.O. Nogueira.

INPA-H 43946, Male, BRAZIL: Acre: Manoel Urbano: Floresta Estadual do Afluente [8°42'16.6"S; 69°32'02.6"W], 23-Nov-2020 by A.T. Mônico, I.Y. Fernandes, U.F. Souza and C.H.O. Nogueira.

INPA-H 43947, Male, BRAZIL: Acre: Manoel Urbano: Floresta Estadual do Afluente [8°42'16.6"S; 69°32'02.6"W], 24-Nov-2020 by A.T. Mônico, I.Y. Fernandes, U.F. Souza and C.H.O. Nogueira.

INPA-H 43948, Male, BRAZIL: Acre: Manoel Urbano: Floresta Estadual do Afluente [8°42'16.6"S; 69°32'02.6"W], 24-Nov-2020 by A.T. Mônico, I.Y. Fernandes, U.F. Souza and C.H.O. Nogueira.

INPA-H 43949, Male, BRAZIL: Acre: Feijó [8°38'46.6"S; 69°43'29.8"W], 25-Nov-2020 by A.T. Mônico, I.Y. Fernandes, U.F. Souza and C.H.O. Nogueira.

INPA-H 43950, Male, BRAZIL: Acre: Manoel Urbano: Floresta Estadual do Afluente [8°42'16.6"S; 69°32'02.6"W], 26-Nov-2020 by A.T. Mônico, I.Y. Fernandes, U.F. Souza and C.H.O. Nogueira.

INPA-H 43951, Male, BRAZIL: Acre: Manoel Urbano: Floresta Estadual do Afluente [8°42'16.6"S; 69°32'02.6"W], 26-Nov-2020 by A.T. Mônico, I.Y. Fernandes, U.F. Souza and C.H.O. Nogueira.

INPA-H 43952, Male, BRAZIL: Acre: Feijó [8°38'46.6"S; 69°43'29.8"W], 29-Nov-2020 by A.T. Mônico, I.Y. Fernandes, U.F. Souza and C.H.O. Nogueira.

Pristimantis ockendeni specimens used exclusively in molecular analyses

MUBI 10538, Unknown sex, PERU: Departamento Madre de Dios: Manu Province: Reserva Comunal Amarakaeri [12°59'25.7"S; 71°00'37.6"W], 25-May-2011 by J.A.D.C. and S.R.M.T.

MUBI13049, Juvenile, PERU: Departamento Cusco: Paucartambo Province: Kosñipata [12°53'10.7"S; 71°26'41.8"W], 28-Aug-2013 by Frank P.C. Ccarhuarupay.

MUBI14568, Juvenile, PERU: Departamento Madre de Dios: Manu Province: Reserva Comunal Amarakaeri [12°46'37.9"S; 70°56'57.8"W], 06-Feb-2015 by J.C. Chaparro, C. Alarcon and G. Huaro.

Pristimantis sp. specimen used exclusively in molecular analyses

MUBI17035, Unknown sex, PERU: La Convencion Province: Cusco: Echarate District: Estacion Ticumpinia [11°59'37.8"S; 72°59'08.0"W], 09-Sep-2018 by J.C. Chaparro and F.P. Condori.

Pristimantis guianensis sp. nov. specimens used exclusively in molecular analyses

MPEG 33750, Male, BRAZIL: Pará: Oriximiná [1°46'07.9"S; 56°12'45.5"W], 27-Sep-2009.

JJLR 007, Male, BRAZIL: Amazonas: Presidente Figueiredo: Reserva Biológica do Uatumã [1°32'19.5"S; 59°32'19.3"W], 28-Nov-2015 by J.J. López-Rojas and A. Ferreira.

JJLR 010, Male, BRAZIL: Amazonas: Presidente Figueiredo: Reserva Biológica do Uatumã [1°32'19.5"S; 59°32'19.3"W], 28-Nov-2015 by J.J. López-Rojas and A. Ferreira.

JJLR 016, Male, BRAZIL: Amazonas: Presidente Figueiredo: Reserva Biológica do Uatumã [1°32'19.5"S; 59°32'19.3"W], 30-Nov-2015 by J.J. López-Rojas and A. Ferreira.

AF2257, Male, SURINAME: Sipaliwini: Savanna Nature Preserve [2°01'27.8"N; 56°07'30.4"W], 27-Apr-2014 by A. Fouquet and J.P. Vacher.

Appendix 2

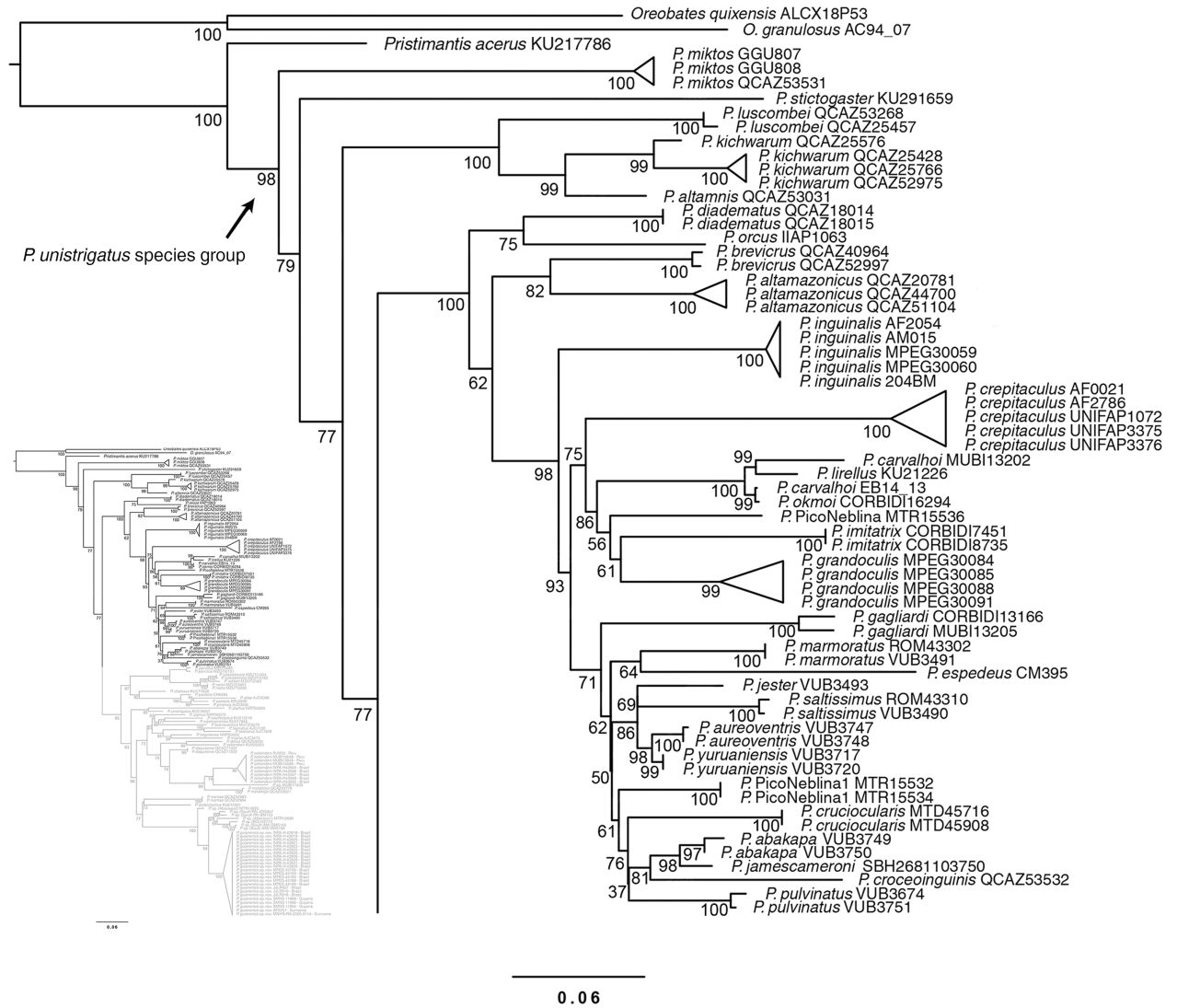
Species of *Pristimantis* and *Oreobates* used in phylogenetic analyses, with respective voucher, Genbank accession number and references.

Species	Voucher	Genbank accession numbers			Reference
		16S	COI	RAG1	
<i>P. abakapa</i>	VUB3749	JQ742162			Kok et al. (2012)
<i>P. abakapa</i>	VUB3750	JQ742163			Kok et al. (2012)
<i>P. acerus</i>	KU217786	EF493678			Heinicke et al. (2007)
<i>P. altae</i>	AJC0398		JN991361	JQ025174	Pinto-Sanchez et al. (2012)
<i>P. ardalonychus</i>	KU212301	EU186664			Hedges et al. (2008)
<i>P. altamazonicus</i>	QCAZ44700	MF118685	MF118717	MF118735	Ortega-Andrade et al. (2017)
<i>P. altamazonicus</i>	QCAZ20781	MF118699	MF118707	MF118734	Ortega-Andrade et al. (2017)
<i>P. altamazonicus</i>	QCAZ51104	MF118678	MF118720	MF118738	Ortega-Andrade et al. (2017)
<i>P. altamnis</i>	QCAZ53031	KP064155	KP064164		Ortega-Andrade and Venegas (2014)
<i>P. aureoventris</i>	VUB3747	JQ742154			Kok et al. (2012)
<i>P. aureoventris</i>	VUB3748	JQ742152			Kok et al. (2012)
<i>P. bogotensis</i>	NRPS0033	JN991432	JN991362		Pinto-Sanchez et al. (2012)
<i>P. brevicrus</i>	QCAZ52997	MF118697	MF118726	MF118744	Ortega-Andrade et al. (2017)
<i>P. brevicrus</i>	QCAZ40964	MF118700	MF118715	MF118751	Ortega-Andrade et al. (2017)
<i>P. buenaventura</i>	MZUTI3270	KU999169			Arteaga et al. (2016)
<i>P. cajamarcensis</i>	KU217845	EF493663			Heinicke et al. (2007)
<i>P. carvalhoi</i>	MUBI13202	OL989869			Ortega-Andrade et al. (2021)
<i>P. carvalhoi</i>	EB14.13	MG820152	MG820177		Catenazzi and Lehr (2018)
<i>P. ceuthospilus</i>	KU212216	EF493520			Heinicke et al. (2007)
<i>P. chalceus</i>	KU177638	EF493675			Heinicke et al. (2007)
<i>P. crepitaculus</i>	AF2786	KDQF01001103			Fouquet et al. (2022)
<i>P. crepitaculus</i>	21AF	JN691315			Fouquet et al. (2022)
<i>P. crepitaculus</i>	UNIFAP 1072	ON908889	ON954784	ON963983	This study
<i>P. crepitaculus</i>	UNIFAP 3375	ON908890	ON954785	ON963984	This study
<i>P. crepitaculus</i>	UNIFAP 3376	ON908891	ON954786	ON963985	This study.
<i>P. croceoiinguinis</i>	QCAZ53532	KP064144	KP064157		Ortega-Andrade and Venegas (2014)
<i>P. cruciocularis</i>	MTD45716	MG820161	MG820186		Catenazzi and Lehr (2018)
<i>P. cruciocularis</i>	MTD45908	MG820162	MG820187		Catenazzi and Lehr (2018)
<i>P. daquilemai</i>	QCAZ71332	MZ430056			Zapata-Brito et al. (2021)
<i>P. daquilemai</i>	QCAZ71333	MZ430057			Zapata-Brito et al. (2021)
<i>P. delius</i>	QCAZ53035	KP064150	KP064162	MF118753	Ortega-Andrade and Venegas (2014)
<i>P. diadematus</i>	QCAZ18014	MH516177			Waddel et al. (2018)
<i>P. diadematus</i>	QCAZ18015	MH516178			Waddel et al. (2018)
<i>P. espedeus</i>	CM395	JN691314			Fouquet et al. (2013)
<i>P. gliardi</i>	CORBIDI13166	OL989857	OL960444		Ortega-Andrade et al. (2021)
<i>P. gliardi</i>	MUBI13205	OL989868	OL960435		Ortega-Andrade et al. (2021)
<i>P. grandoculis</i>	MPEG 30085	KDQF01002880			Fouquet et al. (2022)
<i>P. grandoculis</i>	MPEG 30088	KDQF01002881			Fouquet et al. (2022)
<i>P. grandoculis</i>	MPEG 30084	ON908894	ON964531	ON963988	This study
<i>P. grandoculis</i>	MPEG 30091	ON908895	ON964532	ON963989	This study
<i>P. guianensis sp. nov.</i>	INPA-H 43918	ON897772	ON898573	ON920937	This study
<i>P. guianensis sp. nov.</i>	INPA-H 43919	ON897773	ON898574	ON920938	This study
<i>P. guianensis sp. nov.</i>	INPA-H 43920	ON897774	ON898575	ON920939	This study
<i>P. guianensis sp. nov.</i>	INPA-H 43921	ON897775	ON898576	ON920940	This study
<i>P. guianensis sp. nov.</i>	INPA-H 43923	ON897776	ON898577	ON920941	This study
<i>P. guianensis sp. nov.</i>	INPA-H 43924	ON897777	ON898578	ON920942	This study
<i>P. guianensis sp. nov.</i>	INPA-H 43925	ON897778	ON898579	ON920943	This study
<i>P. guianensis sp. nov.</i>	INPA-H 43943	ON897779	ON898580	ON920944	This study
<i>P. guianensis sp. nov.</i>	MPEG 44183	ON897780	ON898581	ON920945	This study
<i>P. guianensis sp. nov.</i>	MPEG 44184	ON897781	ON898582	ON920946	This study
<i>P. guianensis sp. nov.</i>	MPEG 44188	ON897782	ON898583	ON920947	This study

Species	Voucher	Genbank accession numbers			Reference
		16S	COI	RAG1	
<i>P. guianensis</i> sp. nov.	MPEG 44190	ON897783	ON898584	ON920948	This study
<i>P. guianensis</i> sp. nov.	INPA-H 43926	ON897784	ON898585	ON920949	This study
<i>P. guianensis</i> sp. nov.	INPA-H 43929	ON897785	ON898586	ON920950	This study
<i>P. guianensis</i> sp. nov.	INPA-H 43930	ON897786	ON898587	ON920951	This study
<i>P. guianensis</i> sp. nov.	INPA-H 43931	ON897787	ON898588	ON920952	This study
<i>P. guianensis</i> sp. nov.	INPA-H 43935	ON897788	ON898589	ON920953	This study
<i>P. guianensis</i> sp. nov.	MPEG 33750	ON897789	ON898590		This study
<i>P. guianensis</i> sp. nov.	JJRL 0007	ON897790	ON898591		This study
<i>P. guianensis</i> sp. nov.	JJRL 0010	ON897791	ON898592		This study
<i>P. guianensis</i> sp. nov.	JJRL 0016	ON897792	ON898593		This study
<i>P. guianensis</i> sp. nov.	MNHN-RA-2020.0114	KDQF01000865			Fouquet et al. (2022)
<i>P. guianensis</i> sp. nov.	AF2257	KDQF01000889			Fouquet et al. (2022)
<i>P. guianensis</i> sp. nov.	SMNS 11989	KDQF01004221			Fouquet et al. (2022)
<i>P. guianensis</i> sp. nov.	SMNS 11990	KDQF01004222			Fouquet et al. (2022)
<i>P. guianensis</i> sp. nov.	SMNS 11994	KDQF01004223			Fouquet et al. (2022)
<i>P. imitatrix</i>	CORBIDI7451	OL989854			Ortega-Andrade et al. (2021)
<i>P. imitatrix</i>	CORBIDI8735	OL989855			Ortega-Andrade et al. (2021)
<i>P. inguinalis</i>	204BM	JN691317			Fouquet et al. (2012)
<i>P. inguinalis</i>	AM015	KDQF01001691			Vacher et al. (2020)
<i>P. inguinalis</i>	AF2054	KDQF01000814			Vacher et al. (2020)
<i>P. inguinalis</i>	MPEG 30059	ON908892	ON964824	ON963986	This study
<i>P. inguinalis</i>	MPEG 30060	ON908893	ON964825	ON963987	This study
<i>P. jamescameroni</i>	SBH268110	EU186721			Hedges et al. (2008)
<i>P. jester</i>	VUB3493	JQ742169			Kok et al. (2012)
<i>P. kichwarum</i>	QCAZ52975	KP064154			Ortega-Andrade and Venegas (2014)
<i>P. lirellus</i>	KU212226	EF493521			Heinicke et al. (2007)
<i>P. luscombei</i>	QCAZ53268	KP064156			Ortega-Andrade and Venegas (2014)
<i>P. luscombei</i>	QCAZ25457	EU130618		MH481368	Elmer et al. (2007a)
<i>P. luteolateralis</i>	MZUTI3182	KU999208			Arteaga et al. (2016)
<i>P. luteolateralis</i>	MZUTI1404	KU999205			Arteaga et al. (2016)
<i>P. marmoratus</i>	ROM43302	EU186716			Kok et al. (2018)
<i>P. marmoratus</i>	VUB3491	JQ742167			Kok et al. (2018)
<i>P. martiae</i>	QCAZ52983	KP064148	KP064160	MF118754	Ortega-Andrade and Venegas (2014)
<i>P. martiae</i>	QCAZ52984	KP064149	KP064161	MF118755	Ortega-Andrade and Venegas (2014)
<i>P. matidiktyo</i>	QCAZ 53021	KP064147	KP064159		Ortega-Andrade and Venegas (2014)
<i>P. matidiktyo</i>	QCAZ 52779	KP064146	KP064158		Ortega-Andrade and Venegas (2014)
<i>P. miktos</i>	QCAZ 53531	KP064153			Ortega-Andrade and Venegas (2014)
<i>P. miktos</i>	GGU807	KP064151			Ortega-Andrade and Venegas (2014)
<i>P. miktos</i>	GGU808	KP064152	KP064163		Ortega-Andrade and Venegas (2014)
<i>P. miyatai</i>	AJC3475	KP149490	KP149276		Guarnizo et al. (2015)
<i>P. nietoi</i>	MZUTI3050	KU999214			Arteaga et al. (2016)
<i>P. nietoi</i>	MZUTI3001	KU999212			Arteaga et al. (2016)
<i>P. ockendeni</i>	KU222023	EF493519		EF493434	Heinicke et al. (2007)
<i>P. ockendeni</i> SS	RvM5 12	KY652654	KY672986	KY672970	von May et al. (2017)
<i>P. ockendeni</i> SS	INPA-H 43945	ON897793	ON898594	ON920954	This study
<i>P. ockendeni</i> SS	INPA-H 43946	ON897794	ON898595	ON920955	This study
<i>P. ockendeni</i> SS	INPA-H 43947	ON897795	ON898596	ON920956	This study
<i>P. ockendeni</i> SS	INPA-H 43949	ON897796	ON898597	ON920957	This study
<i>P. ockendeni</i> SS	INPA-H 43952	ON897797	ON898598	ON920958	This study
<i>P. ockendeni</i> SS	MUBI 10538	ON907779			This study
<i>P. ockendeni</i> SS	MUBI 13049	ON907778			This study
<i>P. ockendeni</i> SS	MUBI 14568	ON907780			This study
<i>P. orcus</i>	IIAP1063	OL989865	OL960437		Ortega-Andrade et al. (2021)
<i>P. okmoi</i>	CORBIDI16294	KY652651	KY672983	KY672967	Ortega-Andrade et al. (2021)
<i>P. pardalis</i>	KRL0690	FJ784336	FJ766804	JQ025198	Crawford et al. (2010)
<i>P. pardalis</i>	CH6284	JN991460	JN991390		Pinto-Sanchez et al. (2012)
<i>P. parvillus</i>	MZUTI483	KU999215			Arteaga et al. (2016)

Species	Voucher	Genbank accession numbers			Reference
		16S	COI	RAG1	
<i>P. parvillus</i>	MZUTI2121	KU999216			Arteaga et al. (2016)
<i>P. pirrensis</i>	AJC0594	JN991462	JN991393	JQ025199	Pinto-Sanchez et al. (2012)
<i>P. pulvinatus</i>	VUB3751	JQ742164			Kok et al. (2018)
<i>P. pulvinatus</i>	VUB3674	JQ742165			Kok et al. (2018)
<i>P. saltissimus</i>	VUB3490	JQ742168			Kok et al. (2012)
<i>P. saltissimus</i>	ROM43310	EU186693			Hedges et al. (2008)
<i>P. sp. PicoNeblina1</i>	MTR15532	KDQF01003322			Vacher et al. (2020)
<i>P. sp. PicoNeblina1</i>	MTR15534	KDQF01003323			Vacher et al. (2020)
<i>P. sp. PicoNeblina2</i>	MTR15536	KDQF01003323			Vacher et al. (2020)
<i>P. sp.</i>	MUBI 17035	ON907781			This study
<i>P. sp.</i>	MTR 12855	KDQF01003224			Fouquet et al. (2022)
<i>P. sp.</i>	JOG 847	KDQF01002705			Fouquet et al. (2022)
<i>P. sp.</i>	BM 153	KDQF01001829			Fouquet et al. (2022)
<i>P. sp.</i>	MTR 12690	KDQF01003210			Fouquet et al. (2022)
<i>P. sp.</i>	H2773	KDQF01002605			Fouquet et al. (2022)
<i>P. sp.</i>	SMS155	KDQF01004256			Fouquet et al. (2022)
<i>P. sp.</i>	SMS156	KDQF01004257			Fouquet et al. (2022)
<i>P. taeniatus</i>	AJC1839	JN991470	JN991403	JQ025208	Pinto-Sanchez et al. (2012)
<i>P. taeniatus</i>	AJC1126	JN991472	JN991406	JQ025206	Pinto-Sanchez et al. (2012)
<i>P. stictogaster</i>	KU291659	EF493704		EF493445	Heinicke et al. (2007)
<i>P. unistrigatus</i>	KU218057	EF493387		EF493444	Heinicke et al. (2007)
<i>P. walkeri</i>	MZUTI3183	KU999230			Arteaga et al. (2016)
<i>P. yuruaniensis</i>	VUB3717	JQ742160			Kok et al. (2012)
<i>P. yuruaniensis</i>	VUB3720	JQ742161			Kok et al. (2012)
<i>P. zophus</i>	NRPS0060	JN991479	JN991414	JQ025213	Pinto-Sanchez et al. (2012)
<i>P. zophus</i>	NRPS0072	JN991478	JN991413	JQ025214	Pinto-Sanchez et al. (2012)
<i>O. quixensis</i>	ALCX186P53	KU495404	KU494611		Lyra et al. (2016)
<i>O. granulatus</i>	AC94_07	KY652649	KY672982	KY672965	von May et al. (2017)

Appendix 3



Phylogenetic reconstruction showing the relationships of *Pristimantis guianensis* **sp. nov.** and *P. ockendeni*. Maximum likelihood tree based on genes 16S, COI and RAG1. Non-parametric bootstrap support is shown near branches. Species names are preceded by the specimen voucher number.

Appendix 4

Call recordings list by species and localities: voucher (call deposit number).

Pristimantis guianensis sp. nov.: BRAZIL, Amazonas, Manaus, Reserva Florestal Adolpho Ducke: INPA-H 43918 (FNJV 58730), INPA-H 43919 (FNJV 58731), INPA-H 43920 (FNJV 58732), INPA-H 43921 (FNJV 58733), INPA-H 43923 (FNJV 58734), INPA-H 43924 (FNJV 58735), INPA-H 43925 (FNJV 58736), APL 22797 (FNJV 58737), APL 22798 (FNJV 58738), APL 22799 (FNJV 58739), INPA-H 43942 (FNJV 58761) and INPA-H 43944 (FNJV 58762). — BRAZIL, Amazonas, Presidente Figueiredo, Km 144 of the BR-174 Highway: MPEG 44183 (FNJV 58740), MPEG 44184 (FNJV 58741), INPA-H 43926 (FNJV 58742), APL 22809 (FNJV 58743), INPA-H 43927 (FNJV 58744) and INPA-H 43928 (FNJV 58745). — BRAZIL, Amazonas, Presidente Figueiredo, Balbina: INPA-H 43929 (FNJV 58746-48), INPA-H 43930 (FNJV 58749), INPA-H 43931 (FNJV 58750), INPA-H 43932 (FNJV 58751), INPA-H 43933 (FNJV 58752), MPEG 44185

(FNJV 58753) and MPEG 44186 (FNJV 58754). — BRAZIL, Roraima, São João da Baliza: MPEG 44188 (FNJV 58755), MPEG 44190 (FNJV 58756), INPA-H 43937 (FNJV 58757-58), INPA-H 43938 (FNJV 58759) and INPA-H 43940 (FNJV 58760). — GUYANA, Mabura Hill Forest Reserve: SMNS 11988 (MNHN-SO-2022-784). — SURINAME, Sipaliwini: MNHN-RA-2020.0115 (MNHN-SO-2022-785) and MNHN-RA-2020.0117 (MNHN-SO-2022-786).

Pristimantis ockendeni: BRAZIL, Acre, Manoel Urbano: INPA-H 43945 (FNJV 58763), INPA-H 43946 (FNJV 58764), INPA-H 43947 (FNJV 58765), INPA-H 43948 (FNJV 58766) and INPA-H 43950 (FNJV 58768). — BRAZIL, Acre, Feijó: INPA-H 43949 (FNJV 58767) and INPA-H 43952 (FNJV 58769).

Appendix 5

Acoustic variables of *Pristimantis guianensis* sp. nov. and *Pristimantis ockendeni* summarized according to call arrangement. Abbreviation: SP, species; CD, call duration; ND, note duration (ms); NN, number of notes per call, INI, inter-note interval (ms); LF, HF and DF, minimum, maximum, and dominant frequencies (Hz), respectively.

SP	NN		CD	ND	INI	LF	HF	DF
<i>P. guianensis</i> sp. nov.	4 (n = 57)	mean	204	18	44	3,195	5,492	3,918
		SD	22	10	7	163	368	171
		min	158	5	14	2,827	4,334	3,467
		max	261	79	54	3,696	6,481	4,436
	5 (n = 42)	mean	246	17	43	3,261	5,197	4,025
		SD	21	14	5	132	287	171
		min	188	6	32	2,997	4,414	3,811
		max	284	89	56	3,558	6,688	4,522
	6 (n = 12)	mean	313	14	46	3,098	5,062	4,030
		SD	34	6	3	79	618	184
		min	265	5	39	2,965	4,521	3,709
		max	371	23	51	3,204	6,428	4,414
<i>P. ockendeni</i>	4 (n = 1)		334	43	54	2,610	3,504	3,079
	5 (n = 10)	mean	385	31	58	2,453	3,383	2,933
		SD	31	6	5	25	14	22
		min	365	24	50	2,407	3,355	2,885
		max	467	41	65	2,493	3,398	2,950
	6 (n = 15)	mean	506	36	65	2,461	3,405	2,943
		SD	37	25	8	165	178	176
		min	457	24	52	2,080	2,965	2,519
		max	576	125	74	2,609	3,530	3,079
	7 (n = 13)	mean	700	56	63	2,265	3,278	2,730
		SD	121	44	17	215	267	229
		min	544	32	38	2,047	2,984	2,519
		max	940	158	87	2,610	3,642	3,144
8 (n = 1)		74	40	61	2,072	2,985	2,519	