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Two new cryptic *Pristimantis* (Anura, Craugastoridae) from the southern Amazon basin of Peru with taxonomic comments on *Pristimantis imitatrix* (Duellman, 1978)

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Abstract. Fieldwork in premontane and lowland Amazonian forest (385–1,075 m above sea level) in and around the Amarakaeri Communal Reserve in southeastern Peru led us to describe two new species of the genus *Pristimantis.* We used integrative evidence from several external morphological characters, morphometric and molecular data, geographic distributions, and multivariate analyses to support the identification of these new species. *Pristimantis gagliardi* **sp. nov.** is characterized by lacking a visible tympanic annulus and membrane on skin, having basal webbing between toes, yellow blotches in groin and hidden surfaces of shanks, W-shaped scapular mark, snout subacuminate bearing a very small papilla at tip of snout, and snout–vent length (SVL) = 17.7–21.3 mm in adult males and SVL = 26.7–29.2 mm in females. *Pristimantis okmoi* **sp. nov.** is characterized by having a visible tympanic annulus and membrane on skin, basal webbing between toes, yellow blotches in groin and hidden surfaces of shanks, W-shaped scapular mark, snout rounded with small rostral papilla, and SVL = 13.63–17.04 mm in adult males; females unknown. Both species were initially misidentified as *P. carvalhoi* or *P. imitatrix.* Additionally, we provide new morphological and phylogenetic data on adult specimens of *Pristimantis imitatrix,* a rare Amazonian species described based on a single subadult female.

Keywords. Amarakaeri Communal Reserve; Amphibia; Cryptic diversity; Systematics; Taxonomy.

INTRODUCTION

Amphibian collections made in the past decade in the Amazon basin have resulted in the discovery of new *Pristimantis* Jiménez de la Espada, 1870 (Terrarana: Craugastoridae) species and vast material for further taxonomic analysis (e.g., Lehr et al., 2017; Brito and Almendáriz, 2018; Heinicke et al., 2018; Sánchez-Nivicela et al., 2018; Székely et al., 2018; Waddell et al., 2018; Valencia et al., 2019). Frogs of the genus *Pristimantis* correspond to one of the most diverse and largely understudied groups in the Neotropics (Hedges et al., 2008; Padial et al., 2008; Padial and De la Riva, 2009), with at least 553 nominally described species (Frost, 2020). *Pristimantis,* and all other members of Terrarana, are generally assumed to be direct developing, lacking an aquatic tadpole stage (Hedges et al., 2008).

Similar to other taxonomic groups with diverse radiations in the tropics, these frogs have cryptic behavior, making them difficult to detect, and also display few distinctive morphological differences and high levels of intraspecific polymorphism (Padial et al., 2009; Funk et al., 2011; Guayasamin et al., 2015). The Amazonian lowlands (< 800 m above sea level [a.s.l.]) correspond to a region with high *Pristimantis* diversity, with at least 61 nominal species, comprising over 12% of known species in the genus (Frost, 2020). However, a wide underestimation of their true diversity is suspected (Duellman and Lehr, 2009; Padial and De la Riva, 2009; Ortega-Andrade et al., 2015).

The eastern slopes of the Andes and the tropical forests in southern Peru are recognized as particularly rich in *Pristimantis* species (Duellman and Salas, 1991; Duellman, 2005; Padial et al., 2009), with several waiting to be taxonomically assessed or described (Duellman and Lehr, 2009). An example is the uncommon *Pristimantis imitatrix* (Duellman, 1978), a small cryptic frog known from the type locality (subadult holotype from Panguana, Department Huánuco, Peru) and Cusco Amazónico (Departamento Madre de Dios; Parmelee, 1999; Duellman, 2005). Herein, we describe two new cryptic species from tropical forest in Madre de Dios and Cusco Departments in southeastern Peru, initially misidentified as *Pristimantis*

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carvalhoi (Lutz and Kloss, 1952), and comment about the taxonomic status of the *Pristimantis imitatrix* complex and its relatives in the region.

MATERIALS AND METHODS

Ethics statement

Voucher specimens and tissue samples were obtained following ethical and technical protocols (McDiarmid, 1994). Specimens were transported in plastic bags and euthanized no more than the next day after their capture. Specimens were euthanized with benzocaine or lidocaine hydrochloride 2%, fixed in 10% buffered formalin, and then later transferred to 70% ethanol. Liver and thigh muscle were preserved in 95% ethanol for DNA extraction. Voucher specimens and tissue samples from Peru were deposited at Museo de Biodiversidad del Perú (MUBI) and Centro de Ornitología y Biodiversidad (CORBIDI), whereas specimens from Ecuador were deposited at Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ).

Systematics and species delimitation

Generic names, character definitions, and terminology follow Duellman and Lehr (2009). To assess species delimitation of new species, we followed the integrative protocol proposed by Ortega-Andrade et al. (2015), based on: 1) 1st reduction of taxon sampling by reviewing type series, 2) DNA phylogenetics, 3) 2nd reduction of taxon sampling by qualitative morphological data, and 4) comparative analyses of different lines of evidence. Notes on color, ecology, latitude/longitude/elevation (using GPS Garmin® Montana 650/eTrex 30, set with WGS84 datum), dates, and color photographs of voucher specimens were taken in the field. Voucher specimens were studied at the following Institutions (Tables S1): Museo de Historia Natural de la Universidad Nacional de San Marcos, Lima (MUSM, Peru); Instituto de Investigaciones de la Amazonía Peruana, Iquitos (IIAP, Peru); Centro de Ornitología y Biodiversidad, Lima (CORBIDI, Peru); Museo de Biodiversidad del Peru, Cusco (MUBI, Peru); and the Natural History Museum, University of Kansas, Lawrence (KU, United States).

Type series

As first step of reduction of taxon sampling, we examined the holotypes of *Pristimantis academicus* (MUSM 27634), *P. carvalhoi* (MNRJ 3283), *P. imitatrix* (KU 171892), and *P. lirellus* (KU 212240). We also examined 16 specimens referred to as *P. imitatrix* from Cusco Amazonico (Duellman, 2005; Duellman and Lehr, 2009) to comment on their taxonomic and biogeographic status (Table S1). Photographs of holotypes are provided in Supplementary Figure S1.

Phylogenetic analyses

DNA was extracted from preserved liver or muscle tissue (95% ethanol) using a single-step method with Acid Guanidinium Thiocyanate (Chomczynski and Sacchi, 2006) or by using a UltraClean® Tissue & Cells DNA Isolation Kit (MO-BIO Laboratories, Inc., Carlsbad, CA, USA), following the manufacturer's manual. Two mitochondrial genes—16S rRNA (16S) and Cytochrome Oxidase sub-unit I gene (COI)—were amplified. Polymerase chain reaction (PCR) was carried out under locus-specific optimal annealing temperatures following protocols detailed by Pinto-Sánchez et al. (2012). PCR products were cleaned using the UltraClean PCR Clean-Up Kit (MO-BIO Laboratories, Inc., Carlsbad, CA, USA) or by Exo I/SAP digest, and sequenced in both directions by Macrogen Co. Ltd. (South Korea). Sequences were edited and aligned in Geneious Prime (Drummond et al., 2010). Multiple sequence alignments were generated using MAFFT v7.017 (Katoh et al., 2002) with default gap opening cost and other settings configured in Geneious Prime (Drummond et al., 2010). Leading and lagging ends were trimmed manually to remove any missing data.

To optimize taxon sampling within *Pristimantis,* we blasted (Blast procedure) 31 new sequences of 16S and COI into the GenBank database and carried out an exploratory analysis of phylogenetic similitude, using the 16S matrix performed by Ortega-Andrade and Venegas (2014). Among those sequences, we included the specimen CORBIDI 16294 (*Pristimantis carvalhoi,* accession number KY652651; von May et al., 2017) identified as *P. okmoi* **sp. nov.** (Alessandro Catenazzi, pers. comm.). Sequences and GenBank accession numbers used in the phylogenetic analyses are detailed in Table S2. For the concatenated matrix (54 terminals/1,162 characters), we used PartitionFinder (Lanfear et al., 2012) to select the optimal model of nucleotide substitution for each data partition and coding genes, based on the Bayesian Information Criteria (BIC). We analyzed a concatenated mtDNA matrix with four partitions, one for the 16S non-coding gene and one for each codon position of the COI coding gene. BIC analyses resulted in a substitution GRT+I+G model for all partitions.

Phylogenetic analyses were conducted using Bayesian Methods (BM) and Maximum Likelihood (ML) on the aligned nucleotides. BM analyses were performed in MrBayes v3.2.2 (Ronquist and Huelsenbeck, 2003), with two parallel runs of the Metropolis Coupled Monte Carlo Markov Chain (MCMCMC) algorithm for 10,000,000 generations each, with four heated chains (0.1 heating parameter). Trees were sampled every 1,000 generations; BEAGLE was used to speed up the analyses on the GPU. Convergence rate and stationarity of the Markov process were evaluated with average standard deviation of split frequencies between runs (values < 0.01) and the Potential Scale Reduction Factor (values near 1.0 were considered adequate; Gelman and Rubin, 1992) visualized in the log file of MrBayes. The first 25% of generations were discarded as burn-in, after evaluating the stability and adequate "mixing" of sampled log-likelihood values

assessed from the parameter estimates across generations (ESS > 200) of both runs, visualized using Tracer v1.6 (Rambaut and Drummond, 2013). ML phylogenetic reconstruction was implemented in GARLI v2.0 (Zwickl, 2006) through 10 independent searches, whereas the supports for the nodes were calculated by a search with 1,000 bootstrap replications. Independent searches were performed with stepwise-addition starting trees (streefname = stepwise), setting 5,000,000 generations as maximum to each run (stopgen = 5000000), saving every 100 generations (saveevery = 100), run termination threshold of 20,000 generations without topology improvement (genthreshfortopoterm = 20000), and a termination threshold value of 0.01 on the lnL increase required for any new topology along searches (significanttopochange = 0.01); other parameters were left as default (Zwickl, 2006). A 50% majority-rule consensus was applied on replications to generate a consensus tree with non-parametric bootstrap values in Mesquite (Maddison and Maddison, 2015). Genetic p-distances were calculated on the concatenated matrix with Geneious Prime (Drummond et al., 2010).

Comparative analyses of different lines of evidence

Qualitative and quantitative morphological differences in adult specimens were based on morphological traits. Description of coloration was based on field notes. Sex and maturity were determined by examination of gonads and vocal slits. Bilateral characters were obtained from the right side of specimens. Measurements were taken with digital calipers (to nearest 0.01 mm) for the following traits: (1) snout–vent length (SVL) = distance from snout tip to posterior margin of vent; (2) head width (HW) = greatest width of head measured at level of jaw articulation; (3) head length (HL) = from posterior margin of lower jaw to tip of snout; (4) horizontal eye diameter (ED) = distance between anterior and posterior borders of eye; (5) interorbital distance (IOD) = the breadth of the braincase between the orbits; (6) eye–nostril distance (EN) = distance from posterior margin of nostril to anterior margin of eye; (7) upper eyelid width (EW) = perpendicular distance of the upper eyelid; (8) tympanic length (TYL) = horizontal distance between external anterior and posterior margins of tympanic annulus; (9) femur length (FL) = length of femur from vent to knee; (10) tibia length (TL) = length of flexed leg from knee to heel; (11) foot length (FoL) = from proximal border of inner metatarsal tubercle to tip of fourth toe; (12) hand length (HaL) = from proximal edge of palmar tubercle to tip of Finger III; (13) disc width of Finger III (F3D) = measured across widest part of disc III; (14) disc width of Toe IV (T4D) = measured across widest part of disc IV. Fingers are numbered preaxially to postaxially from I–IV.

Quantitative morphological differences were assessed by multivariate analyses (Principal Component Analysis, PCA) to explore the variation and ordination of specimens based on data from morphological measurements available on adult males and females. We included in this explorative analysis a single specimen of *Pristimantis imitatrix* as no other adults are known in collections. To reduce size-dependent correlation effects, variables were regressed on SVL, and the residuals were then used for PCA based on a variance-covariance matrix. By using residuals from this filtering regression, we aimed to ensure that any relation found among morphological traits was not due to the effect underlying SVL and all other variables. Analyses and statistics were conducted in PAST Statistics v18.0 (Hammer et al., 2001).

We analyzed and described a call from the paratype of *Pristimantis okmoi* **sp. nov.** (MUBI 14547), an adult male recorded on 03 February 2015 by J.C. Chaparro, located between the Dahuene and Colorado rivers in the Reserva Comunal Amarakaeri (RCA; 12°59'25.08"S, 71°0'25.69"W, 955 m a.s.l.), District Huepetue, Province Manu, Department Madre de Dios, Peru. Those calls was recorded with a Tascam DR–07mkII digital audio recorder with built-in adjustable condenser microphones; the digital record was saved with a sample rate of 44.1 kHz, in 16-bit wav format. This specimen was calling ca. 1 m from the microphone, perched on a branch 2.3 m above the ground at 17:50 during a soft rain; air temperature and relative humidity were registered with a thermo-hygrometer IR digital Extech RH101. Five acoustic parameters were measured to describe the structure of the call (Cocroft and Ryan, 1995; Duellman and Lehr, 2009): (1) Call length = time from beginning to end of one call, measured from waveform in milliseconds; (2) dominant frequency = frequency containing the greatest amount of energy, measured along the entire call; (3) call rise time = time from beginning of the call to point of maximum amplitude; (4) interval between calls = time from ending of one call to the beginning of the next; and (5) call rate = number of calls/minute, from beginning of first call to beginning of last call. Measurement of fundamental frequency follows Hutter and Guayasamin (2012). The spectrogram was produced and analyzed using the program Raven Pro 1.5 (Charif et al., 2010). The dominant frequency was calculated with a size of 512 samples in the spectrogram window. Recordings are deposited in the Sound Archive at Museo de Biodiversidad del Perú (MUBI).

RESULTS

Phylogenetic analyses

Genetic samples correspond to specimens identified in the field as *Pristimantis carvalhoi* sensu lato and *P. imitatrix* sensu lato from the upper Amazon Basin in southern Peru. The best topology (log-likelihood −8202.6975) was obtained from a four-partitioned matrix of combined mtDNA, with an extended set of 54 terminals and 1,162 characters. The most relevant results are (Fig. 1): (i) Genetic samples of specimens identified as *P. imitatrix* sensu lato and *P. carvalhoi* sensu lato are polyphyletic; (ii) the phylogeny recovered six divergent lineages resembling two cryptic species related to the *P. carvalhoi* complex and

one confirmed candidate species related to the *P. imitatrix* complex along the upper Amazon basin of Peru (Fig. 1); (iii) The phylogenetic position of *P. carvalhoi* sensu stricto is related to Clade B, whereas *P. okmoi* **sp. nov.** and *Pristimantis gagliardi* **sp. nov.** are related to Clades A and D, respectively; (iv) the phylogenetic position of *P. imitatrix* sensu stricto is related to Clade E, whereas confirmed candidate species 1 (*P.* cf. *imitatrix* KU 215476) and *P. academicus* sensu stricto are related to Clades A and F, respectively; (v) the first cryptic species (*P. okmoi* **sp. nov.**) is part of a well-supported clade, together with a confirmed candidate species (also known as *Pristimantis* cf. *imitatrix*), *P. carvalhoi* sensu stricto, *P. lirellus,* and *Pristimantis* sp. (Fig. 1), whereas two genetic samples of the second cryptic species (*P. gagliardi* **sp. nov.**) are reciprocally highly supported, but related to weakly supported basal clade containing *P. carvalhoi* + *P. lirellus* + *Pristimantis* sp. + *P. okmoi* **sp. nov.** + confirmed candidate species and *P. llojsintuta* (Köhler and Lötters 1999) + *P. platydactylus* (Boulenger 1903) + *P. cruciocularis* (Leher et al., 2006) + *P. croceoinguinis* (Lynch, 1968).

The uncorrected p-distance between individuals of both new species related to *Pristimantis carvalhoi* sensu stricto is 0.02–0.09 (Fig. 2). *Pristimantis imitatrix* sensu stricto has genetic distances of 0.08–0.12, related with the *Pristimantis* confirmed candidate species 1 (*P.* cf. *imitatrix* CCS1).

Multivariate morphometric analyses

The first two principal components of the PCA of morphometric variables separate male specimens of *Pristimantis academicus* from the one male *P. imitatrix* (no ad-

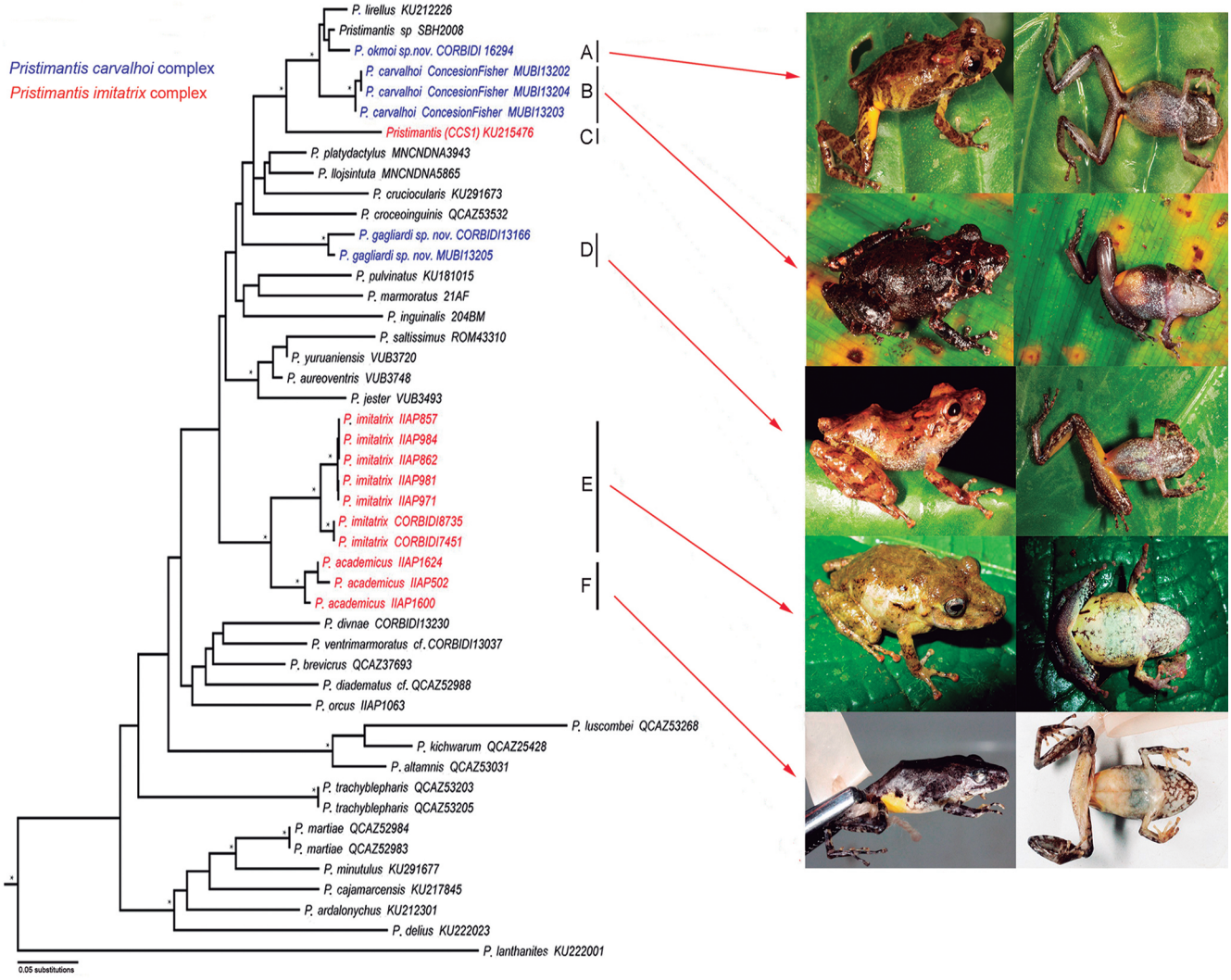


Figure 1. Optimal maximum likelihood tree showing the phylogenetic relationships among 47 sequences of *Pristimantis* (outgroup taxa excluded), scored for 1,162 aligned sites of the 16S mtDNA + COI genes. Asterisks correspond to posterior probabilities resulting from Bayesian phylogenetic analyses (topology not shown; values < 0.99 not shown, * = 1). The phylogenetic position of *P. carvalhoi* sensu stricto is related to Clade B, whereas *P. okmoi* **sp. nov.** and *P. gagliardi* **sp. nov.** are related to Clades A and D, respectively. The phylogenetic position of *P. imitatrix* sensu stricto is related to Clade E, whereas confirmed candidate species 1 (*Pristimantis* cf. *imitatrix* KU 215476) and *P. academicus* sensu stricto are related to Clades C and F, respectively. Photographs: Clade A: Adult male *P. okmoi* **sp. nov.** (MUBI 14461), 14.5 mm snout–vent length (SVL), Pad A in Reserva Comunal Amarakaeri, Lote 76, Huepetue, Madre de Dios, Peru. Clade B: Adult female *P. carvalhoi* (MUBI 13204), 20.8 mm SVL, Concesión Fisher Río Itaya, Loreto, Peru. Clade D: Subadult female *P. gagliardi* **sp. nov.** (MUBI 13205), 17.3 mm SVL, Quincemil, Cusco, Peru. Clade E: Adult female *P. imitatrix* (IIAP 857), 27.0 mm SVL, Buncuya 1, Loreto, Peru. Clade F: Adult female *P. academicus* (IIAP 1624), 21.4 mm SVL.

ditional data from known males in collections) and from *P. okmoi* **sp. nov.** and *P. gagliardi* **sp. nov.,** which overlap one another (Fig. 3A). Femur and tibia lengths are highly related with the first component of the analysis, whereas eye–nostril distance has high loading in the second component (Table S3). Interestingly, the first component narrowly separates both new species when it is contrasted with the third component, highly loaded by the head width and head length (Fig. 3B). In contrast, females of all species are clustered by the two first components, with slightly more separation when contrasting the first and third components (Figs. 3C–D). For example, females of *P. carvalhoi–P. imitatrix* and *P. academicus–P. imitatrix* are separated by the interaction of the first and third components, loaded mainly by tibia and hand lengths. A single female of *P. lirellus* is separated from *P. academicus* and *P. imitatrix* in all the components (Figs. 3C–D), whereas *P. carvalhoi* is separated from *P. imitatrix* by the interaction of femur + foot lengths (component 1), and tibia + hand lengths (component 3). In contrast, females of *P. gagliardi* **sp. nov.** present the greater variation in morphological traits, resulting in an overlap when projected in the PCA. The first three components explained 68.9–80.3% of the variance on data analyzed by sex (Table S3).

Systematics

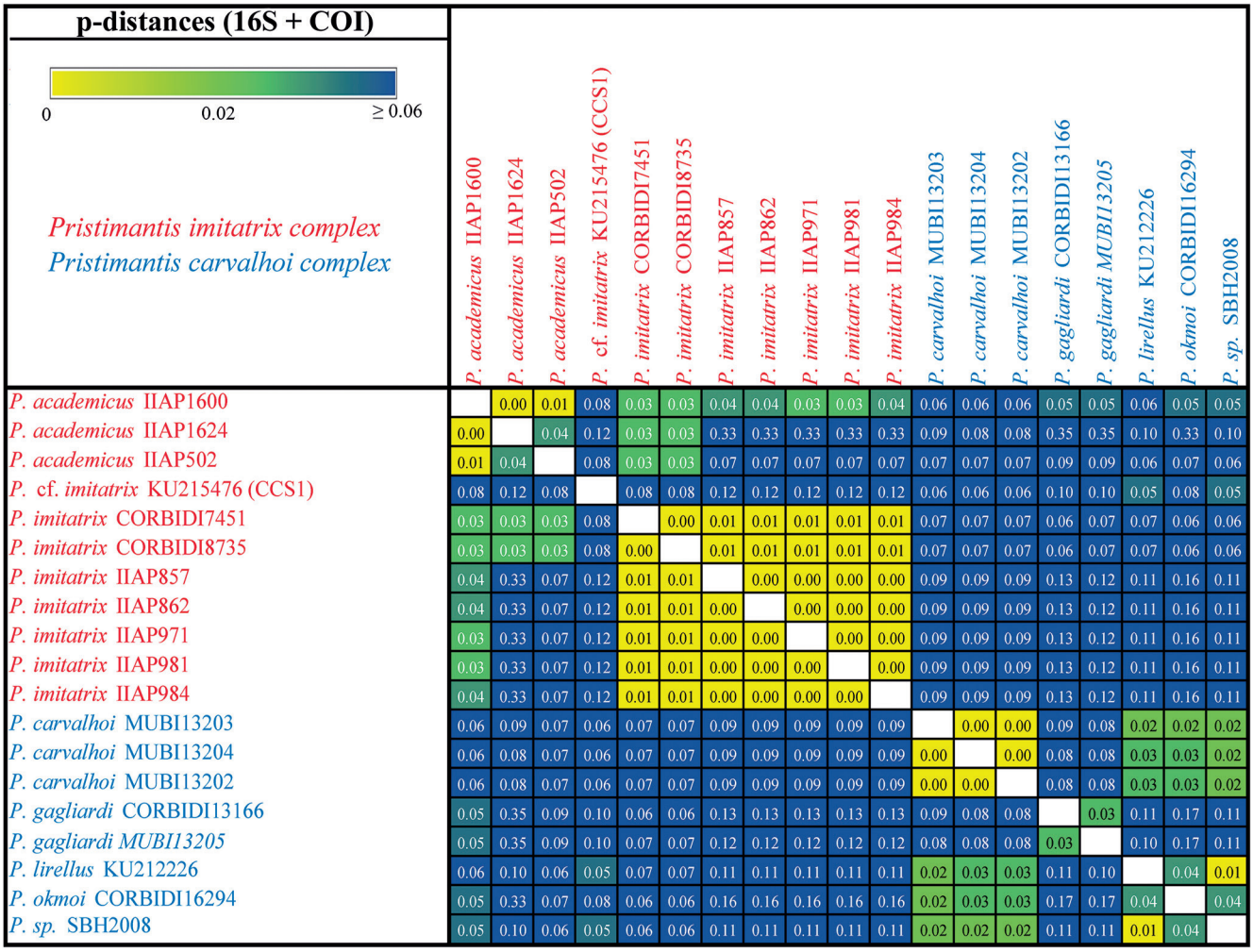
Pristimantis gagliardi sp. nov. (Figs. 4–6)

Holotype

MUBI 14700, an adult female, from between the Dahuene and Colorado rivers in Reserva Comunal Amarakaeri, District Huepetue, Province Manu, Department Madre de Dios, Peru (12°59'40.79"S, 71°0'28.28"W), 1,009 m, collected 19 July 2015 by J.C. Chaparro, F.P. Condori, and J. Arique Moque.

Paratypes

A total of 33 females and 9 males. **Females:** Peru: District Camanti, Province Quispicanchis, Department Cusco: MUBI 13205, subadult female, collected on 18 June 2013 from Quincemil, 13°13'40.01"S, 70°45'13.90"W, 655 m, by H.M. Ortega-Andrade and C. Alarcón; MUBI 6992, 6997, 7011, Santa Isidora, 13°07'58.05"S, 70°51'46.52"W, 800 m, collected on 17 April 2008 by J.C. Chaparro, R. Velasquez, F. Najar, J. Chata, and E. Yabar; MUBI 7016, 7027, 7040,



	P. academicus IIAP1600	*P. academicus* IIAP1624	*P. academicus* IIAP502	*P.* cf. *imitatrix* KU215476 (CCS1)	*P. imitatrix* CORBIDI7451	*P. imitatrix* CORBIDI8735	*P. imitatrix* IIAP857	*P. imitatrix* IIAP862	*P. imitatrix* IIAP971	*P. imitatrix* IIAP981	*P. imitatrix* IIAP984	*P. carvalhoi* MUBI13203	*P. carvalhoi* MUBI13204	*P. carvalhoi* MUBI13202	*P. gagliardi* CORBIDI13166	*P. gagliardi MUBI13205*	*P. lirellus* KU212226	*P. okmoi* CORBIDI16294	*P. sp.* SBH2008
P. academicus IIAP1600		0.00	0.01	0.08	0.03	0.03	0.04	0.04	0.03	0.03	0.04	0.06	0.06	0.06	0.05	0.05	0.06	0.05	0.05
P. academicus IIAP1624	0.00		0.04	0.12	0.03	0.03	0.33	0.33	0.33	0.33	0.33	0.09	0.08	0.08	0.35	0.35	0.10	0.33	0.10
P. academicus IIAP502	0.01	0.04		0.08	0.03	0.03	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.09	0.09	0.06	0.07	0.06
P. cf. *imitatrix* KU215476 (CCS1)	0.08	0.12	0.08		0.08	0.08	0.12	0.12	0.12	0.12	0.12	0.06	0.06	0.06	0.10	0.10	0.05	0.08	0.05
P. imitatrix CORBIDI7451	0.03	0.03	0.03	0.08		0.00	0.01	0.01	0.01	0.01	0.01	0.07	0.07	0.07	0.06	0.07	0.07	0.06	0.06
P. imitatrix CORBIDI8735	0.03	0.03	0.03	0.08	0.00		0.01	0.01	0.01	0.01	0.01	0.07	0.07	0.07	0.06	0.07	0.07	0.06	0.06
P. imitatrix IIAP857	0.04	0.33	0.07	0.12	0.01	0.01		0.00	0.00	0.00	0.00	0.09	0.09	0.09	0.13	0.12	0.11	0.16	0.11
P. imitatrix IIAP862	0.04	0.33	0.07	0.12	0.01	0.01	0.00		0.00	0.00	0.00	0.09	0.09	0.09	0.13	0.12	0.11	0.16	0.11
P. imitatrix IIAP971	0.03	0.33	0.07	0.12	0.01	0.01	0.00	0.00		0.00	0.00	0.09	0.09	0.09	0.13	0.12	0.11	0.16	0.11
P. imitatrix IIAP981	0.03	0.33	0.07	0.12	0.01	0.01	0.00	0.00	0.00		0.00	0.09	0.09	0.09	0.13	0.12	0.11	0.16	0.11
P. imitatrix IIAP984	0.04	0.33	0.07	0.12	0.01	0.01	0.00	0.00	0.00	0.00		0.09	0.09	0.09	0.13	0.12	0.11	0.16	0.11
P. carvalhoi MUBI13203	0.06	0.09	0.07	0.06	0.07	0.07	0.09	0.09	0.09	0.09	0.09		0.00	0.00	0.09	0.08	0.02	0.02	0.02
P. carvalhoi MUBI13204	0.06	0.08	0.07	0.06	0.07	0.07	0.09	0.09	0.09	0.09	0.09	0.00		0.00	0.08	0.08	0.03	0.03	0.02
P. carvalhoi MUBI13202	0.06	0.08	0.07	0.06	0.07	0.07	0.09	0.09	0.09	0.09	0.09	0.00	0.00		0.08	0.08	0.03	0.03	0.02
P. gagliardi CORBIDI13166	0.05	0.35	0.09	0.10	0.06	0.06	0.13	0.13	0.13	0.13	0.13	0.09	0.08	0.08		0.03	0.11	0.17	0.11
P. gagliardi MUBI13205	0.05	0.35	0.09	0.10	0.07	0.07	0.12	0.12	0.12	0.12	0.12	0.08	0.08	0.08	0.03		0.10	0.17	0.11
P. lirellus KU212226	0.06	0.10	0.06	0.05	0.07	0.07	0.11	0.11	0.11	0.11	0.11	0.02	0.03	0.03	0.11	0.10		0.04	0.01
P. okmoi CORBIDI16294	0.05	0.33	0.07	0.08	0.06	0.06	0.16	0.16	0.16	0.16	0.16	0.02	0.03	0.03	0.17	0.17	0.04		0.04
P. sp. SBH2008	0.05	0.10	0.06	0.05	0.06	0.06	0.11	0.11	0.11	0.11	0.11	0.02	0.02	0.02	0.11	0.11	0.01	0.04	

Figure 2. Uncorrected p-distances for sequences in a matrix with 1,162 characters from 16S + COI mtDNA genes, assessed for the *Pristimantis carvalhoi* and *P. imitatrix* complexes. Gradient in color represents p-distances from 0 (yellow) to 6% (blue).

7316, 7322, 7331, 7343, collected on 20 April 2008 from Mabe, 13°06’31.5”S, 70°54’56.0”W, 1,000 m, by J.C. Chaparro, R. Velasquez, F. Najar, J. Chata, and E. Yabar; MUBI 7437, 7441, 7451, 7453, collected from Nusinuscato, 13°08’13.1”S, 70°51’05.8”W, 685 m on 13 September 2008 by J.A. Delgado and R. Velasquez; MUBI 7383, 7389, 7391, 7402–7405, collected from Mabe, 13°06’43.8”S, 70°54’50.7”W, 900 m, on 07 November 2008 by J.A. Delgado and R. Velasquez; MUBI 10311, 10321, 10332 collected from headwaters of Huasoroco river, 13°02’19.97S, 70°51’45.70”W, 776 m, on 07 October 2010 by G. Estrada and J.A. Delgado; MUBI 10417, collected from headwaters of Huasaroco river, 13°2’41.76”S, 70°52’9.33”W, 802 m, on 08 October 2010 by G. Estrada and J.A. Delgado. District Ayapata, Province Carabaya, Department Puno: CORBIDI 13166 collected from Parque Nacional Bahuaja Sonene Alto, Satellite camp, 13°14’23.8”S, 70°08’27.4”W, 1,055 m. District Huepetue, Province Manu, Department Madre de Dios: MUBI 10349 collected from Rio Cupudnoe, 12°46’28.74”S, 70°57’42.25”W, 385 m, on 14 October 2010 by G. Estrada and J.A. Delgado; MUBI 10374, 10376, 10379, 10380, collected from between Colorado and Huasoroco river, 12°58’27.48”S, 70°56’4.54”W, 464 m, on 28 October 2010 by G. Estrada and J.A. Delgado; MUBI 10387 collected from near Inambari Bridge, 12°59’26.65”S, 71°0’45.44”W, 902 m, on 31 October 2010 by G. Estrada and J.A. Delgado; MUBI 10470 collected from near headwaters of Huasoroco river, 12°59’59.25”S, 70°50’31.32”W, 853 m, on November 2010 by G. Estrada and J.A. Delgado; MUBI 14553 from between Dahuene and Colorado river, in RCA, 12°59’32.89”S, 71°0’25.09”W, 1,070 m, on 05 February 2015 by J.C. Chaparro, F.P. Condori, C. Alarcón, and G. Huaro. **Males:** Peru: District Camanti, Province Quispicanchis, Department Cusco: MUBI 6872 collected from San Lorenzo, 13°11’49.1”S, 70°32’31.5”W, 650 m, on 10 April 2008 by J.C. Chaparro, R. Velasquez, F. Najar, J. Chata, and

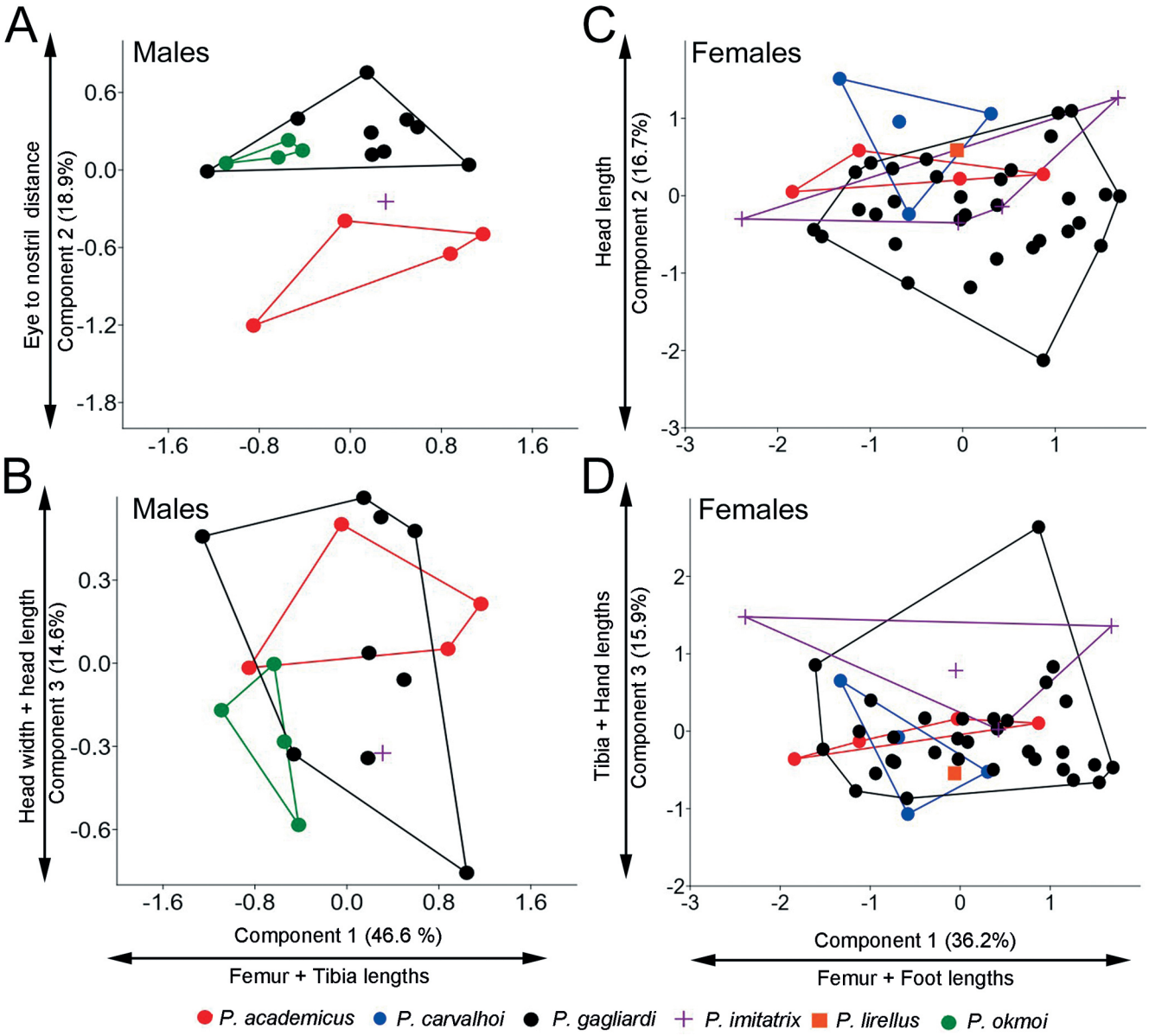


Figure 3. Ordination of specimens assessed by comparison of morphometric data using principal components in **(A, B)** males and **(C, D)** females of *Pristimantis gagliardi* **sp. nov.,** *P. okmoi* **sp. nov.,** and other morphologically similar species in Amazonia. Morphometric variables which best explained the variance in the data are shown accordingly with each axis.

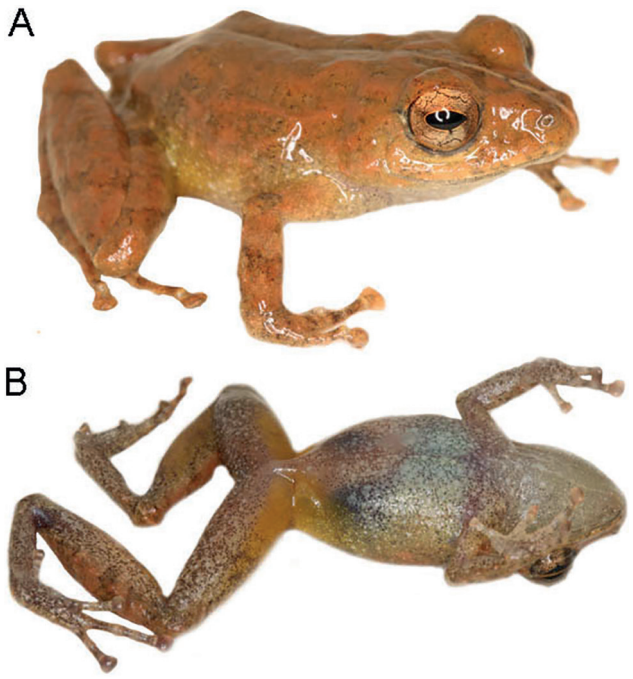


Figure 4. (A) Dorsal and **(B)** ventral view of the holotype of *Pristimantis gagliardi* **sp. nov.** (MUBI 14700, snout–vent length [SVL] = 18.17 mm), an adult female, collected between the Dahuene and Colorado rivers in the Reserva Comunal Amarakaeri, District Huepetue, Province Manu, Department Madre de Dios, Peru. Photos by J.C. Chaparro.

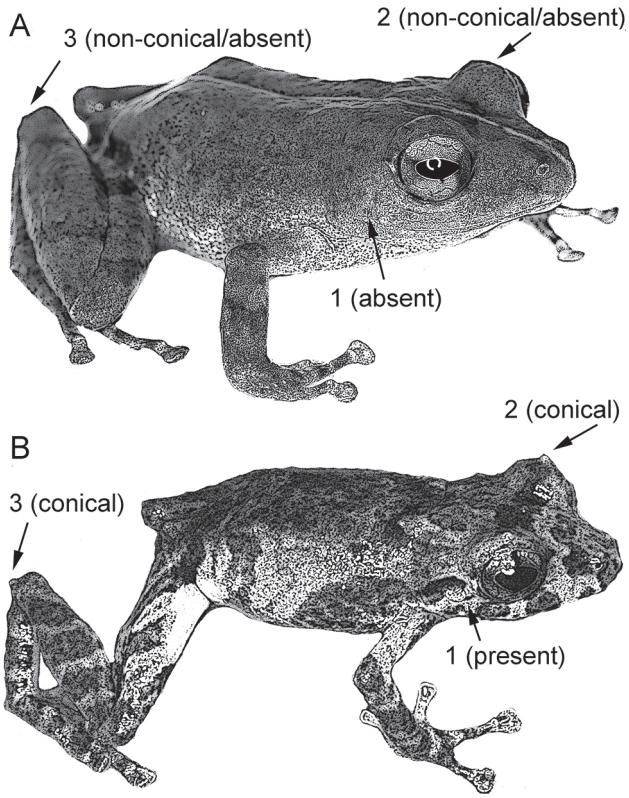


Figure 5. Diagnostic characters to differentiate **(A)** *Pristimantis gagliardi* **sp. nov.** (MUBI 14700, snout–vent length [SVL] = 18.17 mm) and **(B)** *P. okmoi* **sp. nov.** (MUBI 14461, SVL = 14.5 mm): (1) presence/absence of tympanic annulus on skin, (2) tubercles on upper eyelid, and (3) tubercle in heel. Photos by J.C. Chaparro.

E. Yabar; MUBI 7025 collected from Mabe, 13°06’31.5”S, 70°54’56.0”W, 1,000 m, on 20 April 2008 by J.C. Chaparro, R. Velasquez, F. Najar, J. Chata, and E. Yabar; MUBI 7338, 7446 collected from Nusinuscato, 13°08’13.1”S, 70°51’05.8”W, 685 m, collected on 07 August 2008 and 13 July 2008 by J.A. Delgado and R. Velasquez, respectively; MUBI 7401 collected from Mabe, 13°06’43.8”S, 70°54’50.7”W, 900 m, on 11 November 2008 by J.A. Delgado and R. Velasquez; MUBI 7452, 7454 collected from Nusinuscato, 13°08’13.1”S, 70°51’05.8”W, 685 m, on 13 September 2008 by J.A. Delgado and R. Velasquez. District Huepetue, Province Manu, Department Madre de Dios: MUBI 14462, collected from between Dahuene and Colorado river, in RCA, 12°59’33.57”S, 71°0’25.42”W, 1,000 m, on 28 September 2014 by J.C. Chaparro, F.P. Condori, and G. Huaro; MUBI14476 (field number UNMS 154386) 12°59’32.89”S, 71°0’25.09”W, 1,070 m, collected on 05 February 2015 by J.C. Chaparro, F.P. Condori, C. Alarcón, and G. Huaro.

Diagnosis

A new species of *Pristimantis* characterized by: (1) skin of dorsum shagreen with large tubercles or pustules, lateral dermal ridges, and a W-shaped scapular mark (sensu Duellman and Lehr, 2009); dorsolateral folds absent; skin of venter areolate; discoidal fold barely evident; (2) tympanic annulus and membrane not differentiated, covered by muscle under skin; (3) snout sub-acuminate in dorsal view, sub-acuminate in profile, bearing a very small papilla at tip of snout; lips not flared, *canthus rostralis* straight in dorsal view, angular in cross section; (4) upper eyelid ca. 92% of interorbital distance, bearing five to six subconical tubercles; (5) dentigerous processes of vomer narrow, oblique, bearing two small teeth; (6) vocal slits present, vocal sac and nuptial pads absent; (7) fingers long and slender, first shorter than second; discs on outer fingers expanded, truncated, ca. 1.5× the width of digit proximal to pad; supernumerary tubercles large, rounded; (8) fingers with narrow lateral fringes; (9) forearm bearing two or four small non-conical tubercles; (10) heel with a small tubercle; outer border of tarsus shagreen with small subconical tubercles; inner border of tarsus with a small tarsal fold; (11) two metatarsal tubercles; inner elliptical, ca. 4× less than the outer tubercle; supernumerary plantar tubercles barely visible, round; (12) toes with narrow lateral fringes; basal webbing between toes; discs equal in size or slightly larger than those on fingers; Toe V longer than Toe III; (13) in life, dorsum yellowish brown with pale orange tubercles and dermal ridges, with a dark W-shaped mark on scapular region and pale brown inverted chevrons on back; a large bright yellow to orange blotch in the groin extends onto the anterior surfaces of the thigh; a bright yellow or dark brown streak is present on the hidden surface of the shank in some specimens; posterior surfaces of thighs uniform brown; (14) SVL in adult males 14.03 ± 2.02 (12.38–18.81; *n* = 9); females 18.57 ± 2.02 (15.17–23.22, *n* = 32).

Pristimantis gagliardi **sp. nov.** is one of nine small species in the lowlands of the upper Amazon Basin with yellow or orange spots in the groin and hidden surfaces

of shanks (Table 1). *Pristimantis carvalhoi* has a shagreen dorsum with scattered low tubercles, which is in contrast with the large tubercles or pustules and lateral dermal ridges in living specimens of *P. gagliardi* **sp. nov.** In preservative, *P. carvalhoi* specimens have a smoother dorsum, whereas the dorsum is shagreen with scattered pustules

Figure 6. Living specimens of *Pristimantis gagliardi* **sp. nov.** in lateral, ventral, and dorsal views (**A–B,** male, not collected; **C–E,** MUBI 13205, sub-adult female, snout–vent length [SVL] = 17.3 mm). Note the yellow flash colors on hidden parts of the shanks. Photos by J.C. Chaparro, H.M. Ortega-Andrade, and P. Venegas.

Table 1. Comparisons of *Pristimantis okmoi* **sp. nov.** and *P. gagliardi* **sp. nov.** with other species from the Amazonian lowlands having yellow spots or bright marks on hidden surfaces of limbs. Characters were taken from original species descriptions and from the examined material. Presence of a character is indicated by "+", its absence by "–", character unknown is indicated by "?". Snout–vent length = SVL.

Diagnostic character	*P. academicus* Lehr et al., 2010	*P. carvalhoi*, this study	*P. croceoinguinis* (Lynch, 1968)	*P. divnae* Lehr and von May, 2009	*P. gagliardi* sp. nov.	*P. imitatrix*, this study
Females SVL (mm)	20.0–22.0	19.5–22.5	17.4–22.6	20.6–28.7	15.17–23.22	20.7–27.0
Males SVL (mm)	14.9	13.5–14.8	13.9–18.2	21.38–23.4	12.38–18.81	15.7
Dorsal skin texture	Tubercular	Shagreen with scattered low tubercles	Dorsum heavily tuberculated	Shagreen with scattered minute tubercles	Shagreen with large tubercles or pustules, lateral dermal ridges	Dorsum shagreen to glandular with large warts, spiculate pustules, and tubercles; discontinuous dorsolateral folds coalescing in short ridges along the loreal and tympanic region
Ventral skin texture	Areolate	Areolate	Areolate	Areolate	Areolate	Areolate
Tympanic annulus beneath skin	–	–	–	+	–	–
Tympanic membrane beneath skin	–	–	–	–	–	–
Vocal slits	+	–	–	–	+	+
Nuptial pads	–	–	–	–	–	–
Subgular vocal sac	–	–	–	–	–	–
Discoidal fold	Indistinct	–	Indistinct	+	+	+
Ulnar tubercles	+	+	+	–	+	+
Tubercles in heel	+	–	Small	Small	Small	+
Tarsal fold	–	–	–	+	+	–
Relative size inner/outer metatarsal tubercles	4×	6×	4×	6×	6×	3×
Supernumerary plantar tubercles	+	+	–	+	+	+
Basal webbing	–	+	–	+	+	–
Dorsal coloration in life	Dorsum dark red to greenish brown	Dark brown, with or without irregular marks	Tan or brown with dark brown to olive-green marks	Brown with dark brown W-shaped mark on scapula and a dark brown chevron	Yellowish- brown with pale orange tubercles and dermal ridges, with a dark W-shaped mark on scapular region and pale brown inverted chevrons on back	Dorsum greenish brown to brown, with orange tubercles, pustules, and folds
W–shaped mark on scapular region	–	+	+	W-shaped scapular ridge	+	+
Groin and hidden surfaces of thighs	A single yellow spot in anterior surface of groin	A single yellow spot in anterior surface of groin	Two ovoid orange or yellow spots in groin	Yellow with brown marks	A large bright yellow blotch in the groin extends onto the anterior surfaces of the thigh; a bright yellow streak is present on the ventral surface of the shank	Groin with yellow or bluish-cream blotches
Chest and venter coloration in life	Pale grey to white with dark brown flecks or mottling denser on throat	Creamy tan with brown flecks	Creamy tan with brown flecks	Cream with dark brown blotches	Dark brown with white flecks	Pale bluish-white with brown reticulations, flecks, or mottling
Iris coloration	Golden to bronze with fine black vermiculation	Copper with black reticulations	Dull bronze with brown reticulations	Golden with fine black reticulations and a vertical bar above and below the iris forming a cross	Bronze with a wide longitudinal copper band, finely reticulated with brown.	Silver finely reticulated with black

Diagnostic character	***P. lirellus*** **(Dwyer, 1995)**	***P. minutulus*** **(Duellman and Hedges, 2007)**	***P. okmoi*** **sp. nov.**	***P. toftae*** **(Duellman, 1978)**	***P. variabilis*** **(Lynch, 1968)**
Female SVL (mm)	19.4–24.5	17.0–20.1	Unknown	18.9–27.4	18.9–27.5
Male SVL (mm)	14.1–17.0	13.1–17.6	13.63–17.04	17.0–22.8	13.7–22.0
Dorsal skin texture	Shagreen with small, scattered tubercles and low dorsolateral dermal ridges	Shagreen	Shagreen with large tubercles or pustules	Shagreen	Shagreen
Ventral skin texture	Areolate	Areolate	Areolate with scattered enlarged warts	Coarsely areolate	Areolate
Tympanic annulus beneath skin	–	–	+	+	+
Tympanic membrane beneath skin	–	–	–	+	+
Vocal slits	+	–	+	+	+
Nuptial pads	–	–	–	–	–
Subgular vocal sac	+	–	–	+	+
Discoidal fold	Indistinct	Indistinct	Indistinct	+	+
Ulnar tubercles	+	–	+	–	–
Tubercles in heel	Small	–	Conical	–	–
Tarsal fold	–	–	+	–	Tubercle
Relative size inner/outer metatarsal tubercles	45×	3×	3×	2×	6×
Supernumerary plantar tubercles	+	–	+	+	–
Basal webbing	–	–	+	+	–
Dorsal coloration in life	Dark brown to reddish-brown in life, with darker brown markings on back	Brown with dark brown to black markings or with a cream longitudinal median stripe	Yellowish-brown to olive-green with pale orange tubercles and dermal ridges, with a dark W-shaped mark on scapular region and pale brown inverted chevrons on back	Pale yellow-tan, dull olive-green or reddish-brown	Variable; tan, brown, gray, red or greenish-yellow, with or without stripes, marks or blotches.
W-shaped mark on scapular region	+	+	+	–	–
Groin and hidden surfaces of thighs	Groin with a yellow blotch	Groin with a yellow blotch	A large bright yellowish-orange blotch in the groin extends onto the anterior surfaces of the thigh	Pale yellow or orange spot extends onto the proximal anterior surface of the thigh	Pale yellow spot, usually narrowly bordered by black extends from groin onto the proximal anterior surface of the thigh
Chest and venter coloration in life	Grayish-white with brown flecks	Cream with minute brown flecks	Dark brown with cream flecks	Pale cream, commonly with a longitudinal stripe on the throat	Cream with brown or black flecks
Iris coloration	Dull bronze with brown reticulations	Reddish-gray	Gray with a wide longitudinal copper band, finely reticulated with dark brown, with a short vertical bar forming a + cross shape	Pale grayish-bronze	Bronze with a median horizontal reddish-brown streak

in the new species. While we note differences in skin texture, it is important to recognize phenotypic plasticity of this trait in some *Pristimantis,* and caution should be used when employing this as a diagnostic trait (Guayasamin et al., 2015). Also, *P. carvalhoi* only has a single yellow spot on the groin, whereas specimens of *P. gagliardi* **sp. nov.** have a large bright yellow to orange blotch in the groin that extends onto the anterior surfaces of the thigh, and a bright yellow or dark brown streak on the hidden surface of the shank (Fig. 6). The new species lacks a tympanic annulus and membrane, as do *P. academicus, P. antisuyu* Catenazzi and Lehr, 2018, *P. croceoinguinis, P. erythroinguinis* Catenazzi and Lehr, 2018, *P. imitatrix, P. lirellus,* and *P. minutulus.* However, these all lack basal webbing between toes and a bright yellow streak on the ventral surface of the shank, characters which are both present in the new species. *Pristimantis antisuyu* has small conical tubercles in heel and upper eyelid (Catenazzi and Lehr, 2018), which are absent (non-conical) in *P. gagliardi* **sp. nov.** Furthermore, the former species have the groin and hidden portions of the thighs and legs dark brown with yellow spots of variable size, usually larger in the groin, whereas the new species has a large bright yellow to orange blotch in the groin extending onto the anterior surfaces of the thigh and a bright yellow or dark brown streak on the hidden surface of the shank in some specimens. *Pristimantis divnae, P. toftae,* and *P. variabilis* also have yellow spots in the groin but can be distinguished by having a well-differentiated tympanic annulus. Also, *P. divnae* has a cream-colored venter with dark brown vermiculated blotches, while the venter is dark brown with white flecks in the new species. *Pristimantis gagliardi* **sp. nov.** is distinguished from *P. okmoi* **sp. nov.** by lacking a distinguished tympanum, conical tubercles on the eyelid and tarsus, and having a yellow blotch on the hidden surface of the shank (Figs. 5–6).

Other *Pristimantis* in the upper Amazon basin differ from the new species by lacking bright colors on the groin or hidden surfaces of the thighs [*P. delius* (Duellman and Mendelson, 1995), *P. martiae* (Lynch, 1974), *P. ockendeni* (Boulenger, 1912)], having a differentiated tympanum and contrasting colors on the groin and hidden surfaces of the limbs (*P. diadematus* [Jiménez de la Espada, 1875], *P. lythrodes* [Lynch and Lescure, 1980], *P. orcus* Lehr et al., 2009, *P. altamazonicus* [Barbour and Dunn, 1921], *P. divnae,* and *P. eurydactylus* [Hedges and Schlüter, 1992], *P. pseudoacuminatus* [Shreve, 1935], *P. lacrimosus* [Jiménez de la Espada, 1875], and *P. paululus* [Lynch, 1974]), having a green dorsum in life (*P. acuminatus* [Shreve, 1935]), having enlarged conical tubercles on the eyelids (*P. orphnolaimus* [Lynch, 1970], *P. erythroinguinis*), having a yellow interorbital bar and dorsolateral stripes on the body (*P. aureolineatus* Guayasamin et al., 2006), or having the groin and hidden portions of the thighs and legs dark brown with large red markings surrounded by smaller red marks and the iris bronze or gold with fine black reticulations, crossed horizontally by a broad, copper band and vertically by a narrow black streak across the pupil (*P. erythroinguinis*).

Description of holotype (Fig. 4)

Body slender; head wider than body; slightly longer than wide, ca. 40% of SVL; snout short, subacuminate in dorsal view, rounded in profile; distance from nostril to corner of eye slightly larger than diameter of eye; *canthus rostralis* weakly concave in dorsal view, rounded in cross section; lips not flared; internarial area not depressed, nostrils slightly protuberant, directed anterolaterally, situated approximately three-quarters the distance from the eyes to the tip of the snout; interorbital area flat, IOD 30% of head width; eye large, protuberant, diameter ca. 2.5× depth of lip below eye and ca. 32% of head length; upper eyelid ca. 85% of interorbital distance; bearing a single, small nonconical tubercle; no interocular fold; cranial crests absent. Tympanic annulus and membrane not visible on and under skin; postrictal tubercles, low, barely visible in preservative; choana small, triangular, not concealed by the palatal shelf of maxillary arc; dentigerous processes of vomer narrow, oblique, bearing 2–3 small teeth, positioned posterior to level of choanae; tongue elliptical, posterior border notched, not adherent to the floor of the mouth in ca. 47% of its length.

Skin on dorsum shagreen with low granulated tubercles on flanks; no occipital ridges or dorsolateral folds; ventral surfaces of belly, chest, throat, and thighs areolate; discoidal folds barely visible; no thoracic fold. Forearm slender; fingers long and slender, all with circumferential grooves on large oval (broader than long) discs; disc on Finger III ca. 1.5× wider than narrowest portion of penultimate phalanx; disc on Finger I distinctively smaller than those on other fingers; relative length of fingers I < II < IV < III; subarticular tubercles large, subconic; supernumerary tubercles low, elliptical; palmar tubercle elliptic, 1.5× size of oval thenar tubercle; antebrachial tubercle, small; two ulnar tubercles are present on the posterior part of forearm; outer edge of forearm shagreen, tubercles present, small. Hind limbs slender; tibia length ca. 60% of SVL; knee lacking tubercles; heel with a single, subconical tubercle; foot length ca. 46% of SVL; outer and inner border of tarsus shagreen; inner metatarsal tubercle oval, 3× size of round outer metatarsal tubercle; supernumerary tubercles barely visible, rounded, small; subarticular tubercles subconical, rounded; toes lacking lateral fringes; webbing absent between Toes IV−V; pads of Toes III−V large, in all other pads and discs of toes like those of fingers; relative lengths I < II < III < V < IV; Toe III extending to proximal edge of penultimate subarticular tubercle on Toe IV; Toe V extending to distal edge between the penultimate and ultimate subarticular tubercle on Toe IV. Vent opening puckered, shagreen, not extended, with two tubercles on its border, located at upper level of thighs.

Measurements (in mm) of holotype

SVL = 18.17; HL = 7.44; HW = 7.35; ED = 2.42; IOD = 2.20; EN = 3.35; EW = 1.86; TYL = 0; FL = 10.6; TL = 10.90; FoL = 8.42; HaL = 5.34; F3D = 0.77; T4D = 0.74. Proportions: HL/SVL = 0.41; HW/SVL = 0.40; TL/SVL = 0.60; FoL/SVL = 0.46; EN/HL = 0.45; ED/HL = 0.33; IOD/HW = 0.30.

Coloration of holotype in life (Fig. 4)

Dorsum yellowish brown with pale orange tubercles and dermal ridges, with a dark W-shaped mark on scapular region, olive green marks or pale brown inverted chevrons on back; snout with pale yellow stains; upper lips with dark brown diagonal stripes. Dorsal surfaces of arms and thighs with transversal faint brown bars. A large pale yellow blotch in the groin extends onto the anterior surfaces of the thigh; a pale yellow streak is present on the ventral surface of the shank; posterior surfaces of thighs uniform brown. The flanks are pale brown with faint transverse bands. The ventral surfaces are dark brown with white flecks. Iris bronze with a wide longitudinal copper band, finely reticulated with brown.

Coloration of holotype in preservative

All pale yellowish brown surfaces turn gray, whereas the pale orange tubercles and dermal ridges turn cream. Dorsal surfaces of thighs cream with dark brown transversal brands; bright yellow surfaces of groin and thighs, and white flecks of venter turn cream. Posterior surfaces of thighs are brown.

Variation

Measurements of type specimens are listed in Table 2. Male voucher specimens are generally smaller (14.03 ± 2.02 mm; 12.38–18.81 mm, *n* = 9) than females (18.57 ± 2.02 mm; 15.17–23.22 mm; *n* = 32). Furthermore, males and subadult females often have a more tuberculated dorsum, tarsus, and heel (e.g., MUBI 13205 vs. MUBI 14700, Figs. 5–6). Hidden surfaces of shanks are brightly yellow in paratypes and less distinctive in the holotype. Living specimens have tubercles on arms, thighs, heel, and dorsum, but they tend to be less distinct in preserved specimens.

Etymology

The specific name is used as noun in apposition, a patronym for our friend and colleague Giussepe Gagliardi, in recognition of his intensive and dedicated work on Peruvian herpetofauna and its conservation.

Table 2. Descriptive statistics for morphometric measurements (in mm) for males and females of *Pristimantis academicus, P. carvalhoi, P. gagliardi* **sp. nov.,** *P. imitatrix, P. lirellus,* and *P. okmoi* **sp. nov.** Mean ± SD are given, with range in parentheses. The tympanic annulus is absent in the species (–). The measurement of the tympanic length (*) was not used for principal component analyses.

Males				
Morphometrics	***P. academicus* (*n* = 4)**	***P. gagliardi* sp. nov. (*n* = 9)**	***P. imitatrix* (*n* = 1)**	***P. okmoi* sp. nov. (*n* = 4)**
Snout–vent length (SVL)	14.48 ± 0.81 (13.80–15.60)	14.03 ± 2.02 (12.38–18.81)	15.70	14.94 ± 1.47 (13.63–17.04)
Head width (HW)	5.35 ± 0.26 (5.10–5.70)	5.16 ± 1.00 (4.24–7.48)	5.40	5.34 ± 0.42 (4.80–5.80)
Head length (HL)	6.25 ± 0.25 (6.00–6.60)	5.93 ± 0.74 (5.09–7.65)	6.30	6.17 ± 0.39 (5.67–6.62)
Horizontal eye diameter (ED)	1.98 ± 0.13 (1.80–2.10)	2.20 ± 0.35 (1.81–2.81)	2.20	2.09 ± 0.20 (1.87–2.35)
Interorbital distance (IOD)	1.68 ± 0.10 (1.60–1.80)	1.88 ± 0.19 (1.74–2.35)	2.20	2.05 ± 0.24 (1.84–2.31)
Eye–nostril distance (EN)	1.85 ± 0.06 (1.80–1.90)	2.51 ± 0.39 (2.12–3.45)	2.20	2.58 ± 0.31 (2.25–2.99)
Upper eyelid width (EW)	1.43 ± 0.15 (1.30–1.60)	1.20 ± 0.17 (0.95–1.46)	1.60	1.44 ± 0.13 (1.30–1.61)
Tympanic length (TYL)*	–	–	–	0.85 ± 0.11 (0.69–0.94)
Femur length (FL)	7.23 ± 0.52 (6.60–7.70)	7.24 ± 1.20 (5.66–9.84)	7.90	7.21 ± 0.72 (6.73–8.25)
Tibia length (TL)	8.28 ± 0.55 (7.50–8.80)	7.70 ± 1.32 (6.43–10.49)	9.00	7.65 ± 1.02 (6.97–9.16)
Foot length (FoL)	6.20 ± 0.34 (5.70–6.40)	5.94 ± 0.93 (4.86–7.75)	6.70	5.94 ± 0.64 (5.48–6.85)
Hand Length (HaL)	3.83 ± 0.30 (3.50–4.20)	3.51 ± 0.59 (2.86–4.49)	3.90	3.77 ± 0.44 (3.22–4.27)
Disc width of Finger III (F3D)	0.85 ± 0.06 (0.80–0.90)	0.62 ± 0.15 (0.41–0.96)	0.50	0.82 ± 0.05 (0.75–0.87)
Disc width of Toe IV (T4D)	0.83 ± 0.10 (0.70–0.90)	0.58 ± 0.10 (0.41–0.70)	0.40	0.59 ± 0.11 (0.44–0.67)

Females					
Morphometrics	***P. academicus* (*n* = 4)**	***P. carvalhoi* (*n* = 4)**	***P. gagliardi* sp. nov. (*n* = 32)**	***P. imitatrix* (n = 4)**	***P. lirellus* (*n* = 1)**
SVL	21.3 ± 0.54 (20.60–21.90)	20.43 ± 0.5 (19.70–20.80)	18.57 ± 2.02 (15.17–23.22)	23.35 ± 3.04 (20.70–27.00)	23.50
HW	7.98 ± 0.43 (7.50–8.50)	7.88 ± 0.99 (7.20–9.30)	7 ± 0.86 (5.21–8.11)	8.23 ± 0.88 (7.10–9.20)	9.00
HL	8.85 ± 0.42 (8.40–9.30)	8.63 ± 1.03 (7.30–9.80)	7.57 ± 0.87 (6–9.55)	10.15 ± 1.11 (8.70–11.20)	9.60
ED	2.75 ± 0.31 (2.40–3.10)	2.85 ± 0.10 (2.70–2.90)	2.71 ± 0.34 (2.1–3.42)	3.28 ± 0.19 (3.00–3.40)	2.80
IOD	2.60 ± 0.42 (2.00–2.90)	3.10 ± 0.14 (2.90–3.20)	2.22 ± 0.24 (1.68–2.98)	3.00 ± 0.42 (2.50–3.40)	3.10
EN	2.98 ± 0.15 (2.80–3.10)	2.83 ± 0.37 (2.40–3.30)	3.23 ± 0.28 (2.48–3.76)	3.33 ± 0.26 (3.10–3.70)	2.90
EW	1.95 ± 0.10 (1.90–2.10)	1.85 ± 0.25 (1.50–2.10)	1.68 ± 0.26 (1.14–2.16)	2.03 ± 0.28 (1.70–2.30)	2.00
TYD	–	–	–	–	–
FL	9.80 ± 0.96 (8.80–11.10)	9.75 ± 0.87 (9.00–11.00)	9.34 ± 1.02 (7.39–11.17)	10.60 ± 0.57 (10.20–11.40)	11.00
TL	11.03 ± 0.74 (10.10–11.90)	10.83 ± 0.71 (10.20–11.80)	10.26 ± 0.96 (7.39–11.45)	11.83 ± 1.13 (10.60–13.20)	12.40
FoL	8.30 ± 0.81 (7.60–9.00)	7.95 ± 0.37 (7.50–8.30)	7.93 ± 1.06 (5.27–9.38)	8.95 ± 1.59 (6.70–10.40)	9.20
HaL	4.95 ± 0.75 (4.30–5.90)	4.40 ± 0.29 (4.10–4.70)	4.98 ± 0.8 (3.45–7.39)	6.23 ± 0.80 (5.20–7.00)	5.20
F3D	0.95 ± 0.26 (0.60–1.20)	0.90 ± 0.12 (0.80–1.00)	0.84 ± 0.13 (0.42–1.09)	1.28 ± 0.13 (1.10–1.40)	0.90
T4D	0.91 ± 0.27 (0.60–1.20)	0.93 ± 0.10 (0.80–1.00)	0.75 ± 0.12 (0.55–1.04)	1.13 ± 0.17 (0.90–1.30)	0.80

Distribution and natural history

Pristimantis gagliardi **sp. nov.** is known from 11 localities in the Amazonian evergreen lowlands and premontane forests of Peru within an area of approximately 1,135 km² in Cusco and Madre de Dios Departments, ranging in elevation between 340 and 1,050 m a.s.l. (Fig. 7; Rodríguez and Cadle, 1990; Catenazzi et al., 2013; Villacampa

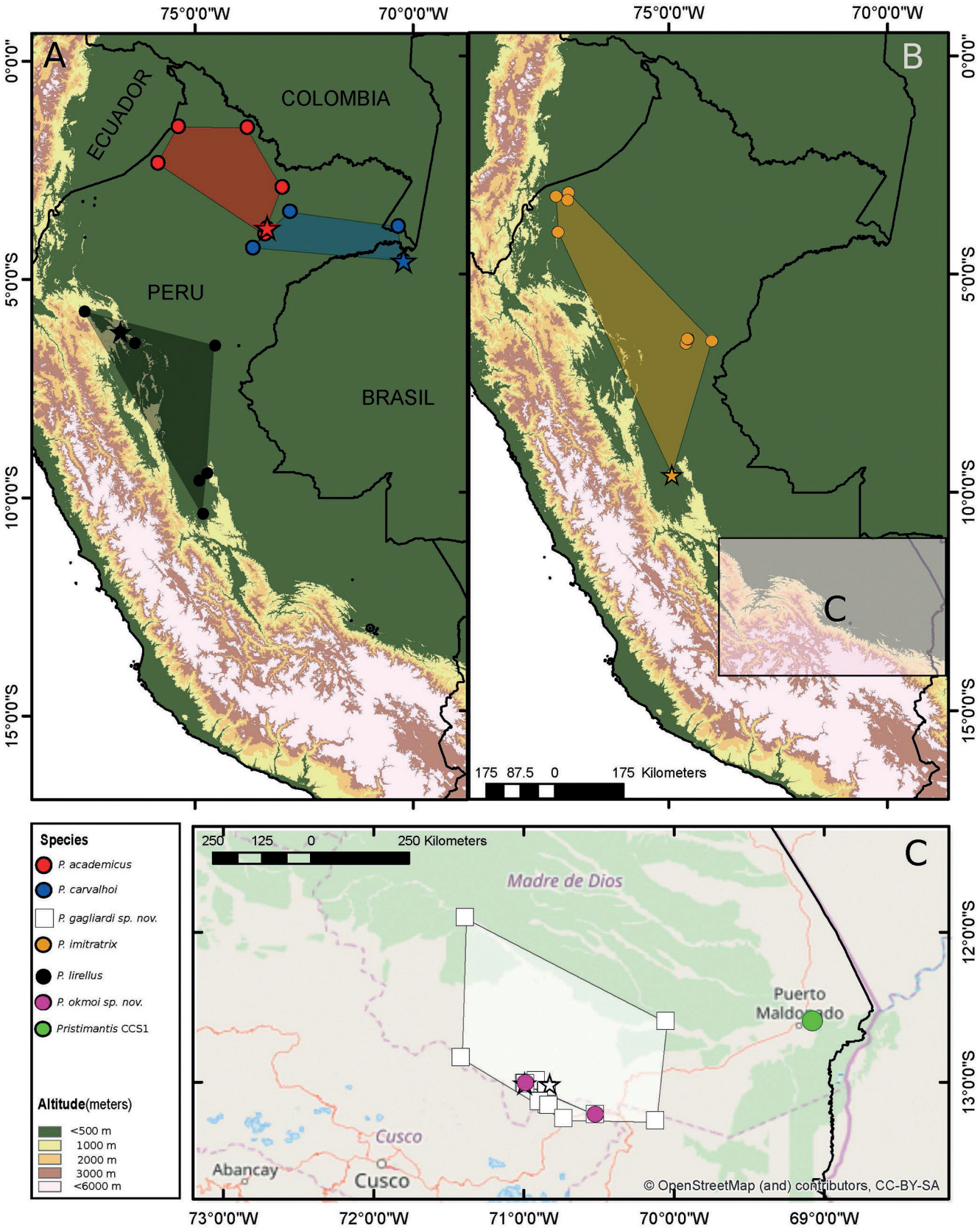


Figure 7. Occurrence areas (convex hull polygons) and localities assessed for **(A)** *Pristimantis academicus, P. carvalhoi, P. lirellus;* **(B)** *P. imitatrix;* **(C)** *P. gagliardi* **sp. nov.,** *P. okmoi* **sp. nov.,** and *P.* confirmed candidate species 1 in the upper Amazon basin. Type localities for each species are represented by stars.

et al., 2017). This species is a forest inhabitant that can be found perching at night on leaves, in the leaf litter, along creeks, bushes, palm trees, herbs, bamboo, aroids, bromeliads, ferns, and fallen trees (up to 1.5 m). The mating call and reproductive behavior are unknown. They were found active and foraging between 18:00–01:50 in February, June, July, September, and October.

Pristimantis okmoi sp. nov.

Holotype (Figs. 5, 8)

MUBI 14461, an adult male from between Dahuene and Colorado, in Reserva Comunal Amarakaeri, District Huepetue, Province Manu, Department Madre de Dios, Peru (12°59'25.08"S, 71°0'25.69"W), 955 m a.s.l., collected 28 September 2014 by J.C. Chaparro, F.P. Condori, C. Alarcón, and G. Huaro.

Paratypes

Three males: Peru. MUBI 14546, 14547 collected at the type locality on 03 February 2015 (12°59'25.08"S, 71°0'25.69"W), 965 m; MUBI 7550, from San Lorenzo, District Huepetue, Province Manu, Department Madre de Dios, Peru (13°11'49.1"S, 70°32'31.5"W), 636 m, collected 21 September 2008 by J.A. Delgado and R. Velásquez.

Diagnosis

A new species of *Pristimantis* characterized by: (1) skin of dorsum shagreen with large tubercles or pustules, lack of lateral dermal ridges, and a W-shaped scapular mark (sensu Duellman and Lehr, 2009); dorsolateral folds absent; skin of venter areolate with scattered enlarged warts; discoidal fold barely evident; (2) tympanic annulus present externally and under skin; membrane not differentiated; (3) snout rounded with small rostral papilla in dorsal view, short rounded in profile; lips not flared, *canthus rostralis* concave in dorsal view, angular in cross section; (4) upper eyelid ca. 79% of interorbital distance, bearing seven to eight small subconical tubercles; and one large subconical tubercle; (5) choanae partly concealed by palatal shelf of maxilla; teeth positioned posterior to level of choanae; (6) males with vocal slits, vocal sac not visible on chest; lack of nuptial excrescences on thumb; (7) fingers medium size and slender, first almost 2× shorter than second; discs on outer fingers expanded, truncated, ca. 1.5× the width of digit proximal to pad; supernumerary tubercles large, rounded; (8) fingers with narrow lateral fringes; (9) forearm bearing two or twelve small nonconical tubercles; (10) heel with a small tubercle; outer border of tarsus shagreen with small subconical tubercles; inner border of tarsus with a small tarsal fold; (11) two metatarsal tubercles; inner elliptical, outer ovoid, ca. 3× the outer tubercle; supernumerary plantar tubercles barely visible, round; (12) toes with narrow lateral fringes; basal webbing between toes; discs equal in size or slightly smaller than those on fingers; Toe V longer than Toe III; (13) in life, dorsum yellowish brown to olive green with pale orange tubercles and dermal ridges, with a dark W-shaped mark on the scapular region and pale brown inverted chevrons on back; a large bright yellowish-orange blotch in the groin extends onto the anterior surfaces of the thigh; posterior surfaces of thighs uniform cream. Iris gray with a wide longitudinal copper band, finely reticulated with dark brown, with a short vertical bar crossing the pupil and forming a cross shape; (14) SVL in adult males 14.94 ± 1.47 mm (13.63–17.04 mm; *n* = 4); females unknown.

Pristimantis okmoi **sp. nov.** is one of nine small species in the lowlands of the upper Amazon basin with yellow or orange spots in the groin. The new species is distinguished from all other congeners in Amazonia by having a visible tympanic annulus and membrane on skin, having basal webbing between toes, and by the yellow blotches in groin (Table 1). Other *Pristimantis* (*P. divnae, P. toftae,* and *P. variabilis*) with colored groins and visible tympanic annulus on skin can be distinguished from the new species by having pale yellow spots on groin, but completely bordered by black and abutted or confluent midventrally (*P. variabilis*), cream venter with dark brown vermiculated blotches (*P. divnae*), or black flecks forming a middle cream longitudinal stripe on throat (*P. toftae*).

Nine small *Pristimantis* also have colored spots on groin (*P. academicus, P. antisuyu, P. carvalhoi, P. croceoinguinis, P. erythroinguinis, P. gagliardi* **sp. nov.,** *P. imitatrix, P. lirellus, P. minutulus*), but all of them lack a tympanic annulus visible on skin, which is characteristic in the new species. *Pristimantis carvalhoi* has a shagreen dorsum with scattered low tubercles, in contrast to the large tubercles or pustules and lateral dermal ridges in living specimens of *P. okmoi.* In addition, *P. carvalhoi* only has a single yellow spot on the groin, whereas *P. okmoi* has two. In preservative, *P. carvalhoi* has a smoother dorsum, whereas the dorsum is shagreening with scattered pustules in the new species.

Other larger *Pristimantis* in the upper Amazon basin with tympanic annulus visible on skin differ from the new species by lacking bright colors in the groin or hidden surfaces of the thighs (*P. delius, P. martiae, P. ockendeni, P. lacrimosus*), having marbled coloration in the groin and hidden surfaces of limbs (*P. altamazonicus, P. diadematus, P. eurydactylus, P. lythrodes, P. orcus*), having a green dorsum in life and acuminated snout (*P. omeviridis* Ortega-Andrade et al., 2015, *P. enigmaticus* Ortega-Andrade et al., 2015, *P. pseudoacuminatus,* and *P. paululus*), having enlarged conical tubercles on eyelids (*P. orphonolaimus*), or having a yellow interorbital bar and dorsolateral stripes on the body (*P. aureolineatus*).

Description of holotype (Fig. 8)

Body moderately robust; head wider than body; slightly longer than wide, ca. 43% of SVL; snout rounded with small rostral papilla in dorsal view, short and rounded in profile; distance from nostril to corner of eye slightly larger than diameter of eye; *canthus rostralis* weakly

concave in dorsal view, rounded in cross section; lips not flared; internarial area slightly depressed, nostrils slightly protuberant, directed anterolaterally, situated approximately three-quarters the distance from the eyes to the tip of the snout; interorbital area flat, IOD 35% of head width; eye large, protuberant, diameter ca. 2.5× depth of

Figure 8. (A–C) Living holotype (MUBI 14461, adult male, snout–vent length [SVL] = 14.5 mm) and **(D–E)** paratype (MUBI 14551); **(F)** froglet (not collected) and **(G)** egg mass (not collected) of *Pristimantis okmoi* **sp. nov.** from between Dahuene and Colorado in Reserva Comunal Amarakaeri, Department Madre de Dios, Peru. Photos by J.C. Chaparro.

lip below eye and ca. 33% of head length; upper eyelid ca. 79% of interorbital distance; bearing a single large tubercle and five small subconical tubercles; no interocular fold; cranial crests absent. Tympanic annulus visible (on and under skin), tympanic membrane not visible on skin; postrictal tubercles low, barely visible in preservative; teeth positioned posterior to level of choanae; choanae small, sub-triangular, partly concealed by the palatal shelf of maxillary arc; dentigerous processes minute, embedded in roof of mouth, barely visible; tongue heart shape, notched posteriorly, not adherent to the floor of the mouth for approximately half its length.

Skin on dorsum shagreen with low granulated tubercles on flanks; no occipital ridges or dorsolateral folds; ventral surfaces of belly, chest, throat, and thighs areolate with scattered enlarged warts; discoidal folds barely visible; no thoracic fold. Forearm slender; fingers long and slender, all with oval pads (broader than long), fingers III–IV with large pads, fingers IV–V with large discs; pad on Finger III ca. 1.5× wider than narrowest portion of penultimate phalanx; disc on Finger II smaller than III–IV, Finger I distinctively smaller than those on other fingers; relative length of fingers I < II < IV < III; subarticular tubercles large, subconical; supernumerary tubercles low, barely visible; palmar tubercle ovoid, 1.5× size of oval thenar tubercle, elliptical; antebrachial tubercle, intermediate size; four ulnar tubercles are present along the forearm; outer edge of forearm shagreen, 12 tubercles present, small.

Hind limbs slender; tibia length ca. 49% of SVL; knee with one small tubercle; heel with three subconical tubercles; foot length ca. 46% of SVL; outer and inner border of tarsus shagreen; inner metatarsal tubercle elliptical, outer ovoid, 3× size of outer; supernumerary tubercles barely visible, rounded, small; subarticular tubercles subconical, rounded; toes with lateral fringes; basal webbing between toes; pads of Toe IV–V large, reduced in all other pads and discs of toes; relative toe lengths I < II < III < V < IV; Toe III extending to proximal edge of antepenultimate subarticular tubercle on Toe IV; Toe V extending to distal edge of penultimate subarticular tubercle on Toe IV. Vent opening puckered, shagreen, not extended, with tubercles on its border, located at upper level of thighs.

Measurements (in mm) of holotype

SVL = 14.50; HL = 6.19; HW = 5.30; ED = 2.06; IOD = 1.84; EN = 2.60; EW = 1.45; TYL = 0.92; FL = 6.74; TL = 7.11; FoL = 5.52; HaL = 3.89; F3D = 0.80; T4D = 0.60. Proportions: HL/SVL = 0.43; HW/SVL = 0.37; TL/SVL = 0.49; FoL/SVL = 0.38; EN/HL = 0.42; ED/HL = 0.33; IOD/HW = 0.35.

Coloration of holotype in life (Fig. 8)

Dorsum yellowish brown to olive green with pale orange tubercles and dermal ridges, with a dark W-shaped mark on scapular region and pale brown inverted chevrons on back; snout with pale yellow stains; upper lips with dark brown diagonal stripes. Dorsal surfaces of arms and thighs with transversal brown bars. A large bright yellowish-orange blotch in the groin extends onto the anterior surfaces of the thigh, posterior surfaces of thighs uniform cream. The flanks are pale brown with poorly defined transverse bands. The ventral surfaces are dark brown with cream flecks. Iris gray with a wide longitudinal copper band, finely reticulated with dark brown, with a short vertical bar forming a + (cross) shape.

Coloration of holotype in preservative

All pale brown areas turn gray, whereas the pale orange tubercles and dermal ridges turn cream. Dorsal surfaces of thighs cream with dark brown transversal bands; bright yellow surfaces of groin and thighs, and white flecks on venter turn cream. Posterior surfaces of thighs are brown.

Variation

Measurements for males are provided in Table 2. In general, males are similar in size (14.94 ± 1.47 mm, 13.63–17.04 mm, *n* = 4); females are unknown. A froglet specimen (not collected) had a dark brown dorsum with a longitudinal dorsal cream stripe and coppery red iris, compared with a yellowish brown dorsum and gray iris with a wide longitudinal copper band with a short vertical bar crossing the pupil and forming a + (cross) shape (Fig. 8F).

Etymology

The specific name is derived from the onomatopoeia "*ogn*" (ok), which resembles several sounds produced from these frogs, and "*moi*" (moi), meaning "to sit or squat in a frog-like position" in Amarakaeri, one of the Harakbut languages. The new species is named in honor of the Harakbut (also known as Arakmbut, Harakmbet, Harakmbut) indigenous people living in the Madre de Dios Department in the southern Peruvian Amazon, where the type series was collected.

The Harakbut people tell a story associated with the species: The sages among the Harakbut people say that these frogs are visionaries with great powers of invisibility when confronted by enemies. Through dreams, the frog gave the power of camouflage, a revered characteristic, to the O'po (a visionary leader) named Sadnba from the Clan Singperi. Other Harakbut O'pos then began to invoke the camouflage power of this frog before encountering their enemies. The Harakbut people also indicate that these frogs are biological indicators of good territory and of climate, noting that their calls announce the rain and forecast the coming of winter.

Distribution and natural history

Pristimantis okmoi **sp. nov.** inhabits *terra firme* premontane and lowland forest in Madre de Dios Department in southern Peru at elevations between 200 and 1,100 m a.s.l. (von May et al., 2010; Catenazzi et al., 2013; Villacampa et al., 2017; von May et al., 2017). This species

is a forest inhabitant that can be found perching at night on tree branches covered with moss (1.0–2.3 m) and has been found active between 17:40–18:50 on 28 September 2014. We also encountered a male individual with a clutch of eggs and froglets (Figs. 8F–G) on 3 February 2015.

Call

We analyzed a call by the paratype MUBI 14547, an adult male recorded calling from a branch 2.3 m above ground at 17:50 during a soft rain on 3 February 2015 (23.5°C air temperature, 100% relative humidity). The recording contained a sequence of trilled calls (*n* = 8) composed of four high-pitched, pulsed notes with length 0.24 ± 0.01 ms, dominant frequency range 4,306.6–4,478.9 Hz (4,403.55 ± 55.20 Hz), call rise time 0.16 ± 0.05 ms, interval between calls 13.45 ± 4.39 ms (10.58–23.19 ms), and a call rate of approximately 5 calls/minute. The interval between pulsed notes is 0.06 ± 0.009 ms (0.045–0.075 ms) per call (Fig. 9).

Taxonomic comments on *Pristimantis imitatrix* (Duellman, 1978)

The holotype (KU 171892, Fig. 10) of *Pristimantis imitatrix* is not well preserved. This subadult female lacks a tympanic annulus and membrane, which are covered by skin and muscle, and seems to have a distinguishable silvery iris in preservation. Those characters are shared with specimens referred erroneously to *P. academicus* by Lehr et al. (2010; Fig. 1C; NMP6F 8, not collected), and new material reviewed herein (Appendix 1) from Buncuya (IIAP 856, 857, 861, 862, 981, 984, 971) and Province Datem del Marañon, Sargento Puño (CORBIDI 8732, 7451, 8735). The specimen KU 171845, erroneously identified as *P. ockendeni,* is diagnosed by lacking a tympanic annulus and membrane, having three barely visible stripes into the hidden surfaces of shanks, and a large bright yellow blotch in the groin extending onto the posterior surfaces of the flanks.

Phylogenetic analyses reveal that specimen KU 215476 referred as *Pristimantis imitatrix* is not related to *P. imitatrix* sensu stricto, but basally related to the clade conformed by *P. carvalhoi* sensu stricto + *P. lirellus* + *Pristimantis* sp. (SBH2008) + *P. okmoi* **sp. nov.** Populations referred by Duellman (2005) as *P. imitatrix* from Cusco Amazónico correspond to a separate cryptic species (CCS1; Fig. 1).

Comparisons with similar species

Pristimantis imitatrix is most similar to *P. academicus* among other lowland Amazonian *Pristimantis* by lacking a tympanic annulus and having a yellow spot on the anterior surfaces of thigh (Table 1). *Pristimantis imitatrix* can be distinguished by having a shagreened dorsum with glandular tubercles and coalescing discontinued dorsolateral folds but fringed fingers, in contrast with the tuberculated dorsum and non-fringed fingers of *P. academicus.* Furthermore, the latter species has brown thighs and hidden surfaces of shanks, compared with the yellow spotted shanks and barred thighs of *P. imitatrix.* Both *P. imitatrix* and *P. gagliardi* **sp. nov.** have large tubercles or pustules on dorsum with dorsolateral discontinuous dermal ridges and lack tympana. However, *P. gagliardi* **sp. nov.** has a tarsal fold and basal webbing between toes, which are absent in *P. imitatrix.* Also, *P. gagliardi* **sp. nov.** specimens have a yellow blotch in hidden surfaces of the shanks, which is absent in *P. imitatrix. Pristimantis okmoi* **sp. nov.** has a visible tympanum on skin, which is absent in *P. imitatrix.* Differences with other brown species of *Pristimantis* in the upper Amazon basin that have bright marks on hidden surfaces of thighs are listed in Table 1.

Coloration in life

Field notes from type specimens IIAP 856–57, IIAP 861–62 describe the following: "Dorsum greenish brown to dull brown, with orange to yellowish cream W-mark on scapula, tubercles, pustules, and folds. Pale bluish-white

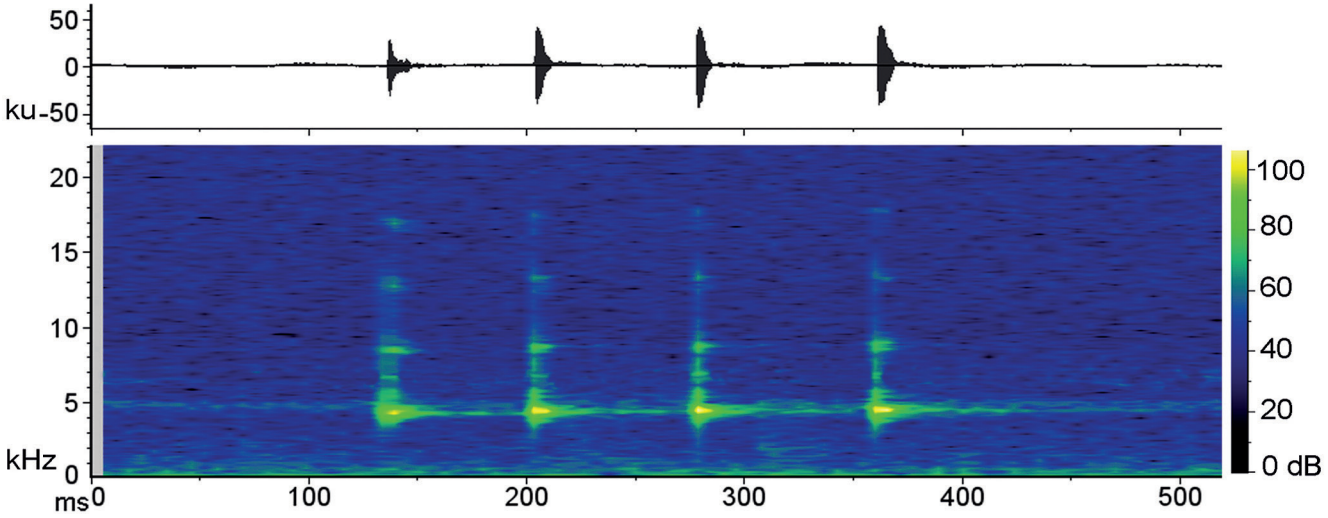


Figure 9. Mating call of *Pristimantis okmoi* **sp. nov.** (MUBI 14547, snout–vent length [SVL] = 13.63 mm). Oscillogram (top) and the respective spectrogram (bottom) showing a call characterized by a trill of four sequenced pulsed notes. Color bars represent the intensity of each call expressed in decibels.

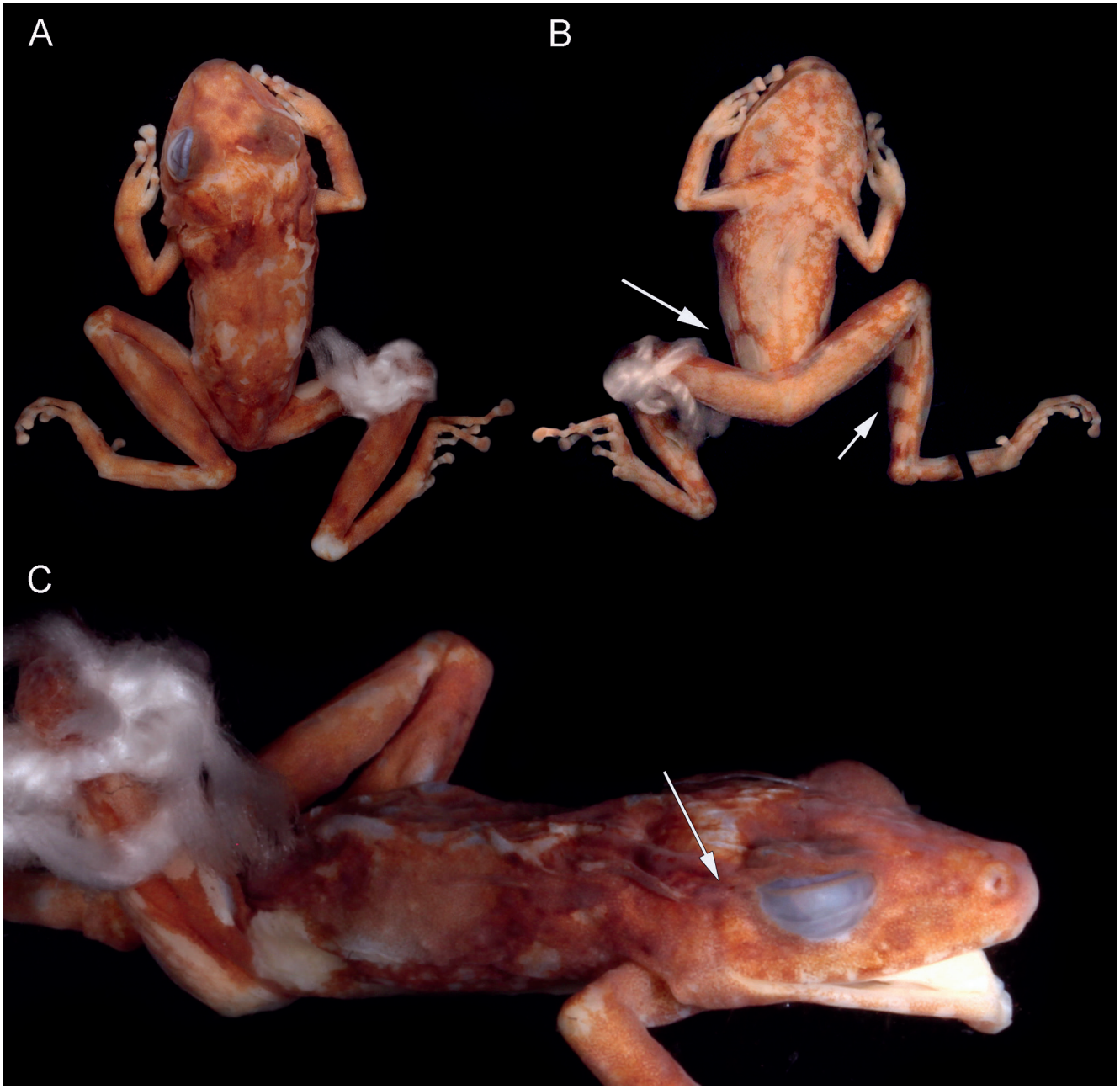


Figure 10. The holotype of *Pristimantis imitatrix* (KU 171892, sub-adult female, snout–vent length [SVL] = 15.7 mm) in **(A)** dorsal, **(B)** ventral, and **(C)** lateral views. Arrows show cream marks in the groin and hidden surfaces of shanks (B) and concealed tympanum (C) in this species. Photos by H.M. Ortega-Andrade.

to white venter and throat, with brown reticulations, flecks or mottling. Groin with a yellow, cream or bluish-cream ovoid blotch, with or without a dark brown border, towards the posterior part of the flanks. Anterior surfaces of thighs and hidden surfaces of shanks dark brown to black with white marks; armpit, ventral and hidden surfaces of limbs bluish-white with brown reticulations, flecks or mottling; posterior surfaces of thighs uniform brown. The flanks are pale greenish brown. Iris silver, finely reticulated with brown; a vertical dark stripe is located from the pupil in the lower half of the iris" (Fig. 11).

Coloration in preservative

In preservation, all greenish brown areas turn into dark brown, whereas the pale orange tubercles and dermal ridges turn into cream. Dorsal surfaces of thighs cream with dark brown transversal brands; bright yellow surfaces of groin and thighs, and white flecks on venter turn into cream. Posterior surfaces of thighs turn to brown.

Variation

Measurements of specimens are listed in Table 2. The single male is smaller (15.70 mm) than the average size of females (23.35 ± 3.04 mm; 20.70–27.00 mm, *n* = 4). Some adult females often have more glandular ridges and tubercles on dorsum, tarsus, and heel (e.g., IIAP 862 vs. IIAP 857, Fig. 11), which is also variable from dull orange to greenish brown. The spot in the groin is variable in color, from bright yellow to cream, with or without a brown border (e.g., IIAP 857 vs. IIAP 856).

Distribution and natural history

Pristimantis imitatrix is known from nine localities from Loreto and Huánuco Departments in northern and central Peru within approximately 105,079 km², at elevations of 130–1,200 m a.s.l. (Fig. 7). This species is a forest inhabitant associated with streams and flooded habitats in premontane and lowland forest in the Peruvian Amazon

Figure 11. (A–B) An amplectant pair (IIAP 861, snout–vent length [SVL] = 15.7 mm; IIAP 862, SVL = 24.7 mm), and **(C–F)** lateral and ventral views of living specimens (C–D: IIAP 857, SVL = 27.0 mm, female; E–F: IIAP 856, male) of *Pristimantis imitatrix* from Peru. Photos by G. Gagliardi.

(Duellman, 1978). An amplectant pair was found in lowland rainforest with old timber trails in Buncuya, perching on a fern frond at night (Lehr et al., 2010; Fig. 11).

DISCUSSION

We have described two new species of *Pristimantis* frogs from Amazonian evergreen lowlands and premontane forests in Cusco and Madre de Dios Departments, southern Peru, that were initially misidentified as *P. carvalhoi.* We used criteria of congruence among phylogenetic analyses, morphometrics, and diagnostic characters to compare the new species with the holotypes of *P. carvalhoi, P. academicus, P. imitatrix, P. lirellus,* and additional material from Amazonia, including other species with yellow spots in the groin and having/lacking an exposed tympanum (Tables 1–2).

The phylogenetic molecular analyses positioned both new species unequivocally within the genus *Pristimantis.* The two species described in this study present differences in diagnostic morphological characters and large genetic distance relative to their sister taxa (Fig. 2). *Pristimantis okmoi* **sp. nov.** is included in a well-supported clade and differentiated morphologically from *P. carvalhoi* sensu stricto and *P. lirellus* (Fig. 1; Table 1). *Pristimantis okmoi* **sp. nov.** was reported as *Eleutherodactylus* "*carvalhoi*" in Rodríguez and Cadle (1990) and as *P. carvalhoi* in Catenazzi et al. (2013), Villacampa et al. (2017), von May et al. (2010), and von May et al. (2017). As part of this lineage, we also identified a confirmed candidate species of *Pristimantis* initially misidentified as *P. imitatrix* in southern Peru that needs further formal description. In contrast, *P. gagliardi* **sp. nov.** is clustered basally with a clade formed by *P. croceoinguinis, P. cruciocularis, P. llojsintuta, P. platydactylus,* and the clade described as *P. okmoi* **sp. nov.** Interestingly, *Pristimantis imitatrix* sensu stricto is sister to *P. academicus* in a well-supported clade located basally to the other species (Fig. 1). As part of the review of additional material collected in Amazonian Peru, and by comparison with the type series, we refer specimens misidentified as *P. academicus* in the original publication (Lehr et al., 2010; Fig. 1C; NMP6F 8, not collected) to *P. imitatrix* sensu stricto.

Multivariate analysis of quantitative morphological data indicated that traits related to limbs (femur, foot, and tibia length) and head (eye–nostril distance, head length, and head width) explain most of the variance and differences between species (Figs. 2–3). While analyses did not show complete separation of all species examined from a morphological perspective, we found high differences in genetic distances in well-supported lineages (Figs. 1–2). This pattern has been reported in several integrative analyses on direct-developing frogs suspected to be cryptic species (i.e., Ortega-Andrade et al., 2015; Ortega Andrade et al., 2017; Rodríguez et al., 2017; Rojas et al., 2018; Páez and Ron, 2019).

Variation in the presence/absence of the tympanic annulus, spots in the groin, body coloration, and, to a lesser extent, skin texture on the dorsum and venter (known to be highly variable; Guayasamin et al., 2015) in living specimens were useful characteristics to identify and separate both species from their congeners (Table 1, Table S3). The absence of a tympanum (condition D sensu Duellman and Lehr, 2009), as occurs in *P. gagliardi* **sp. nov.,** separates it from *P. okmoi* **sp. nov.** (Table 1). The former species is distinguished from all other congeners in Amazonia by the combination of the following characteristics: 1) lack of a visible tympanic annulus and membrane on skin, 2) presence of basal webbing between toes, and 3) presence of yellow blotches in the groin and hidden surfaces of shanks (Table 1). The presence of a tympanic annulus beneath the skin separates *P. okmoi* **sp. nov.** from other *Pristimantis* in the region with yellow spots (i.e., *P. carvalhoi, P. academicus, P. croceoinguinis,* and *P. gagliardi* **sp. nov.**).

Taxonomic status of populations of *P. imitatrix* and *P. carvalhoi* in the Amazon basin

We redefine the taxonomic status of *Pristimantis imitatrix,* a species confused with *P. academicus,* on the basis of additional material from areas near to the type locality and from existing scientific collections (Lehr et al., 2010). *Pristimantis imitatrix* and *P. academicus* are phylogenetically related and morphologically similar to other lowland Amazonian *Pristimantis* by lacking a tympanic annulus and showing a yellow spot on the anterior surfaces of the thighs (Table 1). However, the former species is distinguishable from *P. academicus* by having a shagreen dorsum with glandar tubercles and coalescing discontinued dorsolateral folds, but fingers with lateral fringes, and yellow spotted shanks, and barred thighs in *P. imitatrix* (Table 1).

Pristimantis carvalhoi is considered a widely distributed species in Amazonia, with records from Brazil, Colombia, Peru, and Bolivia (Padial et al., 2004). After reviewing the type material of *P. carvalhoi* and additional specimens from areas near to the type locality in Brazil (Lutz and Kloss, 1952), we hypothesize that southern and eastern Amazonian populations assigned this name (Padial et al., 2004; Frost, 2020) likely correspond to a complex with putative cryptic species. This study reveals latitudinal replacement of several related species with yellow spots in groin, suggesting an allopatric divergence between northern, eastern, and southern Amazonia in Peru (Fig. 7).

The description of two new species and the recognition of an additional confirmed candidate species from southern Amazonia of Peru motivates us to deepen taxonomic, biogeographic, and systematic studies in order to decipher the evolutionary patterns in this widely biodiverse group.

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ONLINE SUPPORTING INFORMATION

The following Supporting Information is available for this article online:

Figure S1. Photographs of dorsal, ventral, and lateral views of the type series from the *Pristimantis carvalhoi* and *P. imitatrix* complexes. **(A)** *P. academicus,* holotype MUSM 27634; **(B)** *P. carvalhoi,* syntype MNRJ 3262; **(C)** *P. imitatrix,* holotype_KU 171892; **(D)** *Pristimantis lirellus,* holotype_KU 212240.

Table S1. Database of examined specimens, collection localities, and geographic coordinates in Amazonia.

Table S2. Sequences and accession numbers to GenBank of specimens used in the phylogenetic analyses.

Table S3. Principal component analysis using morphometric data for the males and females of *Pristimantis gagliardi* **sp. nov.,** *P. okmoi* **sp. nov.,** and other morphologically similar Amazonian species. Loadings and percent of explained variance for principal components I–III are based on residuals from morphometric variables regressed with the snout–vent length. Shaded numbers indicate highest loadings.

APPENDIX 1

Specimens of *Pristimantis imitatrix* and *Pristimantis* Confirmed Candidate Species 1 (CCS1) examined and reported in the text.

Pristimantis imitatrix. **Females:** A total of nine specimens, including two paratypes. Peru: District Yuyapichis, Province Puerto Inca, Department Huanuco: KU 171892, sub-adult female, collected on 20 June 1975 from Rio Llullapichis, 4–5 km upstream from Rio Pachitea, 9°36’00.0”S, 74°56’00.0”W, by Catherine A. Toft; KU 171845, collected on 30 January 1975 from Rio Llullapichis, 4–5 km upstream from Rio Pachitea, Finca Panguana, 9°36’00.0”S, 74°56’00.0”W, by C A. Toft. District Emilio de San Martin, Province Requena, Department Loreto: IIAP 857, gravid female, IIAP 862, gravid female, collected on 10 July 2009 from Buncuya 1, 6°35’19.4”S, 74°36’41.2”W, by G. Gagliardi; IIAP 981, sub-adult female, collected on 21 July 2009 from Buncuya 2, 6°29’33.0”S, 74°33’46.1”W, by G. Gagliardi; IIAP 984, sub-adult female, collected on 24 July 2009 from Buncuya 3, 6°29’12.9”S, 74°34’49.8”W, by G. Gagliardi; IIAP 971, sub-adult female, collected on 20 July 2009 from Buncuya 5, 6°29’00.3”S, 74°33’29.5”W, by G. Gagliardi. District Morona, Province Datem del Marañon: CORBIDI 8732, female, collected on 10 December 2010 from Sargento Puño, 3°13’07.5”S, 77°35’04.2”W, 202 m, by C.Z. Landauro; CORBIDI 7451, juvenile female, collected on 23 September 2010 from Sector 3, 3°08’14.8”S, 77°18’00.4”W, 202 m, by C.Z. Landauro; CORBIDI 8735, gravid female, collected on 23 October 2010 from Sector 4, 3°18’21.2”S, 77°19’17.3”W, 210 m, by C.Z. Landauro. **Male:** Peru: District Emilio de San Martin, Province Requena, Department Loreto:IIAP 861, collected on 10 July 2009 from Buncuya 1, 6°35’19.4”S, 74°36’41.2”W, by G. Gagliardi.

Pristimantis **Confirmed Candidate Species 1 (CCS1).** PERU: Madre de Dios, Cuzco Amazonico, 15 km E Puerto Maldonado, 12°34’60.0”S 69°05’00.0”W: KU 205139, KU 205140, KU 205141, KU 207709, KU 207710, KU 207711, KU 207712, KU 207713, KU 207714, collected on 14 January 1986; KU 215474, KU 215475, KU 215476 collected on 23 June 1989; KU 215477, KU 215478, KU 215746, KU 215747 collected on 21 January 1990.