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## Research article

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# A new minute species of *Pristimantis* (Amphibia: Anura: Craugastoridae) with a large head from the Yanachaga-Chemillén National Park in central Peru, with comments on the phylogenetic diversity of *Pristimantis* occurring in the Cordillera Yanachaga

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**Abstract.** We describe a new minute species of the genus *Pristimantis*, *P. boucephalus* sp. nov., from the Yanachaga-Chemillén National Park, Región Pasco, Peru. The description is based on a freshly collected male specimen found at 2950 m a.s.l. in a cloud forest and four previously unidentified museum specimens consisting of two adult males, one subadult female and a juvenile from the Yanachaga-Chemillén National Park. The new species is mainly characterized by a snout–vent length of 13.4–14.5 mm in adult males (n = 3), and 12.5 mm in the only known subadult female, and is compared morphologically and genetically with other taxonomically and biogeographically relevant species of *Pristimantis*. The new species is characterized by its small size, disproportionally large head with short snout, absence of a tympanic annulus and membrane, and reddish-copper iris. Phylogenetically it belongs to a speciose clade, an as yet unnamed species group, comprising both montane (Andes, Guiana Shield) and lowland (Amazon) taxa from the northern part of South America. The new species is genetically close to the sympatric *P. cruciocularis*. Species of *Pristimantis* occurring in the Cordillera Yanachaga region in the Andes of central Peru are members of six divergent phylogenetic lineages.

**Keywords.** Andes, DNA barcoding, frogs, molecular phylogeny, new species.

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## Introduction

In the area of the Selva Central of Peru (Regions of Pasco and Junín, central Peru), there are twelve natural areas with different levels of legal protection such as national parks, national sanctuaries, national reserves and others of lower protection categories (SERNANP 2010). In 2012 we started surveying the herpetofauna of the Yanachaga-Chemillén National Park (hereafter YCNP; Región Pasco, Fig. 1), with the aim to obtain a better knowledge of the anuran species richness of the YCNP and to provide data needed for an effective protection of the park. The expeditions in the YCNP led to the discovery of several new species of amphibians (e.g., *Phrynopus badius* Lehr, Moravec & Cusi, 2012, *Phrynopus curator* Lehr, Moravec & Cusi, 2012 and *Rhinella yunga* Moravec, Lehr, Cusi, Córdova & Gvoždík, 2014). Among the new findings was an undescribed species of *Pristimantis* Jiménez de la Espada, 1870 readily distinguishable from its local congeners by its small size with a disproportionately large head, short snout, and reddish-copper iris. However, because only a single male specimen was available at that time, we refrained from formally describing it. Revision of specimens from the YCNP housed in the herpetological collection of the Museo de Historia Natural Universidad Nacional Mayor de San Marcos (Lima, Peru) in 2015 led to the discovery of four additional specimens (labeled *Pristimantis* sp.) of the same taxon. Close examination of the five specimens revealed that they bear several morphological characteristics not shared by any other described species of *Pristimantis* in Peru. Therefore, we describe the new species and compare it morphologically and genetically with other taxonomically and biogeographically relevant species of *Pristimantis* from Bolivia, Ecuador, Peru, the Guyanas, and Venezuela. A phylogenetic analysis provided additional insights into the phylogenetic diversity of *Pristimantis* from the area of the Cordillera Yanachaga.

## Material and methods

### *Morphological characters*

The format for the description follows Lynch & Duellman (1997), and diagnostic characters are those of Duellman & Lehr (2009). Taxonomic classification follows Hedges *et al.* (2008) and Heinicke *et al.* (2009), except that we followed Pyron & Wiens (2011) for family placement and Padial *et al.* (2014) for names of *Pristimantis* species groups. We consider minute species of amphibians as having an adult snout–vent length (SVL) below 20 mm, as suggested by Clarke (1996). The holotype was fixed in 96% ethanol and stored in 70% ethanol. Liver tissue of the holotype was taken for genetic analyses. Sex and maturity of specimens were identified by observing secondary sexual characters (vocal slits), and gonads through dissections. Specimens with a SVL  $\leq$  10 mm were considered juveniles when gonads were too small to distinguish between sexes. We used maximum known SVL for males within a species to recognize smallest body size as recommended by Lehr & Coloma (2008). We measured the following variables to the nearest 0.1 mm with digital calipers under a stereo microscope: snout–vent length (SVL, straight length distance from tip of snout to vent), tibia length (TL, distance from the knee to the distal end of the tibia), foot length (FL, distance from proximal margin of inner metatarsal tubercle to tip of Toe IV), head length (HL, from angle of jaw to tip of snout), head width (HW, at level of angle of jaw), horizontal eye diameter (ED), interorbital distance (IOD), upper eyelid width (EW), internarial distance (IND), eye–nostril distance (E–N, straight line distance between anterior corner of orbit and posterior margin of external nares). Fingers and toes are numbered preaxially to postaxially as I–IV and I–V, respectively. We compared the lengths of Toes III and V by adpressing both toes against Toe IV; lengths of Fingers I and II were compared by adpressing the fingers against each other. To avoid reflection, the preserved holotype was photographed immersed in ethanol. All drawings were made by JM using a stereo microscope and a camera lucida. Photographs taken by JM and EL were used for descriptions of coloration in life. Comparisons of congeners focused on species with similar SVL from Ecuador and Peru and genetically close species as recovered in our trees. Information on species for comparative diagnoses was obtained from Duellman & Lehr (2009) and from original species descriptions. For specimens examined see Appendix.

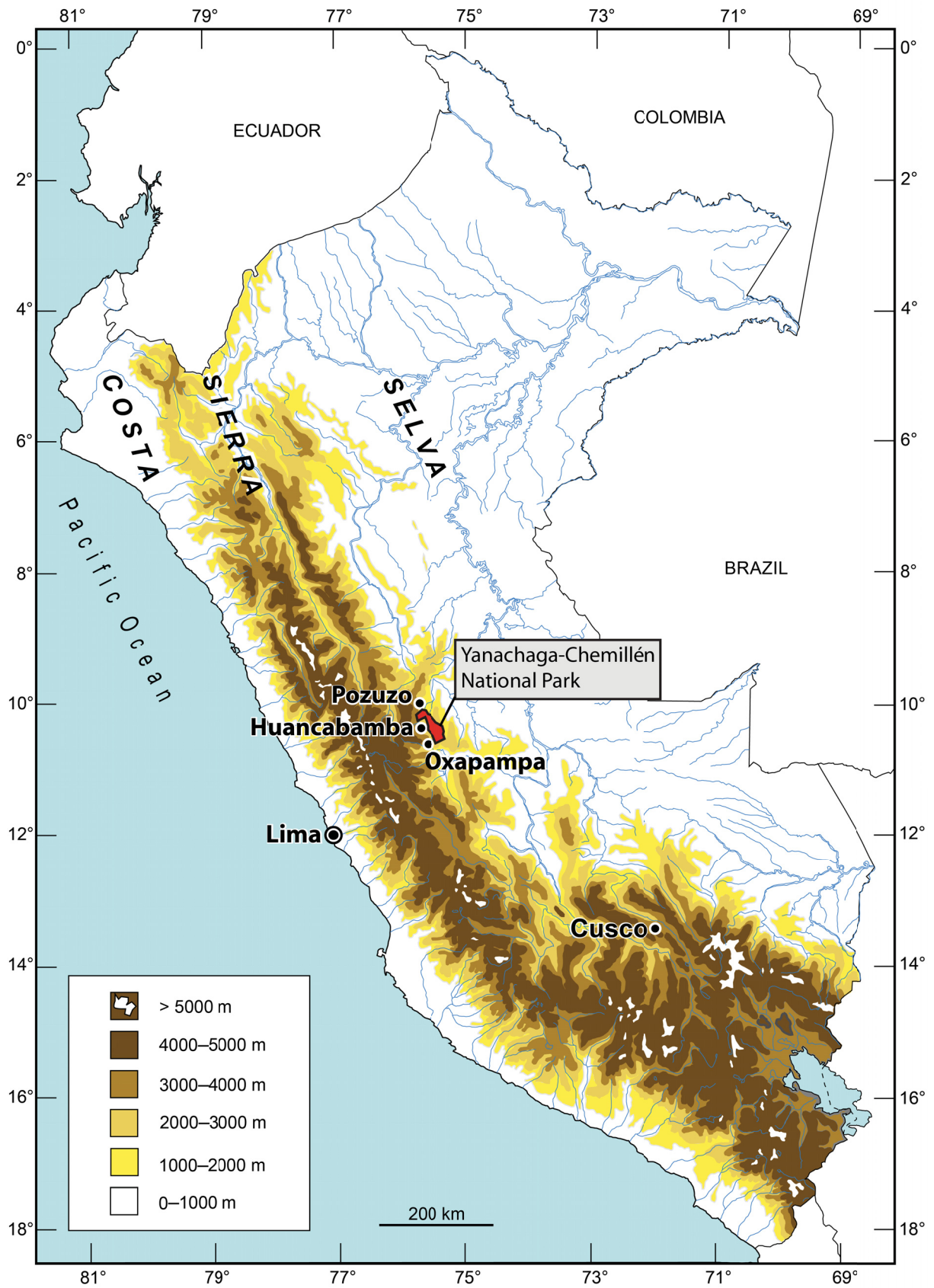


Fig. 1. Map of Peru with the Yanachaga-Chemillén National Park indicated in red.

Codes of collections are:

- KU = University of Kansas, Museum of Natural History, Lawrence, KS, USA  
MUSM = Museo de Historia Natural Universidad Nacional Mayor de San Marcos, Lima, Peru  
NMP6V = National Museum Prague, Prague, Czech Republic  
SMNS = Staatliches Museum für Naturkunde Stuttgart, Stuttgart, Germany  
USNM = National Museum of Natural History, Smithsonian Institution, Washington DC, USA

Threat status was evaluated using the IUCN criteria (2016).

## **Molecular analysis**

### **Taxon sampling**

We included samples of various *Pristimantis* species collected by us in the YCNP during a survey in 2012, and from the nearby Bosque de Shollet Protected Area (BS) and Pui Pui Protected Forest (PPPF) collected between 2012 and 2014. The aim was to detect phylogenetic diversity of *Pristimantis* species distributed in these and nearby montane regions of the Cordillera Yanachaga. Most of the specimens were identified to species level, with the exception of the single ‘bigheaded’ male (mentioned above) and a few specimens from the Pui Pui forest, which are tentatively named *Pristimantis* sp. Pui Pui. A list of the newly genetically investigated material and its GenBank accession numbers is in Table 1. For the final dataset, we retrieved additional sequences conspecific with our samples from GenBank to show phylogenetic positions of our new material in relation to DNA sequences published earlier (most importantly in the review by Hedges *et al.* 2008). We also retrieved and included species known to occur in the Cordillera Yanachaga region (Duellman & Hedges 2005, 2007) but not recorded by us (*P. albertus* Duellman & Hedges, 2007, *P. minutulus* Duellman & Hedges, 2007, *P. stictogaster* (Duellman & Hedges, 2005), *P. sagittulus* (Lehr, Aguilar & Duellman, 2004)). In addition, we specifically focused on the ‘bigheaded’ specimen and applied a BLAST search (Altschul *et al.* 1990) using the BLASTN v. 2.3.1 program (Zhang *et al.* 2000; Morgulis *et al.* 2008) for the most similar DNA sequences available in GenBank based on the 16S rRNA gene fragment (see below). We selected and included into our dataset sequences of species or main evolutionary lineages of species complexes (e.g., the *P. platydactylus* complex) with nucleotide sequence identity in relation to our ‘bigheaded’ specimen  $\geq 90\%$ , and which formed a clade in the BLAST minimum-evolution tree. We also included species from the sister clade, which also contained one species known from the YCNP, *P. minutulus*. As outgroups, we used the craugastorid genera *Oreobates* Jiménez de la Espada, 1872 (*O. cruralis* (Boulenger, 1902)) and *Phrynopus* Peters, 1873 (*P. bracki* Hedges, 1990), retrieved from GenBank. The final dataset was composed of 87 samples of 37 nominal taxa, including the new species and outgroups. All sequences acquired from GenBank can be identified by the GenBank accession numbers as given in Fig. 2.

### **DNA extraction, PCR, sequencing and sequence alignment**

Genomic DNA was extracted from tissues stored in 96% ethanol. A fragment of the mitochondrial gene for 16S rRNA (*16S*), which is commonly used in amphibian DNA barcoding (Vences *et al.* 2012), was targeted using the primers 16SL1 and 16SH1 adapted or directly taken from Palumbi *et al.* (1991). For primer sequences and PCR conditions see Moravec *et al.* (2009). Sequencing was performed by using the PCR primers. Additionally, a newly designed internal primer (prist16SinR1: 5'-ATGTTTAAAGCTCCA-3') had to be used for the ‘bigheaded’ *Pristimantis* to overcome a problem with a G–C homopolymer region in the middle of the fragment. New sequences have been deposited in GenBank (KY006082–006112). The multiple sequence alignment was performed using MAFFT v. 7.1 (Kato & Standley 2013), producing a 561 bp long alignment. Ambiguously aligned positions were eliminated by Gblocks v. 0.91b under options for a less stringent selection (Castresana 2000), producing a final alignment of 510 bp.

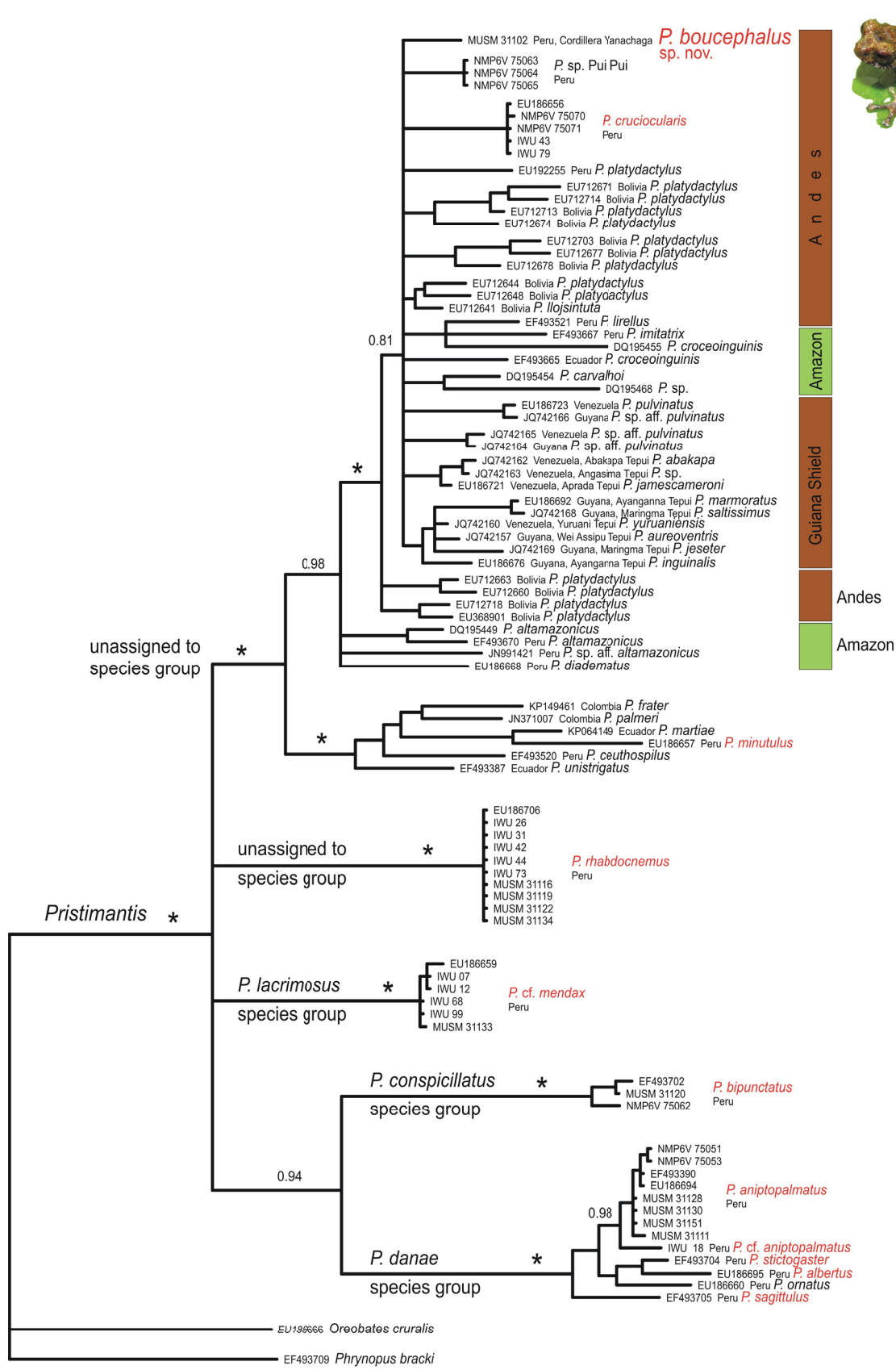


**Table 1.** Names of taxa, field numbers, museum numbers, field data, and GenBank accession numbers of newly genetically investigated material. The new species is indicated in bold. For abbreviations see text.

Taxon	Field numbers	Museum number	Locality	Coordinates	Elevation	GenBank Accession numbers
<i>P. aniptopalmodus</i>	IWU28	MUSM 31111	YCNP	10°23.718' S, 75°28.919' W	2290 m	KY006082
<i>P. aniptopalmodus</i>	IWU58	MUSM 31128	YCNP	10°40.531' S, 75°19.2897' W	2430 m	KY006083
<i>P. aniptopalmodus</i>	IWU64	MUSM 31130	YCNP	10°40.531' S, 75°19.2897' W	2430 m	KY006084
<i>P. aniptopalmodus</i>	IWU97	MUSM 31151	YCNP	10°39'05.7" S, 75°17'39.3" W	2836 m	KY006085
<i>P. aniptopalmodus</i>	IWU272	NMP6V 75051	PPPF	11°07'40.6" S, 75°11'15.7" W	2230 m	KY006086
<i>P. aniptopalmodus</i>	IWU265	NMP6V 75053	PPPF	11°07'40.6" S, 75°11'15.7" W	2230 m	KY006087
<i>P. cf. aniptopalmodus</i>	IWU18	–	YCNP	10°22.772' S, 75°23.717' W	2950 m	KY006088
<i>P. bipunctatus</i>	IWU46	MUSM 31120	YCNP	10°23.718' S, 75°28.919' W	2290 m	KY006089
<i>P. bipunctatus</i>	IWU346	NMP6V 75062	PPPF	11°12'38.5" S, 74°57'28.9" W	1700 m	KY006090
<b><i>P. boucephalus</i> sp. nov.</b>	<b>IWU14</b>	<b>MUSM 31102</b>	<b>YCNP</b>	<b>10°22.772' S, 75°23.717' W</b>	<b>2950 m</b>	<b>KY006091</b>
<i>P. cruciocularis</i>	IWU43	–	YCNP	10°23.718' S, 75°28.919' W	2290 m	KY006092
<i>P. cruciocularis</i>	IWU79	–	YCNP	10°26.688' S, 75°26.773' W	2100 m	KY006093
<i>P. cruciocularis</i>	IWU226	NMP6V 75070	PPPF	11°06'17.0" S, 75°12'26.2" W	1730 m	KY006094
<i>P. cruciocularis</i>	IWU240	NMP6V 75071	PPPF	11°07'37.2" S, 75°10'37.0" W	1970 m	KY006095
<i>P. cf. mendax</i>	IWU07	–	YCNP	10°22.772' S, 75°23.717' W	2950 m	KY006096
<i>P. cf. mendax</i>	IWU12	–	YCNP	10°22.772' S, 75°23.717' W	2950 m	KY006097
<i>P. cf. mendax</i>	IWU67	MUSM 31133	YCNP	10°40.531' S, 75°19.2897' W	2430 m	KY006098
<i>P. cf. mendax</i>	IWU68	–	YCNP	10°40.531' S, 75°19.2897' W	2430 m	KY006099
<i>P. cf. mendax</i>	IWU99	–	YCNP	10°39'05.7" S, 75°17'39.3" W	2836 m	KY006100
<i>P. rhabdocnemus</i>	IWU26	–	YCNP	10°23.718' S, 75°28.919' W	2290 m	KY006101
<i>P. rhabdocnemus</i>	IWU31	–	YCNP	10°23.718' S, 75°28.919' W	2290 m	KY006102
<i>P. rhabdocnemus</i>	IWU37	MUSM 31116	YCNP	10°23.718' S, 75°28.919' W	2290 m	KY006103
<i>P. rhabdocnemus</i>	IWU41	MUSM 31119	YCNP	10°23.718' S, 75°28.919' W	2290 m	KY006104
<i>P. rhabdocnemus</i>	IWU42	–	YCNP	10°23.718' S, 75°28.919' W	2290 m	KY006105
<i>P. rhabdocnemus</i>	IWU44	–	YCNP	10°23.718' S, 75°28.919' W	2290 m	KY006106
<i>P. rhabdocnemus</i>	IWU48	MUSM 31122	YCNP	10°23.718' S, 75°28.919' W	2290 m	KY006107
<i>P. rhabdocnemus</i>	IWU71	MUSM 31134	BS	10°40.531' S, 75°19.2897' W	2430 m	KY006108
<i>P. rhabdocnemus</i>	IWU73	–	BS	10°40.531' S, 75°19.2897' W	2430 m	KY006109
<i>P. sp. Pui Pui</i>	IWU388	NMP6V 75063	PPPF	11°12'38.5" S, 74°57'28.9" W	1700 m	KY006110
<i>P. sp. Pui Pui</i>	IWU377	NMP6V 75064	PPPF	11°12'38.5" S, 74°57'28.9" W	1700 m	KY006111
<i>P. sp. Pui Pui</i>	IWU344	NMP6V 75065	PPPF	11°12'38.5" S, 74°57'28.9" W	1700 m	KY006112

## Phylogenetic analysis

The Bayesian inference (BI) was applied to construct a phylogenetic tree. First, the software jModelTest v. 2.1.7 (Darriba *et al.* 2012) using the PhyML algorithm (Guindon & Gascuel 2003) was used to find the best-fitting model of nucleotide evolution, which was the GTR+I+G model based on both the Akaike and Bayesian information criteria. The BI analysis was run in MrBayes v. 3.2.5 (Ronquist *et al.* 2012), with two runs and four chains in each run for  $6 \times 10^6$  generations, sampling every 100<sup>th</sup> generation. Appropriate sampling was controlled by examining the stationarity of log-likelihood scores against the generation time using Tracer v. 1.6 (Rambaut *et al.* 2013; all parameters had effective sample size > 1000), and convergence between the two simultaneous runs was confirmed by the convergence diagnostics of the average standard deviation of split frequencies and the potential scale reduction factor values. From the sampled trees, 25% were discarded as a burn-in and a 50% majority-rule consensus tree was produced from the remaining post burn-in trees. The posterior probabilities (pp) were calculated as the frequency of samples recovering any particular clade. Clades supported with pp values  $\geq 0.95$  were considered highly supported (Huelsenbeck & Rannala 2004). Genetic uncorrected *p*-distances were calculated in PAUP\* (Swofford 2003).



## Results

### *Molecular analysis*

Our morphology-based species identifications of taxa recorded in the Cordillera Yanachaga were supported by 16S DNA barcodes as they were identical or closely related to haplotypes available in GenBank (*Pristimantis aniptopalmatus* (Duellman & Hedges, 2005), *P. bipunctatus* (Duellman & Hedges, 2005), *P. cruciocularis* (Lehr, Lundberg, Aguilar & von May, 2006), *P. cf. mendax* (Duellman, 1978), *P. rhabdocnemus* (Duellman & Hedges, 2005)). The inferred phylogenetic tree (Fig. 2) showed that species of *Pristimantis* occurring in the Cordillera Yanachaga and nearby regions belonged to at least six deeply divergent lineages (marked as species groups in Fig. 2). Five species (including samples retrieved from GenBank) clustered together forming a well-supported lineage: *P. albertus*, *P. aniptopalmatus*, *P. ornatus* (Lehr, Lundberg, Aguilar & von May, 2006) (from a site near the Cordillera Yanachaga), *P. sagittulus*, and *P. stictogaster*. We also uncovered one distinct lineage within ‘*P. aniptopalmatus*’ (3.8% average genetic uncorrected *p*-distance to other ‘conspecifics’), which we tentatively name *P. cf. aniptopalmatus*. *Pristimantis bipunctatus*, *P. cf. mendax*, *P. minutulus* (taken from GenBank), and *P. rhabdocnemus* are members of four other divergent lineages. The ‘bigheaded’ *Pristimantis* from the YCNP is, together with *P. sp.* Pui Pui from the geographically close PPPF and *P. cruciocularis* from both the YCNP and PPPF, a member of another lineage. This lineage represents a speciose clade, which comprises montane species from the Andes and the Guiana Shield and also lowland species from the intervening Amazon lowlands. The topology within this clade is not resolved, but the clade is highly supported (pp = 0.98). Among the sympatric species, the genetically closest of the ‘bigheaded’ *Pristimantis* is *P. cruciocularis*, with a genetic uncorrected *p*-distance of 5.8%. The genetic uncorrected *p*-distances among the main lineages range between 13 and 17%.

### *Description of new species*

Order Anura Fischer von Waldheim, 1813  
 Superfamily Brachycephaloidea Günther, 1858  
 Family Craugastoridae Hedges, Duellman & Heinicke, 2008  
 Subfamily Ceuthomantinae Heinicke, Duellman, Trueb, Means, MacCulloch & Hedges, 2009  
 Genus *Pristimantis* Jiménez de la Espada, 1870

***Pristimantis boucephalus* sp. nov.**

[urn:lsid:zoobank.org:act:BCF78234-F3F2-4A81-AE80-1B4BE26B1E1F](http://urn:lsid:zoobank.org:act:BCF78234-F3F2-4A81-AE80-1B4BE26B1E1F)

Figs 3–7, Tables 1–4

*Pristimantis* sp. 4 – Angulo *et al.* 2016: 4, figs 76–77.

Suggested English name: Bigheaded Rubber Frog.

Suggested Spanish name: Rana cutín cabeza.

**Fig. 2** (opposite page). The Bayesian phylogenetic tree of selected South American *Pristimantis*; for taxon sampling design see Material and methods. Nodes were collapsed if they appeared in less than 50% of the post burn-in tree samples. Support values of posterior probabilities (pp) given only for discussed lineages (stars indicate full support, pp = 1.00). Taxa in red occur in the Cordillera Yanachaga region and represent six divergent lineages/species groups (uncorrected *p*-distance 13–17%). The new species, *P. boucephalus* sp. nov., is a member of a speciose clade, an as yet unnamed species group, comprising both montane taxa from the Andes and Guiana Shield, and lowland taxa from the intervening Amazon Basin. Species-group names follow Padial *et al.* (2014). DNA sequences of taxa retrieved from GenBank are labelled by standard GenBank accession numbers, all other codes stand for our new material.

### Etymology

The species epithet *boucephalus* is derived from the Greek prefix “bou-“ meaning large, huge, or great, and the Greek noun “cephalo” meaning head. The name refers to the disproportionately large head of the new species.

### Material examined

#### Holotype

PERU: ♂, adult (Figs 3–5), Yanachaga-Chemillén National Park (Sector San Daniel), Distrito de Huancabamba, Provincia de Oxapampa, Región Pasco, Quebrada Yanachaga, 10°22'46.3 S, 75°27'43.0 W, 2950 m a.s.l., Edgar Lehr, Juan C. Cusi and Jiří Moravec leg., 19 Jan. 2012 at 20h30 (MUSM 31102, GenBank *16S rRNA* barcode: KY006091).

#### Paratypes (n = 4)

PERU: 2 ♂♂ (MUSM 24477–78), 1 subadult ♀ (MUSM 24479) and 1 juvenile (MUSM 24474), all from the Yanachaga-Chemillén National Park (based on field numbers, detailed collecting data missing), collected during the 1990s, Javier Icochea leg., see Fig. 6.

### Generic placement

We assign this species to *Pristimantis* based on its general morphological similarity to other members of the genus and our molecular data (Fig. 2).

### Definition

A new species of *Pristimantis*, not assigned to any species group, having the following combination of characters: (1) Skin on dorsum smooth with few low scattered tubercles, skin on venter areolate with low scattered tubercles; discoidal and thoracic folds absent; dorsolateral folds absent; (2) tympanic membrane and tympanic annulus absent; (3) snout short, rounded in dorsal and lateral views; (4) upper eyelid with one enlarged conical tubercle at its center and one enlarged conical tubercle at its posterior end; EW slightly shorter than IOD; cranial crests absent; (5) dentigerous processes of vomers absent; (6) males with vocal slits, nuptial pads absent; (7) Finger I shorter than Finger II; discs of digits expanded, rounded; (8) fingers with lateral fringes; (9) small conical ulnar and tarsal tubercles present; (10) heel with a conical tubercle; inner tarsal fold absent; (11) inner metatarsal tubercle ovoid, 2.5 times as large as outer; outer metatarsal tubercle small, rounded; numerous low, supernumerary plantar tubercles; (12) toes with lateral fringes; basal toe webbing present; Toe V longer than Toe III; toe discs slightly smaller than those on fingers; (13) in life, dorsal ground coloration greenish gray with reddish-brown blotches and scattered brown flecks surrounded by black; canthal and supratympanic stripes absent; groin and anterior surfaces of thighs greenish yellow with black blotches; venter gray with pale reddish and greenish brown mottling and scattered dark gray flecks; iris reddish copper with fine black vermiculation and black narrow vertical streak from pupil across lower half of iris; (14) SVL in adult males 13.4–14.5 mm (n = 3), in adult females unknown (12.5 mm in single subadult female).

### Differential diagnosis

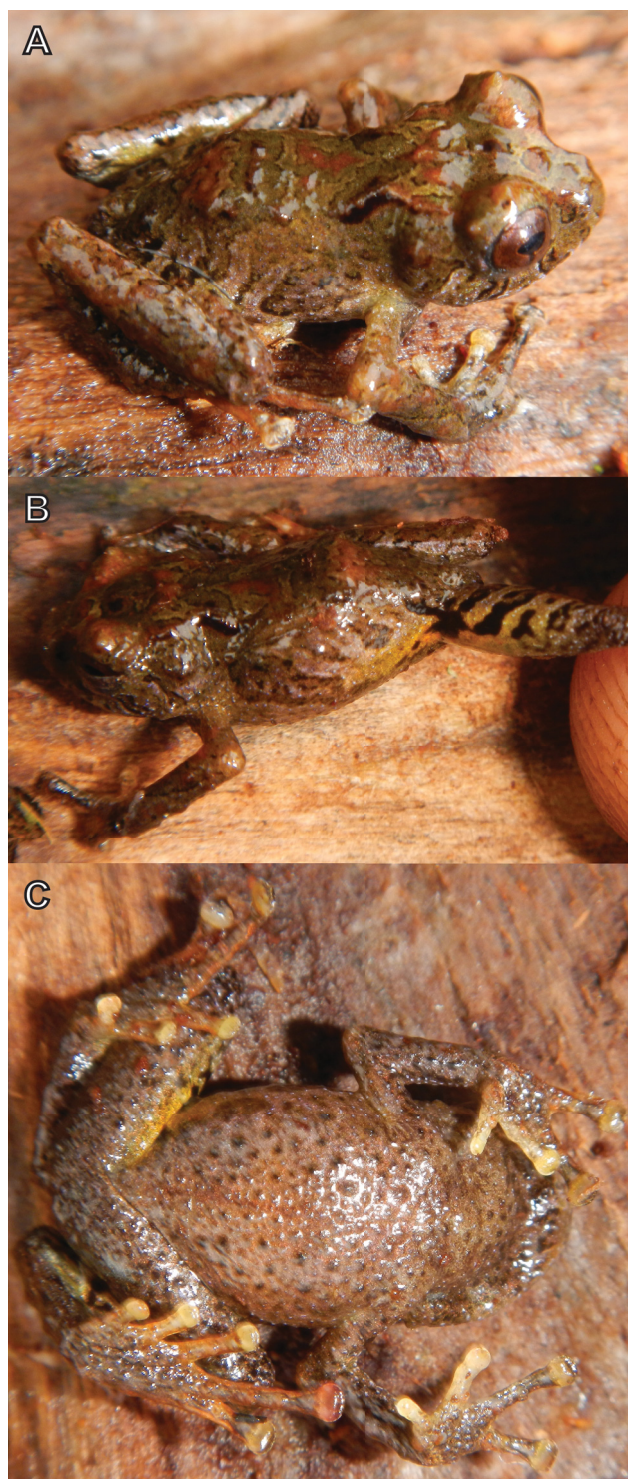
*Pristimantis boucephalus* sp. nov. is readily distinguished from its congeners in Ecuador and Peru by its minute SVL, short snout, absence of a tympanum, large head, groin and anterior surfaces of thighs greenish yellow with black blotches, and reddish-copper iris. It is currently one of the smallest of the 130 species of *Pristimantis* from Peru (AmphibiaWeb 2017) and the 199 species of *Pristimantis* from Ecuador (Ron *et al.* 2017, see Table 2).

*Pristimantis boucephalus* sp. nov. can be distinguished from other minute Andean species of *Pristimantis* from Ecuador (*P. andinognomus*, *P. minimus*, *P. trachyblepharis*) and Peru (*P. caeruleonotus*,



*P. coronatus*, *P. minutulus*, *P. trachyblepharis*) as follows (characters of *P. boucephalus* in parenthesis, if not otherwise stated):

*Pristimantis minutulus* Duellman & Hedges, 2007 from elevations of 250–1200 m a.s.l. in central Peru has larger males (SVL 13.1–17.6 mm [n = 7] vs 13.4–14.5 mm [n = 3] in *P. boucephalus* sp. nov.; Duellman & Hedges 2007). *Pristimantis minutulus* and *P. boucephalus* sp. nov. lack dorsolateral folds, discoidal folds, a tympanum, and dentigerous processes of vomers and both have a smooth dorsum



**Fig. 3.** Live holotype (MUSM 31102, SVL 14.1 mm) of *Pristimantis boucephalus* sp. nov. **A.** Dorsal view. **B.** Dorsolateral view. **C.** Ventral view. Photos by E. Lehr.

**Table 2.** Comparisons of *P. boucephalus* sp. nov. with other small and minute species of *Pristimantis* from Ecuador and Peru. Characters were taken from original species descriptions and the examined material. Presence of a character is indicated by ‘+’, its absence by ‘–’.

Character	<i>boucephalus</i> sp. nov.	<i>andinognomus</i>	<i>caeruleonotus</i>	<i>minimus</i>	<i>minutulus</i>	<i>trachyblepharis</i>
Reference	this paper	Lehr & Coloma (2008)	Lehr <i>et al.</i> (2007)	Terán-Valdez & Guayasamin (2010)	Duellman & Hedges (2007)	Lynch & Duellman (1980)
Male SVL (mm)	13.4–14.5 (n = 3)	10.0–14.5 (n = 21)	11.6–14.6 (n = 4)	9.5–13.7 (n = 46)	13.1–17.6 (n = 7)	12.1–15.8 (n = 20)
Female SVL (mm)	12.5 (n = 1)	12.6–17.9 (n = 19)	20.5–22.6 (n = 4)	15.3–18.9 (n = 25)	17.0–20.1 (n = 3)	15.8–19.2 (n = 19)
Dorsolateral folds	–	+	+	–	–	–
Tympanic membrane and annulus	–	+	+	+	–	+
Vocal slits	+	+	+	+	–	–
Nuptial pads	–	–	–	–	–	–
Lateral fringes	+	+	+	–	– or +	–
Elevational range (m)	2950	2450–2800	2500–2900	1250–1685	250–1200	100–1250

and areolate venter, but *P. minutulus* has the snout moderately long (short), males without vocal slits (present), groin with large yellow spot (greenish yellow with black blotches), and iris reddish gray (reddish copper with fine black vermiculation). *Pristimantis caeruleonotus* Lehr, Aguilar, Siu-Ting & Jordán, 2007 from elevations of 2500–2900 m a.s.l. of humid montane forests in northern Peru has males of similar size (SVL 11.6–14.6 mm [n = 4] vs 13.4–14.5 mm [n = 3] in *P. boucephalus* sp. nov.; Lehr *et al.* 2007). Both *P. caeruleonotus* and *P. boucephalus* sp. nov. have fingers and toes with lateral fringes, heel with conical tubercle, and males that have vocal slits, but lack nuptial pads. However, *P. caeruleonotus* has a distinct tympanum (absent), dorsolateral folds (absent), small dentigerous processes of vomers (absent), a discoidal fold (absent), prominent dorsolateral folds ending in a sacral tubercle (absent), and a long snout with terminal papillae (short snout without papillae). Furthermore, *P. caeruleonotus* has the groin blackish brown with yellow and pale blue blotches (greenish yellow with black blotches). *Pristimantis coronatus* Lehr & Duellman, 2007 is known from a single female specimen of 15.3 mm SVL from 2850 m a.s.l. in northern Peru (Lehr & Duellman 2007). It shares with *P. boucephalus* sp. nov. the absence of dorsolateral folds and a tympanum, and the presence of conical tubercles on the upper eyelid and fingers and toes with lateral fringes. However, *P. coronatus* has dentigerous processes of vomers (absent), groin red and black (greenish yellow with black blotches), and the iris dark brown (reddish copper). *Pristimantis trachyblepharis* (Boulenger, 1918) from elevations of 100–1250 m a.s.l. in southern Ecuador and northern Peru and *P. boucephalus* sp. nov. have smooth dorsum, areolate venter, dorsolateral folds absent, and males that lack nuptial pads. However, *P. trachyblepharis* tends to have larger males (SVL 12.1–15.8 mm [n = 20] vs 13.4–14.5 mm [n = 3] in *P. boucephalus* sp. nov.), tympanum present (absent), males without vocal slits (present), dentigerous processes of vomers



**Fig. 4.** Preserved holotype (MUSM 31102, SVL 14.1 mm) of *Pristimantis boucephalus* sp. nov. A. Dorsal view. B. Ventral view. Photos by E. Lehr.



(absent), and fingers and toes without lateral fringes (present) (Lynch & Duellman 1980). *Pristimantis andinognomus* Lehr & Coloma, 2008 from 2450–2800 m a.s.l. in the Cordillera Oriental in southern Ecuador (Zamora Chinchipe) has males of similar size (SVL 10.0–14.5 mm [n = 20] vs 13.4–14.5 mm [n = 3] in *P. boucephalus* sp. nov.), males that have vocal slits but lack nuptial pads, fingers and toes with lateral fringes, and supernumerary plantar tubercles (Lehr & Coloma 2008). However, *Pristimantis andinognomus* has short dorsolateral folds that end in a conical sacral tubercle (absent), tympanic membrane and tympanic annulus present (absent), a short snout with terminal tubercle (terminal tubercle absent), minute dentigerous processes of vomers (absent), flanks with tubercles coalescing into short ridges (ridges absent), heel with two enlarged tubercles (one heel tubercle), and inner tarsal fold present (absent). *Pristimantis minimus* Terán-Valdez & Guayasamin, 2010 from the Cordillera del Condor in Ecuador at elevations between 1250 and 1685 m a.s.l. has smaller males on average (SVL 9.5–13.7 mm [n = 46] vs 13.4–14.5 mm [n = 3] in *P. boucephalus* sp. nov.; Terán-Valdez & Guayasamin 2010). *Pristimantis minimus* and *P. boucephalus* sp. nov. lack dorsolateral, discoidal and thoracic folds, have a short snout, and males have vocal slits present and nuptial pads absent. However, *P. minimus* has dentigerous processes of vomers (absent), fingers and toes without lateral fringes (present), heel without tubercles (heel with a conical tubercle), upper eyelid without enlarged tubercles (enlarged upper eyelid tubercles present), and has red iris with black reticulations (iris reddish copper with fine black vermiculation and narrow black vertical streak from pupil across lower half of iris).

*Pristimantis boucephalus* sp. nov. can be distinguished from other species of *Pristimantis* from Ecuador and Peru that are on average larger but have males of similar size as follows:

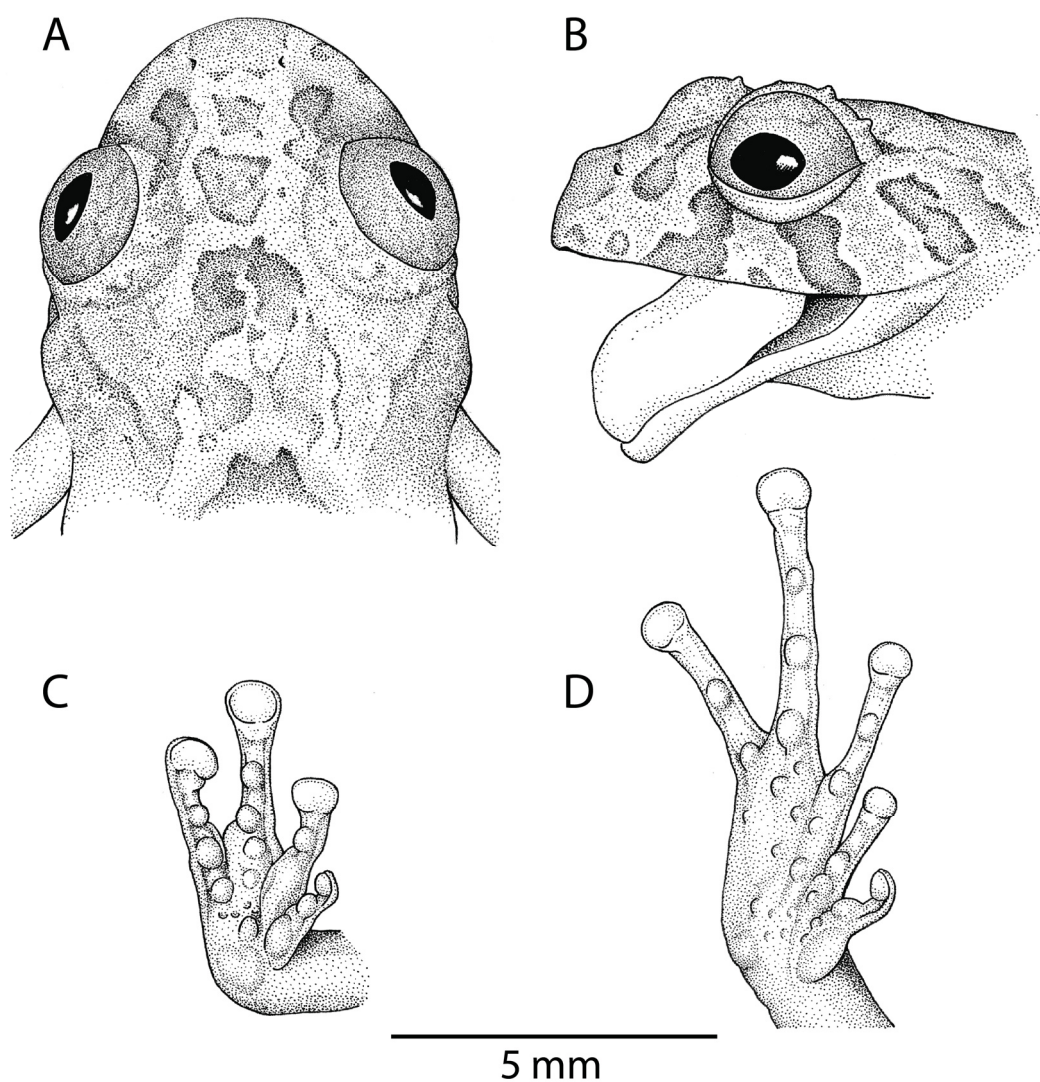
*Pristimantis carvalhoi* (Lutz, 1952) from the Amazonian lowlands and *P. boucephalus* sp. nov. both have males of similar size (SVL 13.4–14.8 mm vs 13.4–14.5 mm [n = 3] in *P. boucephalus* sp. nov.; Lynch 1980), no dorsolateral folds, and a groin with yellow coloration. However, *P. carvalhoi* has a long snout (short), tympanic annulus present (absent), prominent dentigerous processes of vomers (absent), vocal slits absent (present), and fingers and toes without lateral fringes (present). *Pristimantis imitatrix* (Duellman, 1978) from the Amazonian lowlands has slightly smaller males (SVL 13.0–14.0 mm [n = 9] vs 13.4–14.5 mm [n = 3] in *P. boucephalus* sp. nov.; Duellman 2005) and shares with *P. boucephalus* sp. nov. the absence of a tympanum, dorsolateral folds, dentigerous processes of vomers, and nuptial pads, and both species have fingers and toes with lateral fringes. However, *P. imitatrix* lacks vocal slits (present), has the groin mottled black and white (greenish yellow with black blotches) and the iris is bronze (reddish copper). *Pristimantis lirellus* (Dwyer, 1995) from elevations of 470–1200 m a.s.l. in northern Peru and *P. boucephalus* sp. nov. both have males with vocal slits, and fingers and toes with lateral fringes, and both species lack a tympanum, dorsolateral folds, and nuptial pads, but *P. lirellus* has low longitudinal dermal ridges (absent), prominent dentigerous processes of vomers (absent), groin with single pale yellow to orange-yellow spot (greenish yellow with black blotches), and iris bronze to reddish brown (reddish copper). *Pristimantis martiae* (Lynch, 1974) from the Amazonian lowlands up to 1330 m a.s.l. and *P. boucephalus* sp. nov. lack dorsolateral folds, a tympanum, and nuptial pads, and both species have males with vocal slits, fingers and toes with lateral fringes. However, *P. martiae* has larger males (SVL 11.8–16.8 mm vs 13.4–14.5 mm [n = 3], Lynch 1980), small dentigerous processes of vomers (absent), a groin that is dull cream, pale orange or tan with brown to black bars or mottling (greenish yellow with black blotches), and bronze iris (reddish copper). *Pristimantis croceoinguinis* (Lynch, 1968) from the Amazonian lowland forests in Ecuador and Peru has larger males (13.9–18.2 mm vs 13.4–14.5 mm [n = 3], Lynch 1968) which lack vocal slits (present). *Pristimantis llosintuta* (Köhler & Lötters, 1999) from 2000–2200 m a.s.l. in Bolivia has a tympanum (absent) and much larger males (SVL 20.3–21.2 mm [n = 7] vs 13.4–14.5 mm [n = 3], Köhler & Lötters 1999). *Pristimantis cruciocularis* (Lehr, Lundberg, Aguilar & von May, 2006) from the YCNP and its surroundings and *P. boucephalus* sp. nov. are of similar size (SVL 11.4–15.4 [n = 7] vs 13.4–14.5 mm [n = 3]; Lehr *et al.* 2006) and share the absence of a tympanic annulus and membrane, but male *P. cruciocularis* lack vocal slits (present).



Furthermore, *P. cruciocularis* has the iris with a cruciform mark (absent) and a groin that is orange-red (greenish yellow with black blotches). *Pristimantis* sp. Pui Pui and *P. boucephalus* sp. nov. share the absence of a tympanic annulus and membrane, but *P.* sp. Pui Pui is much larger (SVL up to 31.3 mm vs 13.4–14.5 mm [n = 3]), and has a gray groin (greenish yellow with black blotches).

### Description of the holotype

Head broader than body, as long as wide; head length 41.1% of SVL; head width 41.1% of SVL; cranial crests absent; snout short, rounded in dorsal view, rounded in lateral view (Figs 3, 5A–B); eye-nostril distance 50% of eye diameter; nostrils slightly protuberant, directed dorsolaterally; canthus rostralis short, broadly rounded in lateral view, weakly concave in dorsal view; loreal region slightly concave; lips rounded; upper eyelid each with an enlarged conical tubercle at its center and one enlarged conical tubercle at its posterior end; upper eyelid width 57.1% of IOD; few small tubercles on scapular region (see photos in life Fig. 3A–B); supratympanic fold short and broad, extending from posterior margin of upper eyelid slightly curved to insertion of arm; tympanic membrane and annulus absent; small low



**Fig. 5.** *Pristimantis boucephalus* sp. nov., holotype (MUSM 31102). **A.** Dorsal view of head. **B.** Lateral view of head. **C.** Ventral view of hand. **D.** Ventral view of foot. Drawings by J. Moravec.

postrictal tubercles present bilaterally. Choanae small, ovoid, not concealed by palatal shelf of maxilla; dentigerous processes of vomers absent; tongue oval, tongue width is about three-fourths of the tongue length, posterior half free.

Skin on dorsum and flanks smooth, with low scattered tubercles (denser on flanks than on dorsum), dorsolateral folds absent; occipital and scapular region with sinusoidal ridge; skin on throat, chest, and belly areolate, with scattered low tubercles; discoidal and thoracic folds absent; cloacal sheath short.

Outer ulnar surface with minute low tubercles; palmar tubercle partially divided distally; thenar tubercle ovoid; subarticular tubercles well defined, most prominent on base of fingers, round in ventral view, subconical in lateral view; supernumerary tubercles distinct, ovoid, approximately half the size of subarticular tubercles; fingers with lateral fringes; Finger I shorter than Finger II; discs on digits of fingers expanded, round (Fig. 5C).

Hind limbs moderately long, slender, tibia length 54.6% of SVL; foot length 48.9% of SVL; upper surfaces of hind limbs smooth, with low scattered tubercles; inner surface of thighs smooth, posterior and



**Fig. 6.** Paratypes of *Pristimantis boucephalus* sp. nov. in dorsal (upper row) and ventral (lower row) views. From left to right: ♀ (MUSM 24479), ♂ (MUSM 24477), ♂ (MUSM 24478), juvenile (MUSM 24474). Photos by E. Lehr.

ventral surfaces of thighs weakly areolate; heels each with a prominent conical tubercle; outer surface of tarsus with scattered minute low tubercles; inner tarsal fold absent; inner metatarsal tubercle ovoid, two and a half times the size of round outer metatarsal tubercle; subarticular tubercles well defined, round in ventral view, subconical in lateral view; plantar supernumerary tubercles distinct, about half the size of subarticular tubercles; toes with lateral fringes; basal webbing present; discs expanded, round, less expanded than those on fingers; relative length of toes:  $1 < 2 < 3 < 5 < 4$ ; disc on Toe III not reaching distal subarticular tubercle on Toe IV, disc on Toe V reaching distal subarticular tubercle on Toe IV; Fig. 5D.

#### **Measurements (in mm) of the holotype**

SVL 14.1; HL 5.8; HW 5.8; ED 2.4; IOD 2.1; EW 1.2; IND 1.4; E-N 1.2; TL 7.7; FL 6.9.

#### **Coloration of the holotype in life (Fig. 3)**

The dorsal ground coloration is greenish gray with reddish-brown and scattered brown flecks surrounded by black; a reddish-brown blotch mid-dorsally behind the eyes; sinusoidal ridge on occipital and scapular region dark brown; upper lip with two irregularly shaped grayish-brown bars bordered by black on each side of head below eye, bars separated by an irregular pale tan blotch; narrow brown bar laterally in area of canthus rostralis, but not forming a canthal stripe, supratympanic stripe grayish brown, bordered by black; flanks pale greenish and pale reddish brown with scattered dark brown flecks; groin and anterior surfaces of thighs greenish yellow with black blotches and black stripes that extend dorsally to posterior surfaces of thighs; throat, chest, belly and thighs gray with pale reddish and greenish brown mottling and scattered dark gray flecks; palmar and plantar surfaces gray, fingers I and II cream, fingers III and IV gray except for cream discs, toes I and II cream, toes III–V gray with reddish-brown lateral fringes; iris reddish copper with fine black vermiculation and narrow black vertical streak from pupil across lower half of iris.

#### **Coloration of the holotype in preservative (Fig. 4)**

Dorsal coloration grayish tan with scattered brown flecks; brown blotch middorsally behind eyes; sinusoidal ridge on occipital and scapular region dark brown; upper lip with two irregularly shaped grayish-brown bars on each side of head below eye, bars separated by an irregular pale gray blotch; narrow brown bar laterally in area of canthus rostralis, but not forming a canthal stripe, supratympanic stripe grayish brown; flanks tan with scattered brown flecks; groin and anterior surfaces of thighs tan with black blotches and black stripes that extend dorsally to posterior surfaces of thighs; throat, chest, belly and thighs grayish tan with dark gray flecks; palmar and plantar surfaces pale gray, fingers I and II tan, fingers III and IV gray except for tan discs, toes I and II tan, toes III–V gray; iris gray with fine black vermiculation and black narrow vertical streak from pupil across lower half of iris.

#### **Variation**

All paratypes are similar to the holotype regarding morphology (Tables 3–4) and coloration pattern (Fig. 6). They can easily be assigned to the species by having a small size with a disproportionately large head with short snout. The juvenile specimen (MUSM 24474) has two prominent tarsal and two prominent ulnar tubercles on each hind limb. One male specimen (MUSM 24477) has black bars dorsally, laterally and ventrally on the tibia and a brown blotch midventrally on the throat. One specimen (MUSM 24479) is considered a subadult female because of its small size, presence of ovaries, but absence of ovarian eggs.

#### **Distribution, natural history, and threat status**

*Pristimantis boucephalus* sp. nov. is currently only known from the YCNP. The type locality (Figs 1, 7) is located on a mountain crest at 2950 m a.s.l. covered with primary cloud forest. The holotype was found at night, sitting on a leaf at 1 m above the ground. Syntopic anurans included *Pristimantis* cf.



**Table 3.** Measurements (in mm) of the type series of *Pristimantis boucephalus* sp. nov. Abbreviations: M = ♂; SF = subadult ♀; J = juvenile. For other abbreviations see Material and methods.

Character	MUSM 31102	MUSM 24478	MUSM 24477	MUSM 24479	MUSM 24474
sex	M	M	M	SF	J
SVL	14.1	13.4	14.5	12.5	10.0
TL	7.7	7.9	8.2	7.4	5.5
FL	6.9	7.6	7.4	6.3	5.4
HL	5.8	5.2	5.61	5.1	4.5
HW	5.8	5.5	5.8	5.2	4.5
ED	2.4	2.0	2.4	1.9	1.8
IOD	2.1	2.1	2.0	1.9	1.6
EW	1.2	1.8	1.6	1.5	1.5
IND	1.4	1.4	1.6	1.3	1.3
N-E	1.2	1.3	1.6	1.4	1.2

*aniptopalmatus* (MUSM 31103–05), *P. cf. mendax* (MUSM 31101, 31107), and *Phrynopus curator* (MUSM 31106). We classify *Pristimantis boucephalus* sp. nov. as “Data Deficient” according to the IUCN red list criteria and categories (IUCN Standards and Petitions Subcommittee 2016) based on the limited information on its geographic range. Given that *Pristimantis boucephalus* sp. nov. occurs inside the YCNP, a long-term protection of this species should be granted.

## Discussion

The molecular phylogeny (Fig. 2) shows that the new species, *Pristimantis boucephalus* sp. nov., belongs to a well-supported speciose clade, which comprises mainly montane but also some Amazonian lowland taxa from northern South America. The clade contains species from the Andes in the west and the Guiana Shield in the east, and corresponds well to a clade uncovered in other studies (Hedges *et al.* 2008; Pyron & Wiens 2011; Padial *et al.* 2014). In addition, our results point out a surprisingly high phylogenetic diversity of *Pristimantis* associated with the relatively small area (1220 km<sup>2</sup> between 460 and 3643 m elevation, Yallico & Rose 1998) of the Cordillera Yanachaga. The six divergent lineages of *Pristimantis* that we identified in this study correspond with lineages detected in previous phylogenetic reconstructions of the genus (Hedges *et al.* 2008; Pyron & Wiens 2011). Three lineages were assigned to species groups (Fig. 2), whereas the three other lineages remain unassigned to species groups (Padial *et al.* 2014). The high phylogenetic diversity indicates that members of *Pristimantis* probably colonized this region of the central Peruvian Andes through several independent events or underwent unusually high diversification in the region. Today, 18 species of *Pristimantis* (including three unnamed species) are known to occur in the cloud forests of the YCNP and its buffer zone (see Angulo *et al.* 2016).

In recent years, several new species of minute *Pristimantis* have been described from the Andes of Ecuador (Lehr & Coloma 2008: *P. andinognomus*; Terán-Valdez & Guayasamin 2010: *P. minimus*), and Peru (Duellman & Hedges 2007: *P. minutulus*; Lehr *et al.* 2007: *P. caeruleonotus*; this paper), indicating that the diversity of small and minute species of this genus is highly underestimated due to difficulties in discovering them. With the description of *P. boucephalus* sp. nov., the number of *Pristimantis* known from Peru rises to 131 species (updated from AmphibiaWeb 2017).

Miniaturization of frogs has been documented in many species of different families (Wells 2007; Zimkus *et al.* 2012), and miniaturized frogs often have lost (e.g., digits) or reduced (e.g., phalanges) elements of





**Fig. 7.** Type locality of *Pristimantis boucephalus* sp. nov. in the Yanachaga-Chemillén National Park. Photo by E. Lehr.



**Table 4.** Measurements (in mm) and proportions of the male type series of *Pristimantis boucephalus* sp. nov.; ranges followed by means and one standard deviation in parentheses. For other abbreviations see Materials and methods.

Characters	Males (n = 3)
SVL	13.4–14.5 (14.0 ± 0.5)
TL	7.7–8.2 (7.9 ± 0.2)
FL	6.9–7.6 (7.3 ± 0.3)
HL	5.2–5.8 (5.5 ± 0.2)
HW	5.5–5.8 (5.7 ± 0.1)
ED	2.2–2.4 (2.3 ± 0.2)
IOD	2.0–2.1 (2.1 ± 0.0)
EW	1.2–1.8 (1.5 ± 0.2)
IND	1.4–1.6 (1.5 ± 0.1)
E–N	1.2–1.6 (1.4 ± 0.2)
TL/SVL	0.55–0.59
FL/SVL	0.49–0.57
HL/SVL	0.39–0.41
HW/SVL	0.40–0.41
HW/HL	1.00–1.06
E–N/ED	0.50–0.67
EW/IOD	0.57–0.86

their skeleton, but rarely lose bones of the skull (Yeh 2002). Body proportions of *P. boucephalus* sp. nov. indicate that the process of miniaturization affected various body structures, leaving the size of the head less affected. However, whether the head of *P. boucephalus* sp. nov. experienced increased growth or the postcranial body experienced increased miniaturization cannot be determined at this moment.

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## Appendix

Comparative specimens examined:

*Phrynopus curator* Lehr, Moravec & Cusi, 2012 (1): Peru: Pasco, Yanachaga-Chemillén National Park (Sector San Daniel), Distrito de Huancabamba, Provincia de Oxapampa, 2950 m, MUSM 31106.

*Pristimantis* cf. *aniptopalmatus* (Duellman & Hedges, 2005) (3): Peru: Pasco, Yanachaga-Chemillén National Park (Sector San Daniel), Distrito de Huancabamba, Provincia de Oxapampa, 2950 m, MUSM 31103–05.

*Pristimantis cruciocularis* (Lehr, Lundberg, Aguilar & von May, 2006) (2): Peru: Pasco, Yanachaga-Chemillén National Park (Sector San Daniel), Distrito de Huancabamba, Provincia de Oxapampa, ca 2900 m, MUSM 31140, MUSM 31146.

*Pristimantis imitatrix* (Duellman, 1978) (2): Peru: Madre de Dios, Cusco Amazónico, 15 km E of Puerto Maldonado, 200 m, MUSM 7348, MUSM 14605.

*Pristimantis* cf. *mendax* (Duellman, 1978) (2): Peru: Pasco, Yanachaga-Chemillén National Park (Sector San Daniel), Distrito de Huancabamba, Provincia de Oxapampa, 2950 m, MUSM 31101, MUSM 31107.

*Pristimantis minutulus* Duellman & Hedges, 2007 (10): Peru: Huánuco, Panguana, SMNS 13017–22; Pasco: 0.0–1.5 km W of Cacazu, 900 m, KU 291677, KU 291679, KU 291680, KU 308608.

*Pristimantis trachyblepharis* (Boulenger, 1918) (7): Ecuador: Pastaza, Puyo, 5 km SSE of Hacienda Madrid, USNM 233386; Puyo, USNM 233388; Tungurahua, Río Negro, USNM 233391, USNM 233394, USNM 233397–99.

*Pristimantis* sp. Pui Pui (3): Peru: Junín, Pui Pui Protected Forest, 1700–1800 m, NMP6V 75063–65.