A new species of terrestrial-breeding frog (Amphibia, Craugastoridae, Pristimantis) from high elevations of the Pui Pui Protected Forest in central Peru

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Abstract
We describe a new species of Pristimantis from upper montane forests and high Andean grasslands of the Pui Pui Protected Forest and its close surroundings, Región Junín, central Peru. The description of the new species is based on 34 specimens found at elevations between 3400 and 3936 m a.s.l. Pristimantis attenboroughi sp. n. is characterized by a snout–vent length of 14.6–19.2 mm in adult males (n = 21), 19.2–23.0 mm in adult females (n = 10), and is compared morphologically and genetically with other taxonomically and biogeographically relevant species of Pristimantis. The new species is characterized by having narrow digits that lack circumferential grooves, irregularly shaped, discontinuous dorsolateral folds, and absence of both tympanic membrane and tympanic annulus. The high similarity in morphology between P. attenboroughi sp. n. and members of the Andean genera Phrynopus and Bryophryne provides an example for convergent evolution, and highlights the importance of using molecular data to justify generic assignment. Pristimantis attenboroughi sp. n. is most similar to Phrynopus chaparroi from the Región Junín, suggesting that the generic placement of this species needs to be revised. Phylogenetically the new species belongs to the Pristimantis danae species Group, a clade that includes several Pristimantis species distributed in the montane forests of central Peru, including P. albertus, P. aniptopalmatus, P. ornatus, and P. stictogaster.

Keywords
Andes, DNA barcoding, frogs, molecular phylogeny, montane forest, Pristimantis attenboroughi new species, Puna
Introduction

The Pui Pui Protected Forest (Bosque de Protección Pui Pui, hereafter PPPF, Fig. 1) is located in the Selva Central of Peru and is one of twelve natural protected areas with different levels of legal protection such as national parks, national sanctuaries, and national reserves in the regions of Pasco and Junín (SERNANP 2010). The PPPF, located in the Región Junín, was established in 1985 and covers 60,000 hectares encompassing montane forest (30%) and high Andean grassland (Puna; 70%) habitats (SERNANP 2010). The area protects the upper watershed of several rivers and includes elevations between 1700 and 4500 m a.s.l. (SERNANP 2010).

In 2012–2014, we conducted herpetological surveys in montane forests and Puna of the PPPF to catalog the amphibian and reptile species and to evaluate their conservation status. As a result, we found several new species of frogs (Craugastoridae) as well as new species of lizards (Gymnophthalmidae). All new species were compared morphologically and genetically with other taxonomically and biogeographically relevant taxa mostly from Ecuador, Peru, and Bolivia. Herein we describe a new species of Pristimantis from upper montane and Puna habitats collected between 2012 and 2013.

Materials and methods

Fieldwork. Because of its remote location, the PPPF is difficult to reach and is only accessible through a few entrances located ca. 1–2 days of walking distance from the nearest villages. The upper montane forests and Puna of the PPPF were reached from Toldopampa (11°30'15.4"S, 74°55'32.7"W, 3670 m a.s.l., ca. 45 km SW from Satipo) with the help of local guides by walking in 1.5 days (ca. 11 km airline). In 2012 fieldwork was conducted between May 8 and 21 by EL and RvM, and in 2013 between June 21 and July 8 by EL, J. Moravec, and J.C. Cusi. Amphibians were preserved in 96% ethanol and stored in 70% ethanol. Deposited eggs were stored in 70% ethanol.

Morphological characters. The format for the description follows Lynch and Duellman (1997), except that the term dentigerous processes of vomers is used instead of vomerine odontophores (Duellman et al. 2006), and diagnostic characters are those of Duellman and Lehr (2009). Taxonomic classification follows Hedges et al. (2008), except that we followed Pyron and Wiens (2011) for family placement and Padial et al. (2014) for names of Pristimantis species groups. Sex and maturity of specimens were identified by observing gonads through dissections. Specimens were considered juveniles when gonads were too small to distinguish between sexes. The tympanic region of two specimens (MUSM 31199, NMP6V 75534) was opened to see if a tympanic annulus is present under the skin. We measured the following variables to the nearest 0.1 mm with digital calipers under a stereomicroscope: 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
New terrestrial-breeding frog

Figure 1. Map of Peru with the Pui Pui Protected Forest indicated in red.

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 narial opening), and egg diameter. Fingers and toes are numbered preaxially to postaxially from 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. All drawings were made by EL using a stereomicroscope and a camera lucida. Photographs taken by EL and RvM were used for descriptions of coloration in life. Comparisons of congeners focus on species in similar habitats from Ecuador and Peru and those with close phylogenetic relationships as recovered in our phylogenetic trees. Information on species for comparative diagnoses was obtained from Duellman and Lehr (2009) and from original species descriptions. For specimens examined see Appendix. Codes of collections are: MUSM = Museo de Historia Natural Universidad Nacional Mayor de San Marcos, Lima, Peru; NMP6V = National Museum Prague, Prague, Czech Republic; UMMZ = University of Michigan Museum of Zoology, Ann Arbor, USA. Field number code is: IWU = Illinois Wesleyan University, Bloomington, USA. Conservation status was evaluated using the criteria in IUCN (2001). Maps were designed with ArcGIS 10.0 by J.C. Cusi.

**Molecular phylogenetic analysis.** The phylogenetic position of the new species with respect to other morphologically similar species was determined through analysis of DNA sequence data. This analysis included two mitochondrial genes, 16S rRNA (16S) and 12S rRNA (12S). We used tissue samples from specimens collected in central Peru (Región Junín) to obtain DNA sequences for the new species and several other *Pristimantis* species (Table 1). Additionally, we downloaded selected sequences of morphologically similar taxa (*Bryophyrne*, *Lynchius*, *Phrynopus*, *Oreobates*) distributed at high elevations (> 2000 m a.s.l.) from Genbank (Table 1). We included *Hamptophryne boliviana*, *Ischnocnema guentheri*, and *Bufo melanostictus* as outgroup taxa (Padial et al. 2014).

Extraction, amplification, and sequencing of DNA followed protocols previously used for Neotropical terrestrial breeding frogs (Lehr et al. 2005, Hedges et al. 2008). We used the 16SA (forward) primer (5’-3’ sequence: CGCCTGTTTATCAAAAAACAT) and the 16SB (reverse) primer (5’-3’ sequence: CCGGTCTGAACTCAGATCACGT) to amplify a fragment of the 16S gene (Palumbi et al. 1991), and we employed the following thermocycling conditions to amplify DNA using the polymerase chain reaction (PCR): 1 cycle of 96°C/3 min; 35 cycles of 95°C/30 s, 55°C/45 s, 72°C/1.5 min; 1 cycle 72°C/7 min. Additionally, we used the L25195 (forward) primer (5’-3’ sequence: AAACGTGGATTAGATACCCACTA) and the H2916 (reverse) primer (5’-3’ sequence: GAGGGTGACGGGCGGTGTGTG) to amplify a fragment of the 12S gene (Palumbi et al. 1991, Vences et al. 2000), and we employed the following thermocycling conditions to amplify DNA using PCR: 1 cycle of 94°C/1.5 min; 35 cycles of 94°C/45 s, 50°C/1 min., 74°C/2 min; 1 cycle 72°C/10 min. We completed the cycle sequencing reactions by using the corresponding PCR primers and the Big-Dye Terminator 3.1 (Applied Biosystems), and obtained sequence data by running the purified reaction products in an ABI 3730 Sequence Analyzer (Applied Biosystems). The newly obtained sequences are deposited in GenBank (Table 1).
Table 1. GenBank accession numbers for taxa and genes sampled in this study.

| Taxon                        | 16S     | 12S     | Voucher_Nbr | Reference                           |
|------------------------------|---------|---------|-------------|-------------------------------------|
| Bryophryne bakersfield       | KT276289| na      | MHNC5999    | Chaparro et al. 2015                |
| Bryophryne bakersfield       | KT276287| KT276281| MHNC6022    | Chaparro et al. 2015                |
| Bryophryne bakersfield       | KT276290| KT276282| MHNC6023    | Chaparro et al. 2015                |
| Bryophryne bakersfield       | KT276291| KT276283| MHNC6007    | Chaparro et al. 2015                |
| Bryophryne bakersfield       | KT276288| KT276284| MHNC6009    | Chaparro et al. 2015                |
| Bryophryne bustamantei       | KT276293| KT276286| MHNC6019    | Chaparro et al. 2015                |
| Bryophryne cophites          | EF493537| EF493537| KU173497    | Heinicke et al. 2007               |
| Bufo melanostictus           | FJ882791| FJ882791| VUB 0052    | Van Bocxlaer et al. 2009           |
| Hamptophryne boliviana       | DQ283438| DQ283438| na          | Frost et al. 2006                  |
| Ichtyophrynus guentheri       | EF493533| EF493533| na          | Heinicke et al. 2007               |
| Lynchius flavomaculatus       | EU186667| EU186667| KU218210    | Hedges et al. 2008                 |
| Lynchius nebulanastes         | EU186704| EU186704| KU181408    | Hedges et al. 2008                 |
| Lynchius oblitas             | AM039640| AM039708| MUSM19914   | Lehr et al. 2005, Motta et al. 2016|
| Lynchius oblitas             | AM039639| AM039707| MTD45954    | Lehr et al. 2005, Motta et al. 2016|
| Lynchius parkeri             | EU186705| EU186705| KU181307    | Hedges et al. 2008                 |
| Lynchius simmonsi            | JF809904| JF809940| QZ41639     | Padial et al. 2014                 |
| Oreobates amarakaeri         | JF809996| JF809934| MHNC6975    | Padial et al. 2014                 |
| Oreobates ayacucho           | JF809970| JF809939| MNCN_IDR5024| Padial et al. 2014                |
| Oreobates cruralis           | EU186666| EU186666| KU215462    | Hedges et al. 2008                 |
| Oreobates gemcare            | JF809960| JF809930| MHNC6687    | Padial et al. 2014                 |
| Oreobates granulosus         | EU368897| JF809929| MHNC3936    | Padial et al. 2014                 |
| Phrynopus auriculatus        | EF493708| EF493708| KU291634    | Heinicke et al. 2007               |
| Phrynopus barthlenae         | AM039653| AM039721| SMF81720    | Lehr et al. 2005                   |
| Phrynopus bracki             | EF493709| EF493709| USNM286919  | Heinicke et al. 2007               |
| Phrynopus bufoide            | AM039645| AM039713| MUSM19860   | Lehr et al. 2005                   |
| Phrynopus heimorum           | AM039635| AM039703| MTD45621    | Lehr et al. 2005                   |
| Phrynopus horstapuli         | AM039651| AM039719| MTD44333    | Lehr et al. 2005                   |
| Phrynopus horstapuli         | AM039647| AM039715| MTD44334    | Lehr et al. 2005                   |
| Phrynopus kantorei           | AM039650| AM039718| MTD44332    | Lehr et al. 2005                   |
| Phrynopus kaunorei           | AM039655| AM039723| MUSM20595   | Lehr et al. 2005                   |
| Phrynopus peanetti           | AM039656| AM039724| MTD45072    | Lehr et al. 2005                   |
| Phrynopus tautzorum          | AM039652| AM039720| MUSM20613   | Lehr et al. 2005                   |
| Phrynopus tribolosus         | EU186725| EU186707| KU291630    | Hedges et al. 2008                 |
| Pristimantis acuminatus      | EU130579| na      | QCAZ19664   | Elmer et al. 2007                  |
| Pristimantis albertus        | EU186695| EU186695| KU291675    | Hedges et al. 2008                 |
| Pristimantis albertus        | KY594749| na      | RVM41_14    | This study                          |
| Pristimantis albertus        | KY594750| na      | RVM42_14    | This study                          |
| Pristimantis albertus        | KY594751| na      | RVM527      | This study                          |
| Pristimantis altamazonicus   | EF493670| EF493670| KU215460    | Heinicke et al. 2007               |
| Pristimantis altamazonicus   | DQ195449| na      | MC11717     | Mahecha et al., unpublished         |
| Pristimantis anitopalmatus   | EF493390| EF493390| KU291627    | Heinicke et al. 2007               |
| Pristimantis anitopalmatus   | EU186694| EU186694| KU291666    | Padial et al. 2014                 |
| Taxon                        | 16S  | 12S  | Voucher_Nbr | Reference                  |
|-----------------------------|------|------|-------------|----------------------------|
| *Pristimantis attenboroughi* sp. n. | KY594752 | na   | MUSM31186   | This study                 |
| *Pristimantis attenboroughi* sp. n. | KY594753 | KY594761 | NMP6V75522  | This study                 |
| *Pristimantis attenboroughi* sp. n. | KY594754 | KY594762 | NMP6V75524  | This study                 |
| *Pristimantis attenboroughi* sp. n. | KY594755 | KY594763 | NMP6V75525  | This study                 |
| *Pristimantis attenboroughi* sp. n. | KY594756 | KY594764 | NMP6V75528  | This study                 |
| *Pristimantis attenboroughi* sp. n. | KY594757 | na   | NMP6V75529  | This study                 |
| *Pristimantis aureoventris*   | JQ742152 | na   | VUB3748     | Kok et al. 2012            |
| *Pristimantis bipunctatus*    | EF493702 | EF493702 | KU291638    | Heinicke et al. 2007      |
| *Pristimantis cf. mendax*     | KY594758 | na   | MUSM31179   | This study                 |
| *Pristimantis cf. mendax*     | KY628996 | na   | MUSM31157   | This study                 |
| *Pristimantis croceinguisinis* | EU186659 | na   | MTD45080    | Hedges et al. 2008        |
| *Pristimantis diadematus*     | EU186668 | EU186668 | KU291673    | Hedges et al. 2008        |
| *Pristimantis llojintuta*     | EU712641 | na   | MNCN67314   | Padial and De la Riva 2009|
| *Pristimantis melanogaster*   | EF493664 | EF493826 | na          | Heinicke et al. 2007      |
| *Pristimantis ornatus*        | EU186660 | EU186660 | MTD45073    | Hedges et al. 2008        |
| *Pristimantis petrobardus*    | EF493367 | EF493825 | KU212293    | Heinicke et al. 2007      |
| *Pristimantis platydactylus*  | EU712653 | na   | MNCN63943   | Padial et al. 2009        |
| *Pristimantis sagittulus*     | EU712671 | na   | MNCN6377    | Padial et al. 2009        |
| *Pristimantis schultei*       | EF493681 | na   | KU212220    | Heinicke et al. 2007      |
| *Pristimantis simonbolivari*  | EF493671 | EF493671 | KU212854    | Heinicke et al. 2007      |
| *Pristimantis simonsii*       | EU186665 | EU186665 | KU212350    | Heinicke et al. 2007      |
| *Pristimantis skydmainos*     | EF493393 | EF493393 | MUSM10071   | Heinicke et al. 2007      |
| *Pristimantis sp.*            | AM039658 | na   | MTD45201    | Lehr et al. 2005          |
| *Pristimantis stictogaster*   | EF493704 | EF493704 | KU291659    | Heinicke et al. 2007      |
| *Pristimantis tofiae*         | EF493353 | EF493353 | KU215493    | Heinicke et al. 2007      |
| *Pristimantis tofiae*         | EU192294 | na   | MNCN43246   | Padial and De la Riva 2009|
| *Pristimantis viensi*         | EF493668 | EF493377 | KU219796    | Heinicke et al. 2007      |

Geneious R6, version 6.1.8 (Biomatters 2013; http://www.geneious.com/) was used to align the sequences. Within Geneious, we used the MAFFT, version 7.017 (Katoh and Standley 2013) alignment program. Prior to conducting phylogenetic analysis, we used PartitionFinder, version 1.1.1 (Lanfear et al. 2012) to select the appropriate models of nucleotide evolution and used the Bayesian information criterion (BIC) to
determine the best partitioning scheme and substitution model for each gene. According to PartitionFinder, the best scheme included one partition combining both 12S and 16S and the best model of nucleotide substitution was GTR + I + Γ. Phylogenetic analysis was done using Maximum Likelihood (ML) approach using RaxML version 8.2.4 (Stamatakis 2006), where the “f-a” function was employed to conduct a bootstrap analysis and search for the optimal likelihood tree. Our analysis included 82 terminals and a 922 bp concatenated alignment that included the 16S and 12S dataset. The GTR + I + Γ model of nucleotide substitution was used to perform 200 trees searches; node support was assessed using 1000 bootstrap replicates. Additionally, we used the R package ‘APE’ (Paradis et al. 2004) to estimate uncorrected p-distances (i.e., the proportion of nucleotide sites at which any two sequences are different).

Results

Molecular phylogenetic analysis. The Maximum Likelihood (ML) tree (Fig. 2) was generally congruent with a previous molecular phylogeny (Padial et al. 2014) and supported the distinctiveness of the new species from other closely related taxa. Placement of Pristimantis attenboroughi sp. n. in the genus Pristimantis Jiménez de la Espada, 1871 was strongly supported and, based on the available data, the new species is most closely related to P. albertus Duellman and Hedges, 2007, P. aniptopalampus (Duellman and Hedges, 2005), P. ornatus (Lehr, Lundberg, Aguilar, and von May, 2006), and P. stictogaster (Duellman and Hedges, 2005) (Fig. 2). Table 2 compares uncorrected p-distances of a 542 bp (including gaps) fragment of the 16S mitochondrial rRNA gene of Pristimantis species included in our analyses. The lowest distance occurs between the new species and P. aniptopalampus (uncorrected p-distance 4.3 %) while the uncorrected p-distances between the new species and the other three species in the same clade of the Pristimantis danae species Group (P. albertus, P. ornatus, P. reichlei Padial and De la Riva, 2009, P. rhabdolaemus [Duellman, 1978a], P. stictogaster [Duellman and Hedges, 2005], P. sagittulus [Lehr, Aguilar, and Duellman, 2004], P. toftae [Duellman, 1978b]) vary between 5.2 to 11.8 %.

Pristimantis attenboroughi sp. n.
http://zoobank.org/DCE88D49-0EB1-4DA4-A672-5341763B3236

Common name. English: Attenborough’s Rubber Frog. Spanish: Rana cutín Attenborough.

Holotype. MUSM 31196 (IWU 178, Figs 3, 4), adult male from the Pui Pui Protected Forest, Provincia Satipo, Región Junín, Peru, Upper part of Quebrada Tarhuish, “Laguna Udrecocha”, Puna, open area on east side of Laguna Udrecocha, 11°23’24.1”S, 74°58’32.5”W, 3936 m a.s.l. (Fig. 8A), collected on 17 May 2012 by E. Lehr and R. von May.
Figure 2. Maximum Likelihood (ML) phylogeny based on the combined 16S + 12S dataset (ML bootstrap values >50 are indicated at each node).
Table 2. Uncorrected p-distances of the 16s mitochondrial rRNA gene for six specimens of *Pristimantis attenboroughi* sp. n. (in bold) and other *Pristimantis* species from GenBank.

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| **Pristimantis albertus** KU291675 |  |  |  |  |  |  |  |  |
| *Pristimantis albertus* RsM41_14 | 0.000 |  |  |  |  |  |  |  |
| *Pristimantis albertus* RsM42_14 | 0.000 | 0.000 |  |  |  |  |  |  |
| *Pristimantis albertus* RsM527 | 0.000 | 0.000 | 0.000 |  |  |  |  |  |
| **Pristimantis attenboroughi** sp. n. NMP6V 75522 | 0.062 | 0.065 | 0.062 | 0.066 |  |  |  |  |
| **Pristimantis attenboroughi** sp. n. NMP6V 75529 | 0.062 | 0.065 | 0.062 | 0.066 | 0.000 |  |  |  |
| **Pristimantis attenboroughi** sp. n. NMP6V 75524 | 0.062 | 0.065 | 0.062 | 0.066 | 0.000 | 0.000 |  |  |
| **Pristimantis attenboroughi** sp. n. NMP6V 75525 | 0.062 | 0.065 | 0.062 | 0.066 | 0.000 | 0.000 | 0.000 |  |
| **Pristimantis attenboroughi** sp. n. MUSM 31186 | 0.062 | 0.065 | 0.062 | 0.066 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Pristimantis attenboroughi** sp. n. NMP6V 75528 | 0.062 | 0.065 | 0.062 | 0.066 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Pristimantis ornatus** MTD45073 | 0.056 | 0.059 | 0.056 | 0.059 | 0.052 | 0.052 | 0.052 | 0.052 |
| **Pristimantis stictogaster** KU291659 | 0.041 | 0.043 | 0.041 | 0.043 | 0.049 | 0.049 | 0.049 | 0.049 |
| **Pristimantis aniptopalmatus** KU291627 | 0.056 | 0.059 | 0.056 | 0.059 | 0.043 | 0.043 | 0.043 | 0.043 |
| **Pristimantis aniptopalmatus** KU291666 | 0.056 | 0.059 | 0.056 | 0.059 | 0.043 | 0.043 | 0.043 | 0.043 |
| **Pristimantis rhabdolemus** KU173492 | 0.093 | 0.097 | 0.093 | 0.097 | 0.058 | 0.058 | 0.058 | 0.058 |
| **Pristimantis tofae** KU215493 | 0.110 | 0.115 | 0.110 | 0.115 | 0.074 | 0.074 | 0.074 | 0.074 |
| **Pristimantis tofae** MNCN43246 | 0.105 | 0.110 | 0.105 | 0.110 | 0.070 | 0.070 | 0.070 | 0.070 |
| **Pristimantis sagittulus** KU291635 | 0.093 | 0.097 | 0.093 | 0.097 | 0.066 | 0.066 | 0.066 | 0.066 |
| **Pristimantis reichlei** MHNSM9267 | 0.132 | 0.135 | 0.132 | 0.136 | 0.118 | 0.118 | 0.118 | 0.118 |

Paratypes. A total of 33 (Figs 5–7, 8C), all from inside the PPPF (except for: MUSM 31199–31202, NMP6V 75526–29), Provincia Satipo, Región Junín: 10 adult females (MUSM 31977, 31980, 31987, 31201, NMP6V 75076, 75522 [GenBank accession numbers KY594753, KY594761], 75523, 75528 [GenBank accession numbers KY594756, KY594764], 75529 [GenBank accession number KY594757], 75534), 20 adult males (MUSM 31186 [GenBank accession number KY594752], 31195, 31199, 31202, 31975, 31979, 31988, 31989, 31992, 31993, NMP6V 75077–75079, 75524 [GenBank accession numbers KY594754, KY594762], 75525 [GenBank accession numbers KY594755, KY594763], 75526, 75527, 75533, UMMZ 244726, 244727), 3 juveniles (MUSM 31187, 31990, 31200).

MUSM 31186, MUSM 31187, NMP6V 75522, 75523: Quebrada Tarhuish, left bank of Antuyo River, “Shiusha”, upper montane forest, 11°22’3.9”S, 74°56’12.7’’W, 3414 m a.s.l. collected on 12 May 2012 by E. Lehr and R. von May. MUSM 31195, NMP6V 75524, 75524: collected at the type locality along with the holotype. MUSM 31199, 31200, MUSM 31201, 31202, NMP6V
Table 2. Continued.

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Pristimantis albertus | KU291675 |   |   |   |   |   |   |   |   |   |   |   |   |
| 2 | Pristimantis albertus | ReM41_14 |   |   |   |   |   |   |   |   |   |   |   |   |
| 3 | Pristimantis albertus | ReM42_14 |   |   |   |   |   |   |   |   |   |   |   |   |
| 4 | Pristimantis albertus | ReM527 |   |   |   |   |   |   |   |   |   |   |   |   |
| 5 | Pristimantis attenboroughi sp. n. | NMP6V 75522 |   |   |   |   |   |   |   |   |   |   |   |   |
| 6 | Pristimantis attenboroughi sp. n. | NMP6V 75529 |   |   |   |   |   |   |   |   |   |   |   |   |
| 7 | Pristimantis attenboroughi sp. n. | NMP6V 75524 |   |   |   |   |   |   |   |   |   |   |   |   |
| 8 | Pristimantis attenboroughi sp. n. | NMP6V 75525 |   |   |   |   |   |   |   |   |   |   |   |   |
| 9 | Pristimantis attenboroughi sp. n. | MUSM 31186 |   |   |   |   |   |   |   |   |   |   |   |   |
|10 | Pristimantis attenboroughi sp. n. | NMP6V 75528 |   |   |   |   |   |   |   |   |   |   |   |   |
|11 | Pristimantis ornatus | MTD45073 | 0.052 |   |   |   |   |   |   |   |   |   |   |   |
|12 | Pristimantis stictogaster | KU291659 | 0.049 | 0.037 |   |   |   |   |   |   |   |   |   |   |
|13 | Pristimantis aniptopalmatus | KU291627 | 0.043 | 0.048 | 0.049 |   |   |   |   |   |   |   |   |   |
|14 | Pristimantis aniptopalmatus | KU291666 | 0.043 | 0.048 | 0.049 | 0.000 |   |   |   |   |   |   |   |   |
|15 | Pristimantis rhabdolaeus | KU173492 | 0.058 | 0.082 | 0.076 | 0.074 | 0.074 |   |   |   |   |   |   |   |
|16 | Pristimantis toftae | KU215493 | 0.074 | 0.091 | 0.091 | 0.083 | 0.083 | 0.070 |   |   |   |   |   |   |
|17 | Pristimantis toftae | MNCN43246 | 0.070 | 0.099 | 0.088 | 0.082 | 0.082 | 0.074 | 0.055 |   |   |   |   |   |
|18 | Pristimantis sagittulus | KU291635 | 0.066 | 0.084 | 0.080 | 0.068 | 0.068 | 0.066 | 0.078 | 0.095 |   |   |   |   |
|19 | Pristimantis danae | MNCN44234 | 0.094 | 0.107 | 0.107 | 0.100 | 0.100 | 0.082 | 0.101 | 0.100 | 0.083 |   |   |   |
|20 | Pristimantis reichlei | MHNSM9267 | 0.118 | 0.124 | 0.113 | 0.117 | 0.117 | 0.103 | 0.126 | 0.114 | 0.117 | 0.113 |   |   |

75526, 75527: Upper part of Quebrada Tasta, “Laguna Luichococha”, Puna, 11°27’23.7”S, 74°55’10.6”W, 3708 m a.s.l. collected on 20 May 2012 by E. Lehr and R. von May. NMP6V 75528, 75529: near trail from Tasta to Tarhuish (first mountain peak), Polylepis forest patch, 11°26’8.6”S, 74°53’56.5”W, 3886 m a.s.l. collected on 20 May 2012 by E. Lehr and R. von May. MUSM 31975: Antuyo, 11°20’03.7”S, 74°59’49.1”W, 3700 m a.s.l. collected on 27 June 2013 by E. Lehr, J. Moravec, and J.C. Cusi. MUSM 31977, 31979, MUSM 31980, NMP6V 75076, UMMZ 244726: Hatunpata, 11°18’07.9”S, 75°01’35.0”W, 3710 m a.s.l. collected on 28 June 2013 by E. Lehr, J. Moravec, and J.C. Cusi. MUSM 31987–31990, NMP6V 75077, 75078, 75533, UMMZ 244727: Trancapampa, 11°17’49.2”S, 75°00’46.3”W, 3550 m a.s.l. collected on 2 July 2013 by E. Lehr, J. Moravec, and J.C. Cusi. MUSM 31992, 31993, NMP6V 75079, 75534: Antuyo Bajo, 11°18’53.4”S, 74°59’34.8”W, 3400 m a.s.l. collected on 4 July 2013 by E. Lehr, J. Moravec, and J.C. Cusi.
Generic placement. We assign this species to *Pristimantis* based on our molecular data (Fig. 2).

Diagnosis. A new species of *Pristimantis* assigned to the *danae* species Group having the following combination of characters: (1) Skin on dorsum shagreen with low scattered tubercles, skin on flanks tuberculate, skin on venter areolate; discoidal fold absent, thoracic fold present; irregularly shaped, discontinuous dorsolateral folds present; (2) tympanic membrane and tympanic annulus absent; (3) snout short, rounded in dorsal and in lateral views; (4) upper eyelid without enlarged conical tubercles; EW shorter...
Figure 4. Ventral views of right hand (A) and right foot (B) of holotype of *Pristimantis attenboroughi* sp. n. (MUSM 31196). Drawings by E. Lehr.

than IOD; cranial crests absent; (5) dentigerous processes of vomers present; (6) males without vocal slits, nuptial pads absent; (7) Finger I shorter than Finger II; tips of digits narrow, rounded, lacking circumferential grooves; (8) fingers without lateral fringes; (9) small conical ulnar and tarsal tubercles present; (10) heel with a small conical tubercle; inner tarsal fold usually absent; (11) inner metatarsal tubercle ovoid, 1.5 times as large as outer; outer metatarsal tubercle small, rounded; vie low supernumerary plantar tubercles; (12) toes without lateral fringes; basal toe webbing absent; Toe V longer than Toe III; tips of digits narrow, rounded, lacking circumferential grooves, toe tips slightly smaller than those on fingers; (13) in life, dorsal ground coloration pale or dark gray, reddish brown or brownish olive with dark gray scattered flecks, some with X-shaped mark on scapular and ill-defined diagonal bars on flanks; dark grayish-brown canthal and supratympanic stripes usually present; groin dark gray or pale reddish brown with a pale
red to pink tint in some; venter dark gray, pale gray, grayish brown or pale grayish green and in some dark gray mottled; iris pale grayish green with fine black vermiculation and brownish-orange horizontal streak across pupil and lower half of iris; (14) SVL in adult males 14.6–19.2 mm (n = 21), in adult females 19.2–23.0 mm (n = 10).

Comparisons. *Pristimantis attenboroughi* is readily distinguished from its congeners in Ecuador (176 species, AmphibiaWeb 2016), Peru (128 species, AmphibiaWeb 2016), and Bolivia (17 species, AmphibiaWeb 2016) by having narrow digits without circumferential grooves, by lacking a tympanic annulus and tympanic membrane, and by having irregularly shaped, discontinuous dorsolateral folds. In Peru 18 species of *Pristimantis* lack a tympanum; these are *P. academicus* Lehr, Moravec, and Gagliardi Urrutia, 2010, *P. altamazonicus* (Barbour and Dunn, 1921), *P. ashaninka* Lehr and Moravec, 2017, *P. colodactylus* (Lynch, 1979), *P. coronatus* Lehr and Duellman, 2007a, *P. croceoinguinis* (Lynch, 1968), *P. cruciocularis* (Lehr, Lundberg, Aguilar, and von May, 2006), *P. flavobracatus* (Lehr, Lundberg, Aguilar, and von May, 2006), *P. imitatrix* (Duellman, 1978b), *P. lirellus* (Dwyer, 1995), *P. leucorrhinus* Boano, Mazzotti, and Sindaco, 2008, *P. martiae* (Lynch, 1974), *P. minutulus* Duellman and Hedges, 2007, *P. rhabdocnemus* (Duellman and Hedges, 2005), *P. simonsii* (Boulenger, 1900), *P. tantanti* (Lehr, Torres-Gastello,
and Suárez-Segovia, 2007), *P. ventrimarmoratus* (Boulenger, 1912), and *P. vikabamba* Lehr, 2007. Of these, only *Pristimantis simonsii* from northern Peru has narrow digits without circumferential grooves. *Pristimantis attenboroughi* and *P. simonsii* lack circumferential grooves and a tympanum, and both have dorsolateral folds, but *P. attenboroughi* is smaller than *P. simonsii* (female SVL 26.2–33.3 mm in *P. simonsii*), and male *P. attenboroughi* lack nuptial pads which are present in *P. simonsii*.

Members of the *Pristimantis orestes* species Group are terrestrial and inhabit high elevations in southern Ecuador and in Peru (Duellman and Lehr, 2009) and have narrow digits, and only one of the 17 species (Guayasamin and Artega 2013) lacks circumferential grooves (*P. simonsii*), and only two (*P. seorsus, P. simonsii*) lack a tympanum. Furthermore *P. attenboroughi* is phylogenetically distant from members of this group which is considered to be not monophyletic (Duellman and Lehr 2009, Fig. 2).

Among the three other new species of *Pristimantis* from the upper montane forests and Puna of the PPPF, only *Pristimantis* sp. n. E lacks circumferential grooves and a tympanum. However, *P. attenboroughi* and *P. sp. n. E* both differ regarding other morphological traits, coloration, and genetically.

*Pristimantis attenboroughi* shares with *P. stipa* Venegas and Duellman, 2012 from the Puna of northern Peru (Venegas and Duellman 2012) narrow digits without circumferential grooves and dorsolateral folds. However, *P. attenboroughi* is smaller (female SVL 19.2–23.0 mm [n = 10] vs. 35.1 mm [n = 1]), lacks a tympanum (present in *P. stipa*), and has ulnar tubercles not coalesced into fold (coalesced into low fold in *P. stipa*), Venegas and Duellman (2012).

The new species shares narrow digits without circumferential grooves and the absence of a tympanic annulus and tympanic membrane with the Andean genera *Phrynopus* Peters, 1873 (except for *Phrynopus auriculatus* Duellman and Hedges, 2008, and *P. peruanus* Peters, 1873), 28 species from elevations between 2200 and 4400 m a.s.l. in central and northern Peru (Duellman and Lehr, 2009) and *Bryophryne* Hedges, Duellman, and Heinicke, 2008 (8 species from elevations between 2900 and 4120 m a.s.l. in southern Peru, Duellman and Lehr 2009), AmphibiaWeb (2016). *Pristimantis attenboroughi* is most similar with *Phrynopus chaparroi* Mamani and Malqui, 2014 which was described based on morphological characters and found at elevations between 4205 and 4490 m a.s.l. in southern Región Junín (Mamani and Malqui 2014). Both *Pristimantis attenboroughi* and *Phrynopus chaparroi* lack a tympanum and have narrow digits without circumferential grooves. However, *P. attenboroughi* is smaller than *P. chaparroi* (female SVL 19.2–23.0 mm [n = 10] vs. 30.0–32.2 [n = 4]), lacks protuberant subconical posttrical tubercles (present in *P. chaparroi*), has dorsolateral folds (absent in *P. chaparroi*), dentigerous processes of vomers present (absent in *P. chaparroi*), and males lack nuptial pads (present in *P. chaparroi*). *Phrynopus chaparroi* might belong to *Pristimantis*, but molecular characters need to be applied to confirm our suspicion.

**Description of the holotype.** Head about as long as wide; head length 39.7% of SVL; head width 38.6% of SVL; cranial crests absent; snout short, rounded in dorsal view, rounded in lateral view (Fig. 3A, B); eye-nostril distance 70% of eye diameter; nostrils slightly protuberent, directed dorsolaterally; canthus rostralis short, rounded
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in lateral view, weakly concave in dorsal view; loreal region concave; lips rounded; outer margin of upper eyelid each with few slightly enlarged conical tubercles; upper eyelid width 51.9% of IOD (see photo in life Fig. 3); supratympanic fold short and broad, extending from posterior margin of upper eyelid slightly curved to insertion of arm; tympanic membrane and annulus absent; distinct conical postrictal tubercles present bilaterally. Choanae small, ovoid, not concealed by palatal shelf of maxilla; dentigerous processes of vomers positioned posterior to level of choanae, oblique, narrowly separated; tongue long, oval, about three times as long as wide, not notched posteriorly, posterior half free.

Skin on dorsum shagreen with low scattered tubercles, skin on flanks tuberculate, irregularly shaped, discontinuous dorsolateral folds present extending from posterior level of tympanic area to level of hind limb insertion; skin on throat, chest, and belly areolate; discoidal fold absent, thoracic fold present; cloacal sheath short.

Outer ulnar surface each with a row of four minute low tubercles; palmar tubercle bifid; thenar tubercle ovoid; subarticular tubercles well defined, most prominent on base of fingers, round in ventral view, subconical in lateral view; supernumerary tubercles indistinct; fingers short and stout lacking lateral fringes, Finger I shorter than Finger II; tips of digits of fingers narrow, round, lacking circumferential grooves (Fig. 4A).

Hind limbs short, slender, tibia length 40.2% of SVL; foot length 41.3% of SVL; dorsal surfaces of hind limbs tuberculate; inner surface of thighs smooth, posterior surfaces of thighs tuberculate, ventral surfaces of thighs areolate; heels each with a small conical tubercle; outer surface of tarsus with few scattered minute low tubercles; inner tarsal fold absent, but small tubercle proximal to metatarsal tubercle; inner metatarsal tubercle ovoid, one and a half times the size of round outer metatarsal tubercle; subarticular tubercles well defined, round in ventral view, subconical in lateral view; few plantar supernumerary tubercles, about one third the size of subarticular tubercles; toes without lateral fringes; basal webbing absent; tips of digits narrow, round, less expanded than those on fingers, lacking circumferential grooves; relative length of toes: 1<2<5<3<4; Toe V slightly longer than Toe III (tip of digit of Toe III and Toe V not reaching distal subarticular tubercle on Toe IV; Fig. 4B).

**Measurements (in mm) of the holotype.** SVL 18.9; tibia length 7.6; foot length 7.8; head length 7.5; head width 7.3; eye diameter 2.0; inter orbital distance 2.7; upper eyelid width 1.4; internarial distance 1.9; eye–nostril distance 1.4.

**Coloration of the holotype in life (Fig. 3).** The dorsal ground coloration is pale reddish brown with few dark brown flecks; narrow dark brown canthal and supratympanic stripes; flanks pale reddish brown with dark brown flecks forming irregularly shaped diagonal bars; groin and anterior surfaces of thighs reddish brown with dark brown flecks and pale reddish tint; chest, belly, and ventral surfaces of thighs dark grayish brown, throat pale reddish brown and pale gray mottled; palmar and plantar surfaces, and fingers and toes dark grayish brown; iris pale grayish green with fine black vermiculation and brownish-orange horizontal streak across pupil and lower half of iris.

**Coloration of the holotype in preservative.** The dorsal ground coloration is pale brown with few dark brown flecks; narrow dark brown canthal and supratympanic
stripes; flanks pale brown with many dark brown flecks forming irregularly shaped diagonal bars; groin and anterior surfaces of thighs brown with dark brown flecks; chest, belly, and ventral surfaces of thighs dark brown, throat pale brown and pale gray mottled; palmar and plantar surfaces, and fingers and toes dark brown; iris pale gray.

**Variation.** All paratypes (Figs 5–7) are similar to the holotype regarding morphology and proportions (Tables 3, 4). Besides differences in SVL, notable morphological variation includes prominence of dorsolateral folds (e.g., prominent dorsolateral folds in MUSM 31186, 31192, 31195, Fig. 5D–F, G–I; weak dorsolateral folds in MUSM 31186, 31197, NMP6V 75527, 75528, 75529, Fig. 6G–I), and coarseness of tuberculate skin texture on flanks and hind limbs (skin coarsely tuberculate in MUSM 31186, 31192, 31195, NMP6V 75525, Fig. 5; skin weakly tubercular MUSM 31987, 31997, NMP6V 75528, 75529). Two specimens (NMP6V 75529, 75534) have a tubercle-like inner tarsal fold present. *Pristimantis attenboroughi* demonstrates a remarkable polymorphism in coloration (Figs 5–7).

The dorsal coloration ranges from pale gray (MUSM 31987, NMP6V 75533, Fig. 6D–F), dark gray (MSUM 31186, 3199, NMP6V 75522, 75523, 75528, 75529,
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Figure 7. Variation of juvenile paratypes of *Pristimantis attenboroughi* sp. n. in dorsolateral, dorsal, and ventral views. A–C (MUSM 31990, SVL 14.0 mm) D–F (MUSM 31187, SVL 12.5 mm) G–I (MUSM 31200, SVL 14.0 mm). Photos by E. Lehr.

Fig. 6A–C), reddish brown (MUSM 31195, 31975, NMP6V 75525, Figs 5D–F) to brownish olive (MUSM 31992, 31997, Figs 5G–I, 6G–I) with dark gray scattered flecks. Some have an X-shaped mark on scapular (MUSM 31200, 31975, 31990), some ill-defined diagonal bars on the flanks (MUSM 31195). Dark grayish-brown canthal and supratympanic stripes are usually present except for dark gray specimens (MSUM 31186, 3199, NMP6V 75522, 75523, 75528, 75529). The groin is dark gray (MSUM 31186, 3199, NMP6V 75522, 75523, 75528, 75529) or pale reddish brown with a pale red to pink tint in some specimens (MUSM 31195, 31196). The venter is dark gray (NMP6V 75522, 75523, 75528, 75529, Fig. 6C), pale gray (MUSM 31987, Fig. 6F), grayish brown (MUSM 31186, 31195, NMP6V 75525, Fig. 5C, F) or pale grayish green and gray mottled (MUSM 31197, Fig. 6I) or dark gray and pale gray mottled (MUSM 31199, 31975, 31992, NMP6V 75533, Fig. 5I).

Juveniles (MUSM 31187, 31990, 31200, Fig. 7) have a paler coloration (yellowish to reddish brown) with contrasting dark brown flecks and distinct canthal and supratympanic stripes. All have the iris pale grayish green with fine black vermiculation and brownish-orange horizontal streak across pupil and lower half of iris, and usually a narrow vertical dark gray streak from pupil through middle of lower iris.
Table 3. Measurements (in mm) of selected adult type specimens of *Pristimantis attenboroughi* sp. n. M = male, F = female. For other abbreviations see methods.

| Characters | MUSM 31988 | MUSM 31992 | MUSM 31186 | UMMZ 244727 | NMP6V 75523 | MUSM 31980 | MUSM 31977 | MUSM 75076 | MUSM 31987 |
|------------|------------|------------|------------|--------------|-------------|------------|------------|------------|------------|
| sex        | M          | M          | M          | M            | F           | F          | F          | F          | F          |
| SVL        | 14.6       | 15.9       | 18.6       | 19.2         | 20.1        | 21.5       | 21.9       | 22.9       | 23.0       |
| TL         | 6.0        | 6.2        | 7.3        | 6.8          | 8.3         | 8.4        | 8.1        | 8.3        | 8.8        |
| FL         | 5.8        | 6.1        | 7.7        | 7.3          | 9.4         | 8.8        | 8.8        | 9.2        | 10.2       |
| HL         | 5.3        | 6.2        | 6.2        | 6.8          | 7.5         | 7.6        | 7.3        | 8.4        | 7.1        |
| HW         | 5.0        | 5.7        | 6.3        | 6.6          | 7.4         | 7.8        | 7.8        | 7.9        | 7.9        |
| ED         | 1.6        | 1.7        | 1.9        | 1.9          | 2.0         | 2.2        | 2.4        | 2.4        | 2.2        |
| IOD        | 1.8        | 2.1        | 2.4        | 2.1          | 2.7         | 2.5        | 2.3        | 2.6        | 2.9        |
| EW         | 0.9        | 1.4        | 1.2        | 1.3          | 1.6         | 1.6        | 1.6        | 1.6        | 1.3        |
| IND        | 1.3        | 1.5        | 1.7        | 2.0          | 2.0         | 1.9        | 2.1        | 2.3        | 2.1        |
| N-E        | 1.1        | 1.0        | 1.3        | 1.3          | 1.7         | 1.5        | 1.8        | 1.7        | 1.7        |

Table 4. Measurements (in mm) and proportions of adult male and adult female type specimens of *Pristimantis attenboroughi* sp. n.; ranges followed by means and one standard deviation in parentheses. For abbreviations see methods.

| Characters | Males (n = 21) | Females (n = 10) |
|------------|----------------|------------------|
| SVL        | 14.6–19.2 (17.1 ± 1.2) | 19.2–23.0 (21.6 ± 1.1) |
| TL         | 5.8–7.6 (6.7 ± 0.5) | 8.0–8.8 (8.4 ± 0.2) |
| FL         | 5.8–7.8 (7.0 ± 0.5) | 8.8–10.2 (9.3 ± 0.4) |
| HL         | 5.3–7.3 (6.3 ± 0.5) | 7.1–8.4 (7.6 ± 0.4) |
| HW         | 5.0–6.9 (6.0 ± 0.5) | 7.3–8.3 (7.9 ± 0.3) |
| ED         | 1.6–2.1 (1.9 ± 0.2) | 1.8–2.4 (2.1 ± 0.2) |
| IOD        | 1.8–2.5 (2.1 ± 0.1) | 2.3–2.9 (2.7 ± 0.2) |
| EW         | 0.9–1.9 (1.3 ± 0.2) | 1.3–1.7 (1.5 ± 0.1) |
| IND        | 1.3–2.1 (1.6 ± 0.2) | 1.9–2.3 (2.1 ± 0.1) |
| E–N        | 0.8–1.4 (1.2 ± 0.1) | 1.3–1.8 (1.5 ± 0.2) |
| TL/SVL     | 0.34–0.44 | 0.36–0.42 |
| FL/SVL     | 0.35–0.46 | 0.40–0.47 |
| HL/SVL     | 0.33–0.41 | 0.31–0.39 |
| HW/SVL     | 0.31–0.38 | 0.34–0.39 |
| HW/HL      | 0.84–1.02 | 0.94–1.11 |
| E–N/ED     | 0.47–0.71 | 0.62–0.89 |
| EW/IOD     | 0.45–0.70 | 0.45–0.70 |

**Etymology.** We dedicate this species to Sir David Frederick Attenborough in honor for his educational documentaries on wildlife, especially on amphibians (e.g., *Life in Cold Blood*, *Fabulous Frogs*), and for raising awareness about the importance of wildlife conservation. The specific epithet is used as noun in apposition.

**Distribution, natural history, and conservation status.** *Pristimantis attenboroughi* is known from six localities inside the PPPF (Puna of Quebrada Tarhuish at
Figure 8. Habitats of *Pristimantis attenboroughi* sp. n. in the PPPF: A type locality in the upper Tarhuish valley at Laguna Udrecoca, Puna at 3936 m a.s.l., 17 May 2012 B upper montane forest at 3550 m a.s.l. where *P. attenboroughi* sp. n. was found in moss pads C female *P. attenboroughi* sp. n. (MUSM 31980, SVL 21.5 mm) guarding a clutch in a moss pad. Photos by E. Lehr.
Laguna Udrecocha, Fig. 8A; upper montane forest of Quebrada Tarhuish on the left bank “Shiusha” of Antuyo River; Antuyo; Antuyo Bajo; Hatunpata, and Trancapampa, Figs 8B, 9) and from two outside the PPPF (upper part of Quebrada Tasta close to Laguna Luichococha; in *Polylepis* forest of first mountain peak next to trail from Tasta to Tarhuish), and is distributed at elevations between 3400 and 3936 m a.s.l., Fig. 9. The type locality (Figs 8A, 9), upper part of Quebrada Tarhuish, on the east side of Laguna Udrecocha at 3936 m a.s.l., belongs to the Puna ecoregion (Brack 1986). The vegetation consists of Peruvian feather grass (*Stipa ichu*), mosses, and small bushes. The holotype was found inside moss in the afternoon on 17 May 2012. No sympatric anurans were found at the type locality. At the upper montane forest of Quebrada Tarhuish on the left bank “Shiusha” of Antuyo River, *Pristimantis attenboroughi* was found deep inside large moss layers. Sympatric anurans are *Gastrotheca griswoldi* (MUSM 31193),

| Location                  | Elevation (m a.s.l.) |
|---------------------------|----------------------|
| Laguna Udrecocha          | 3936                 |
| Upper part of Quebrada Tasta | 3708                |
| Polylepis forest patch    | 3886                 |
| Quebrada Tarhuish, left bank of Antuyo River | 3414 |
| Antuyo                    | 3700                 |
| Antuyo Bajo               | 3400                 |
| Hatunpata                 | 3710                 |
| Trancapampa               | 3550                 |
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Pristimantis sp. n. C (MUSM 31190–92), Pristimantis sp. n. D (MUSM 31197–98), and Phrynopus sp. n. A (MUSM 31203).

A female Pristimantis attenboroughi (MUSM 31980, Fig. 8C) guarding 20 eggs was found at Hatunpata inside moss, 3710 m a.s.l., on 28 June 2013. The eggs were pale cream colored and had an average diameter of 3.5 ± 0.1 mm (3.3–3.6 mm, n = 20).

The IUCN Red List criteria (IUCN 2001) consider that if a species occurs in fewer than 10 threat-defined locations and the extent of occurrence (EOO) is < 20,000 km², it should be classified as Vulnerable or Endangered. Pristimantis attenboroughi is known from seven localities distributed in the PPPF and its buffer zone (Fig. 9), with an estimated EOO of 66.54 km². As such, this new species might be classified as Vulnerable if we take into account these criteria. However, given that the PPPF may host a greater number of locations and most of them are inside the protected area, we propose that Pristimantis attenboroughi should likely be categorized as Near Threatened (NT).

Given that the known distribution of Pristimantis attenboroughi overlaps with the PPPF, a substantial portion of the habitat of this species is formally protected. However, other factors such as fungal infections, climate change, pollution, and man-made fires (used to expand grazing areas for livestock) continue to be threats for many Andean amphibians even inside protected areas (Catenazzi and von May 2014).

Discussion

When we encountered the first specimen of Pristimantis attenboroughi in the field both of us were sure that we had found a new species of Phrynopus because of its overall morphological appearance: most species in the genus Phrynopus usually lack tympanum, have narrow digits without circumferential grooves and are distributed at high elevations. However, following an integrative taxonomy approach that included molecular and morphological data, we realized that Pristimantis attenboroughi is not a Phrynopus species. Our analysis also revealed that Pristimantis attenboroughi is not closely related to other Pristimantis species that have narrow digits (e.g., members of the P. orestes species group), an assumption that could have been made if only morphological data were available. In other words, Pristimantis attenboroughi displays convergence that easily could have led to an incorrect generic assignment. Pristimantis attenboroughi is morphologically most similar to Phrynopus chaparroi (Mamani and Malqui 2014) and we assume that the latter species might belong to Pristimantis and to the danae species group. Thus, molecular data are needed to determine whether the current generic placement of Phrynopus chaparroi is correct.

With Pristimantis attenboroughi, seven species of Pristimantis are known from the Puna (> 3000 m a.s.l.) of Peru. Of these, six occur in northern Peru (P. atrabracus [Duellman and Pramuk, 1999], 2963–3330 m a.s.l.; P. bellator Lehr, Aguilar, Siu-Ting, Jordán, 2007, 1900–3100 m a.s.l.; P. cordovae [Lehr and Duellman, 2007b], 3400–4100 m a.s.l.; P. mariaelenae Venegas and Duellman, 2012, 3596 m a.s.l.; P. pinguis
[Duellman and Pramuk, 1999], 3000–3916 m a.s.l.; *P. stipa* Venegas and Duellman, 2012, 3596 m a.s.l.), and only one species in central Peru (*P. attenboroughi*, 3400–3936 m a.s.l.), Duellman and Lehr 2009. Navarrete et al. (2016) pointed out the disparity in species richness of *Pristimantis* at high elevation between Ecuador (18 species of *Pristimantis*) and Peru (5 species of *Pristimantis*). Whilst the Páramo in Ecuador is more humid than the drier Puna in Peru, it is likely that, besides climatic differences between the two regions, the lower species richness of *Pristimantis* in the Puna of Peru is an artifact of lower survey effort and the presence of other high-elevation clades not present in Ecuador. Thus, we hypothesize that the occurrence of the genus *Phrynopus* at high elevations (28 species from elevations between 2200–4400 m a.s.l., AmphibiaWeb 2016, Duellman and Lehr 2009) in central Peru might restrict the number of niches available for *Pristimantis* at high elevations.

Additional new species of terrestrial-breeding frogs from montane forests and Puna of the PPPF will be described in the near future.

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Appendix

Comparative specimens examined

Pristimantis mariaelenae: Peru: Lambayeque: Cañaris, 3406–3494 m: MUSM 26478.
Pristimantis simonsii: Peru: Cajamarca: 23.5 km NE Encanada, 3510 m: MUSM 1163–1179.
Pristimantis stipa: Peru: Lambayeque: Cañaris, 3406–3494 m: MUSM 26481, 26482.
Phrynopus sp. n. A: Peru: Junín: Pui Pui Protected Forest: near trail from Tasta to Tarhuish (first mountain peak), Polylepis forest patch, 3886 m: MUSM 31203.
Pristimantis sp. n. C: Peru: Junín: Pui Pui Protected Forest: Quebrada Tarhuish on the left bank „Shiusha“ of Antuyo River, 3414 m: MUSM 31190–92.
Pristimantis sp. n. D: Peru: Junín: Pui Pui Protected Forest: Quebrada Tasta, Runda, 3463 m: MUSM 31197–98.
Pristimantis sp. n. E: Peru: Junín: Peru: Junín: Pui Pui Protected Forest: Laguna Sinchon, 3890 m: MUSM 31981–83.