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A new species of terrestrial-breeding frog of the genus *Psychrophrynella* (Anura: Strabomantidae) from the Cordillera de Vilcabamba, southeastern Peru

^{1,2,*}F. Peter Condori, ³Aldemar A. Acevedo, ^{1,2,4}Luis Mamani, ²J. Amanda Delgado, and ^{1,2}Juan C. Chaparro

¹Museo de Biodiversidad del Perú, Urbanización Mariscal Gamarra A-61, Zona 2, Cusco, PERU ²Museo de Historia Natural de la Universidad Nacional de San Antonio Abad del Cusco, Paraninfo Universitario (Plaza de Armas s/n), Cusco, PERU ³Laboratorio de Biología Evolutiva, Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Alameda 340, Santiago 6513677, CHILE ⁴Programa de Doctorado en Sistemática y Biodiversidad, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, CHILE

Abstract.—A new frog of the genus *Psychrophrynella* is described based on specimens from the Cordillera de Vilcabamba, in the department of Cusco in southeastern Peru. The new species inhabits the humid puna and is only known from its type locality in Challcha, near the road between Vilcabamba and Pampaconas, at 3,707 m asl. This new taxon is assigned to the genus *Psychrophrynella* based on a narrowest genetic distance of 16S rRNA with *P. glauca* (8.3%) and the presence of a fold-like tubercle on the inner edges of the tarsus. The description of *Psychrophrynella vilcabambensis* sp. nov. is based on three individuals. This new species can be differentiated from other members of the genera *Psychrophrynella* and *Noblella* by the combination of the following characters: light reddish-brown to tan coloration on the dorsum with dark brown markings, the presence of a thoracic fold, ulnar tubercles, a tubercle on the heel, three tubercles on outer edge of tarsus, and toes with lateral fringes. The SVL of male and female specimens are 16.5 and 16.6 mm, respectively.

Keywords. Amphibia, Andes, Cusco, Holoadeninae, *Noblella*, *Psychrophrynella vilcabambensis* sp. nov.

Resumen.—Describimos una nueva rana del género *Psychrophrynella* de la Cordillera de Vilcabamba, en el departamento de Cusco al sudeste del Perú. La nueva especie habita la puna húmeda y sólo se conoce de su localidad tipo en Challcha, cerca de la carretera entre Vilcabamba y Pampaconas, a 3,707 m snm. El nuevo taxón se asigna al género *Psychrophrynella*, basándose en la distancia genética de 16S ARNr más estrecha con *P. glauca* (8.3%), y la presencia de un tubérculo alargado similar a un pliegue en el borde interior del tarso. *Psychrophrynella vilcabambensis* sp. nov. fue descrita en base a tres individuos. Esta nueva especie se puede diferenciar de otros miembros de los géneros *Psychrophrynella* y *Noblella* por la siguiente combinación de caracteres: coloración marrón rojiza clara a marrón bronceada en el dorso con manchas marrón oscuras, presencia de pliegue torácico, tubérculos cubitales, un tubérculo en el talón, tres tubérculos en el borde exterior del tarso y dedos con rebordes laterales. El SVL del macho y de la hembra es 16.5 y 16.6 mm, respectivamente.

Palabras clave. Andes, Anfibia, Cusco, Holoadeninae, *Noblella*, *Psychrophrynella vilcabambensis* sp. nov.

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Introduction

The subfamily Holoadeninae Hedges, Duellman, and Heinicke, 2008 was constructed based on molecular data, and includes the genera *Barycholos*, *Bryophryne*,

Euparkerella, *Holoaden*, *Microkayla*, *Noblella*, *Psychrophrynella*, and *Qosqophryne* (Catenazzi et al. 2020; Heinicke et al. 2018). *Psychrophrynella bagrecito* Hedges, Duellman, and Heinicke, 2008 is the type species of the genus *Psychrophrynella*, proposed by Hedges et al.

Correspondence. *cfrankpeter@gmail.com

(2008). In the same study, Hedges et al. (2008) include one Peruvian and 18 Bolivian species in the genus *Psychrophrynella*. Subsequent studies increased the diversity of the genus to 22 species distributed in humid puna of the Andes of southeastern Peru and northwestern Bolivia (De la Riva and Burrowes 2014; De la Riva and Aparicio 2016). However, in 2017, additional molecular studies by De la Riva et al. (2017) divided the genus *Psychrophrynella* into two genera, *Psychrophrynella* and the new genus *Microkayla*. In this new genus, these authors included 24 species from Bolivia and three from Peru. Moreover, De la Riva et al. (2017) supported the monophyly and validity of the genus *Psychrophrynella*, reducing the number of species in this genus to three (*P. bagrecito*, *P. chirihampatu*, and *P. usurpator*), while the other species were moved to the genus *Microkayla*. Later, Catenazzi and Ttito (2018) described *P. glauca* from Peru.

Thus, the Peruvian endemic genus *Psychrophrynella* currently contains four formally described species (*P. bagrecito*, *P. usurpator*, *P. chirihampatu*, and *P. glauca*). This genus is distributed along the eastern slopes of southeastern Peruvian Andes, between the departments of Cusco and Puno at 2,225–3,400 m asl, where these frogs are typically found in the leaf litter and under stones and terrestrial mosses (Catenazzi and Ttito 2016, 2018; De la Riva et al. 2008; Lynch 1986).

Despite the advancements in the systematics and taxonomy of the subfamily Holoadeninae (De la Riva 2020; De la Riva et al. 2017; Heinicke et al. 2018), our understanding of some lineages is still precarious. One example is the uncertainty regarding the phylogenetic relationship between *Psychrophrynella* and *Noblella* (Catenazzi and Ttito 2016, 2018; Catenazzi et al. 2020). The genus *Noblella* is polyphyletic and includes two divergent lineages: one containing five species with distribution ranges from central Peru to Ecuador, and the other with four species from southeastern Peru (Catenazzi and Ttito 2019; Reyes-Puig et al. 2019; Santa Cruz et al. 2019). The type species of *Noblella* (*N. peruviana*) and *Psychrophrynella* (*P. bagrecito*) are both distributed in southeastern Peru, but genetic sequences are not available for these two species and the synapomorphies that support *Noblella* and *Psychrophrynella* are inconclusive (Catenazzi and Ttito 2016, 2018; De la Riva et al. 2008; Santa Cruz et al. 2019). Herein, a morphological description is provided for a new species of terrestrial-breeding frog from the Cordillera de Vilcabamba, department of Cusco in southeastern Peru. This species was recognized as a new species in some earlier studies, being listed as *Psychrophrynella* sp. by Lehr and Catenazzi (2010) and Catenazzi et al. (2020).

Materials and Methods

Data collection. Specimens were collected by hand and euthanized by application of 8% benzocaine paste on the

dorsal and ventral regions. Tissue samples (muscle) were stored in 2 mL cryogenic tubes filled with 96% ethanol, and specimens were fixed in 10% formalin and preserved in 70% ethanol. Specimens were deposited in the herpetological collection of the Museo de Biodiversidad del Peru (MUBI).

Morphology. The description of morphological characters of *Psychrophrynella* follows Duellman and Lehr (2009), and Catenazzi and Ttito (2016, 2018). The taxonomic classification follows Heinicke et al. (2018). Morphometric measurements were taken using a digital caliper and a stereoscope. Abbreviations of measurements are as follows: snout-vent length (SVL), tibia length (TL), foot length (FL, distance from the 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), eye diameter (ED), tympanum diameter (TY), interorbital distance (IOD), upper eyelid width (EW), internarial distance (IND), and eye-nostril distance (E-N, straight line distance between the anterior corner of orbit and posterior margin of external nares). Fingers and toes are numbered preaxially to post axially from I–IV and I–V, respectively. To determine the lengths of Toes III and V, both toes were pressed against Toe IV, and the lengths of fingers I and II were determined by pressing these fingers against each other. The variation in coloration in life is based on the field notes and photographs by the third author (LM). This new species was compared with the descriptions as published in the literature for the four valid and formally described species of the genus *Psychrophrynella* (Catenazzi and Ttito 2016, 2018; De la Riva et al. 2008; Lynch 1986), and five species of the genus *Noblella* from southern Peru (Catenazzi and Ttito 2019; Catenazzi et al. 2015; Lehr and Catenazzi 2009; Noble 1921; Santa Cruz et al. 2019). Examined material is listed in **Appendix 1**.

DNA extraction, amplification, and sequencing.

Genomic DNA was extracted from the voucher specimen MUBI 13485 using the QIAGEN DNeasy Blood and Tissue extraction kit following the manufacturer's protocol. Fragments of the mitochondrial long subunit rRNA gene (16S) were amplified by Polymerase Chain Reaction (PCR) with the following conditions: an initial 2 min at 93 °C, followed by 35 cycles of 30 sec at 95 °C, 1 min at 42 °C, 1.5 min at 72 °C, and a final extension step of 6 min at 72 °C. The primers used were 16Sar (CGC CTG TTT ATC AAA AAC AT) and 16Sbr (CCG GTC TGA ACT CAG ATC ACG T) [Palumbi et al. 1991]. Purified PCR products were sent to Macrogen Inc. (Seoul, Republic of Korea) for sequencing in both directions with the amplification primers. Raw sequence chromatographs for sequences generated in this study were edited using AliView 1.14 (Larsson 2014). One new gene sequence of this locus was produced with GenBank accession number MT818174.

Table 1. GenBank codes for sequences of the species of the subfamily Holoadeninae used in this study.

Species and voucher specimens	GenBank accession (16S)	Source
<i>Barycholos ternetzi</i> CFBHT 04408	KU495152	Lyra et al. 2016
<i>Bryophryne bakersfield</i> MUBI 6022	MF186341	De la Riva et al. 2017
<i>Bryophryne cophites</i> AC 270.07	KY652641	von May et al. 2017
<i>Bryophryne hanssaueri</i> MUSM 27567	KY652642	von May et al. 2017
<i>Bryophryne nubilosus</i> MUSM 27882	KY652643	von May et al. 2017
<i>Bryophryne phuyuhampatu</i> CORBIDI 18226	MF419256	Catenazzi et al. 2017
<i>Holoaden luederwaldti</i> CFBHT 07810	KU495249	Lyra et al. 2016
<i>Microkayla chilina</i> MNCN 43774	MF186416	De la Riva et al. 2017
<i>Microkayla iatamasi</i> MNCN 42054	MF186368	De la Riva et al. 2017
<i>Microkayla katantika</i> CBF 6012	MF186380	De la Riva et al. 2017
<i>Noblella lochites</i> KU 177356	EU186699	Hedges et al. 2008
<i>Noblella losamigos</i> MUSA 6973	KY652644	von May et al. 2017
<i>Noblella madrevelva</i> CORBIDI 15770	MN056356	Catenazzi and Ttito 2019
<i>Noblella myrmecoides</i> QCAZ 40180	JX267542	Canedo and Haddad 2012
<i>Noblella pygmaea</i> MUSM 24536	KY652645	von May et al. 2017
<i>Noblella thiuni</i> CORBIDI 18723	MK072732	Catenazzi and Ttito 2019
<i>Phrynopus peruanus</i> MUSM 38316	MG896582	von May et al. 2018
<i>Psychrophrynella chirihamptu</i> MUBI 14664	KU884560	Catenazzi and Ttito 2016
<i>Psychrophrynella glauca</i> CORBIDI 18729	MG837565	Catenazzi and Ttito 2018
<i>Psychrophrynella usurpator</i> AC186.09	KY652662	von May et al. 2017
<i>Psychrophrynella vilcabambensis</i> sp. nov. MUBI 13485	MT818174	This study
<i>Psychrophrynella</i> sp. MUSM 27619	MT437065	Catenazzi et al. 2020

Genetic distances. Uncorrected *p*-distances were estimated using the 16S rRNA mitochondrial gene, comparing the new species to some representative species of the genera *Barycholos*, *Bryophryne*, *Holoaden*, *Microkayla*, *Noblella*, *Phrynopus*, and *Psychrophrynella*, which were available in GenBank (Table 1). The DNA sequences were aligned in MUSCLE (Edgar 2004) and uncorrected *p*-distances were estimated in MEGAX (Kumar et al. 2018). Following Catenazzi and Ttito (2016, 2018), phylogenetic analyses were not performed because taxonomic uncertainty exists in the genera *Noblella* and *Psychrophrynella*; and molecular information about the type species, *Noblella peruviana* and *Psychrophrynella bagrecito*, are needed for taxonomic resolution.

Results

Generic placement. The new species is placed in the genus *Psychrophrynella* (Hedges et al. 2008) on the basis of morphological and molecular data. The main diagnostic phenotypic traits of *Psychrophrynella* are: (1) tympanic membrane and annulus differentiated (annulus and membrane visible beneath skin); (2) tips of digits narrow and rounded, not expanded, lacking circumferential grooves and pads; and (3) inner edge of tarsus bearing a prominent, elongate, sigmoid-shaped or fold-like tubercle (De la Riva et al. 2017). These aforementioned characteristics are shared by the new species. Additionally, analyses of the

uncorrected *p*-distances for 16S rRNA showed that the new species has wide genetic distances from all species that were compared (Table 2), the narrowest being with *P. glauca* (8.3%) and *N. thiuni* (9.4%).

Taxonomy

Psychrophrynella vilcabambensis **sp. nov.**

Psychrophrynella sp. Lehr and Catenazzi 2010: 317

Psychrophrynella sp. MUSM 27619 Catenazzi et al. 2020: 10.

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Holotype. MUBI 13485, an adult male (Fig. 1) from Challcha (13°05'44"S, 73°01'37.7"W) [WGS84], 3,707 m asl, district of Vilcabamba, province of La Convención, department of Cusco, Peru; collected on 8 August 2016, by F.P. Condori, L. Mamani, and J.A. Delgado.

Paratypes. Two specimens: one adult female, MUBI 13486 (Fig. 2A–B), and one juvenile, MUBI 13484 (Fig. 2C–D), same data as holotype.

Diagnosis. *Psychrophrynella vilcabambensis* **sp. nov.** is characterized by having: (1) skin on dorsum shagreen with small warts, coalescing into linear ridges at midbody; dorsolateral fold visible on half of the body

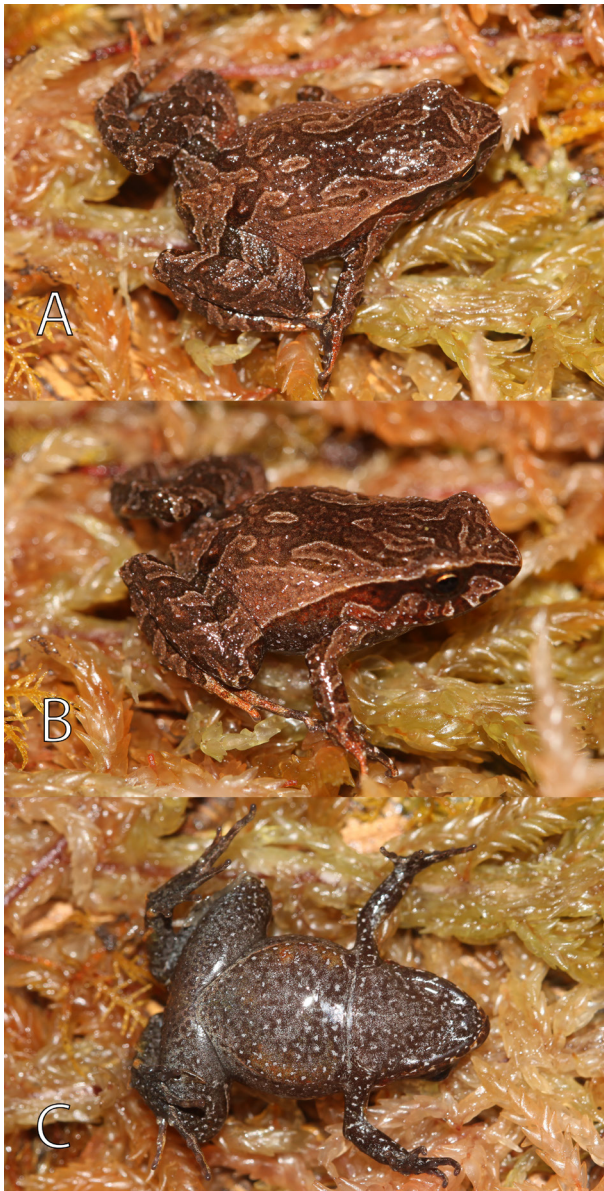


Fig. 1. Photographs in life of the holotype of *Psychrophrynella vilcabambensis* sp. nov., adult male MUBI 13485 (SVL = 16.5 mm.). (A–B) Dorsolateral views; (C) ventral view.

and ending posteriorly in a sacral tubercle; skin on venter smooth, discoidal, and thoracic fold present; (2) tympanic membrane not differentiated, anteroventral part of tympanic annulus barely visible below skin; (3) snout short, rounded in dorsal view and in profile; (4) upper eyelid narrower than IOD, bearing small tubercles; cranial crests absent; (5) dentigerous processes of vomers absent; (6) vocal slits present; nuptial pads absent; (7) fingers lacking lateral fringes; Finger I shorter than Finger II; tips of digits rounded, not expanded laterally; (8) ulnar tubercles present; (9) heel with one tubercle; inner edge of tarsus bearing an elongate, oblique fold-like tubercle; outer edge of tarsus with some tubercles; (10) inner metatarsal tubercle prominent elliptical, 1.25 times larger than ovoid outer metatarsal tubercle; supernumerary plantar tubercles small, poorly defined; (11) toes with lateral fringes; webbing absent; Toe V slightly

shorter than or equal to Toe III; tips of digits rounded, not expanded; (12) dorsum light reddish brown to tan, with or without a pale middorsal line extending from tip of snout to the cloaca, and with dark brown markings, inside of which there are dermal protuberances; interorbital blotch present; flanks dark reddish brown; chest and throat dark brown with moderate or abundant pale gray flecks; palmar and plantar surfaces dark brown with tiny pale gray flecks; belly and legs grayish brown with pale gray flecks; (13) SVL of males 16.5 mm (based on a single adult specimen), SVL of females 16.63 mm (based on a single adult specimen) [Table 3].

Comparative diagnosis. The new species differs morphologically from species of *Noblella* in southern Peru (*N. losamigos*, *N. madreSelva*, *N. peruviana*, *N. pygmaea*, and *N. thiuni*) based on discoidal fold. It is also different from *N. losamigos*, *N. madreSelva*, *N. peruviana*, and *N. pygmaea* due to the absence of elongate acuminate toe tips. Relative to all species of *Psychrophrynella*, it differs in having unique characters such as light reddish-brown to tan coloration on dorsal surfaces with dark brown marks and in presenting a thoracic fold, toes with lateral fringes (Fig. 3A), one tubercle on the heel and some tubercles on the outer edge of the tarsus, and ulnar tubercles (Fig. 3B). Morphologically, *P. vilcabambensis* sp. nov. is similar to *P. chirihampatu* in having a large fold-like tubercle on the inner edge of the tarsus, a prominent elliptical inner metatarsal tubercle larger than the ovoid outer metatarsal tubercle, and shagreen dorsum skin with small warts forming linear ridges at the middorsum. However, *P. vilcabambensis* sp. nov. can be distinguished from *P. chirihampatu* by having a visible discoidal fold (not visible in *P. chirihampatu*), small upper eyelid tubercles (lacking in *P. chirihampatu*), and an inner metatarsal tubercle 1.25 times larger than the outer metatarsal tubercle (versus 1.5 times). From *P. bagrecito*, the new species differs by having smooth (areolate) skin on venter, short snout (moderately long), tarsus with an elongate fold-like tubercle (short), inner metatarsal tubercle larger than outer metatarsal tubercle (equal), poorly defined small supernumerary plantar tubercles (lacking), and Toe V slightly shorter or equal to Toe III (Toe V shorter than Toe III). From *P. glauca*, it differs by having a dorsolateral fold (absent), short snout (snout very short), small upper eyelid tubercles (lacking), tarsus elongate with fold-like tubercle (short), and inner metatarsal tubercle larger than outer metatarsal tubercle (equal). From *P. usurpator*, the new species differs by having shagreen dorsum skin (smooth), small upper eyelid tubercles (lacking), inner metatarsal tubercle larger than outer metatarsal tubercle (inner metatarsal tubercle same size as outer metatarsal tubercle), and Toe V slightly shorter than or equal to Toe III (Toe V shorter than Toe III).

Description of holotype. Adult male (SVL 16.5 mm); head narrower than the body, its length 28.30% of SVL;



Fig. 2. Photographs in life of paratypes of *Psychrophrynella vilcabambensis* sp. nov. (A–B) Dorsolateral and ventral views of adult female, MUBI 13486 (SVL = 16.63 mm); (C–D) dorsolateral and ventral views of juvenile MUBI 13484 (SVL = 12.99 mm).

head slightly wider than long, HW 117.8% of HL; HW 33.3% of SVL; snout short, rounded in dorsal and lateral views, ED 38.97% of HL, its diameter 1.6 times as large as its distance from the nostril; nostrils not protuberant, close to snout, directed laterally; canthus rostralis concave in dorsal view, slightly convex in profile; loreal region flat; lips rounded; upper eyelids with small tubercles; EW 62.6% of IOD; interorbital region flat, lacking cranial crests; E–N distance 62.1% of ED; supratympanic fold absent; tympanic membrane not differentiated, anteroventral part of tympanic annulus visible below skin; postrictal tubercles present. Choanae round, small; dentigerous processes of vomers and vomerine teeth absent; the tongue covers almost the entire floor of the mouth, and it is large and ovoid.

Skin on dorsum shagreen with small warts, which are equally distributed on the dorsum, at middorsum these warts conform linear ridges; dorsolateral folds present only anteriorly and terminate posteriorly in a sacral tubercle; skin on flanks shagreen; venter smooth; pectoral and discoidal fold present; cloaca not protuberant, cloacal region with small tubercles. Ulnar tubercles present; circular outer palmar tubercle approximately the same length but twice the width of oval thenar tubercle; supernumerary palmar tubercles present; subarticular tubercles prominent, rounded in ventral and lateral view; fingers lacking lateral fringes, not webbed; relative lengths of fingers $3 > 4 > 2 > 1$; tips of digits bulbous, not expanded laterally.

Hindlimbs moderately long, TL 44.4% of SVL; FL 51.5% of SVL, upper surface shagreen with moderately small tubercles; posterior surfaces smooth; heel with one tubercle; inner edge of tarsus bearing a large, oblique fold-like tubercle, outer edge of tarsus with tubercles; elliptical inner metatarsal tubercle larger than ovoid outer metatarsal tubercle; plantar supernumerary tubercles weakly defined; subarticular tubercles rounded in ventral view and ovoid in profile view; toes with lateral fringes, not webbed; toe tips weakly pointed, not expanded laterally; relative lengths of toes $4 > 3 > 5 > 2 > 1$ (Fig. 2A).

Measurements of holotype (in mm). SVL 16.5, TL 7.33, FL 8.49, HL 4.67, HW 5.5, ED 1.82, TY 0.89, IOD 2.19, EW 1.37, IND 1.8, E–N 1.13.

Coloration of holotype in life. Dorsal surfaces of head, body, and extremities reddish brown, with dark brown markings bordered by a poorly defined cream stripe. Lateral surface of the head with three dark brown labial bars, the middle one in contact with the eye; and a dark reddish-brown stripe, extending from the tip of the snout to the border of the eye, crossing above the tympanum and extending to the insertion of the forelimb. This stripe becomes redder and lighter as it approaches the insertion of the forelimb. Iris dark reddish brown with abundant black reticulations and gold pallid stripe on

Table 2. Uncorrected *p*-distances for 16S rRNA sequences between *Psychrophrynella vilcabambensis* **sp. nov.** and related taxa in the subfamily Holadeninae. The genetically most similar species are highlighted in blue.

<i>Barycholos ternetzi</i>	19.8																										
<i>Bryophryne bakersfield</i>																											
<i>Bryophryne cophites</i>		1																									
<i>Bryophryne hanssaueri</i>	20.8	3.1	4.2																								
<i>Bryophryne nubilosus</i>	19.8	5.2	6.3	2.1																							
<i>Bryophryne phyuuhampatu</i>	18.8	4.2	4.2	3.1	5.2																						
<i>Holoaen ludewaldti</i>	18.8	17.7	16.7	14.6	12.5	14.6																					
<i>Microkayla chilina</i>	20.8	12.5	12.5	11.5	11.5	9.4	14.6																				
<i>Microkayla iatamasi</i>	18.8	11.5	11.5	12.5	12.5	10.4	15.6	3.1																			
<i>Microkayla katantika</i>	18.8	12.5	12.5	9.4	9.4	9.4	11.5	5.2	5.2																		
<i>Noblella lochites</i>	12.5	17.7	17.7	15.6	13.5	13.5	12.5	16.7	15.6	16.7	15.6																
<i>Psychrophrynella vilcabambensis</i> sp. nov.	16.7	11.5	10.4	12.5	13.5	11.5	18.8	13.5	12.5	12.5	13.5	16.7	15.6														
<i>Noblella losamigos</i>	16.7	16.7	15.6	15.6	16.7	16.7	14.6	16.7	15.6	15.6	15.6	14.6	16.7	15.6	11.5	17.7	21.9										
<i>Noblella myrmecoides</i>	16.7	15.6	15.6	15.6	15.6	13.5	16.7	14.6	15.6	13.5	13.5	13.5	15.6	13.5	11.5	11.5	21.9										
<i>Noblella pygmaea</i>	15.6	16.7	15.6	16.7	17.7	15.6	15.6	13.5	13.5	13.5	14.6	14.6	14.6	14.6	14.6	12.5	8.3	21.9									
<i>Noblella madreseha</i>	15.6	15.6	14.6	16.7	15.6	15.6	14.6	15.6	14.6	16.7	16.7	14.6	14.6	14.6	11.5	11.5	20.8										
<i>Noblella thiuni</i>	13.5	11.5	12.5	12.5	13.5	12.5	15.6	10.4	9.4	13.5	13.5	13.5	13.5	13.5	9.4	8.3	18.8	7.3	9.4								
<i>Psychrophrynella chirihampatu</i>	11.5	14.6	13.5	15.6	16.7	14.6	14.6	14.6	12.5	12.5	12.5	14.6	14.6	14.6	13.5	9.4	19.8	7.3	8.3	8.3							
<i>Psychrophrynella glauca</i>	15.6	13.5	12.5	14.6	14.6	13.5	18.8	12.5	10.4	11.5	11.5	11.5	12.5	12.5	12.5	12.5	18.8	12.5	11.5	9.4	10.4						
<i>Psychrophrynella usurpator</i>	14.6	17.7	16.7	14.6	13.5	15.6	11.5	15.6	15.6	12.5	12.5	12.5	12.5	12.5	11.5	9.4	19.8	11.5	5.2	11.5	7.3	11.5					
<i>Phrynopus peruans</i>	20.8	13.5	12.5	13.5	13.5	13.5	16.7	10.4	9.4	9.4	9.4	9.4	19.8	13.5	17.7	12.5	17.7	17.7	15.6	15.6	16.7	13.5	18.8				
<i>Psychrophrynella</i> sp. MUSM27619	16.7	11.5	10.4	12.5	13.5	11.5	18.8	13.5	12.5	12.5	13.5	13.5	13.5	13.5	16.7	11.5	17.7	12.5	11.5	9.4	13.5	8.3	13.5	13.5			

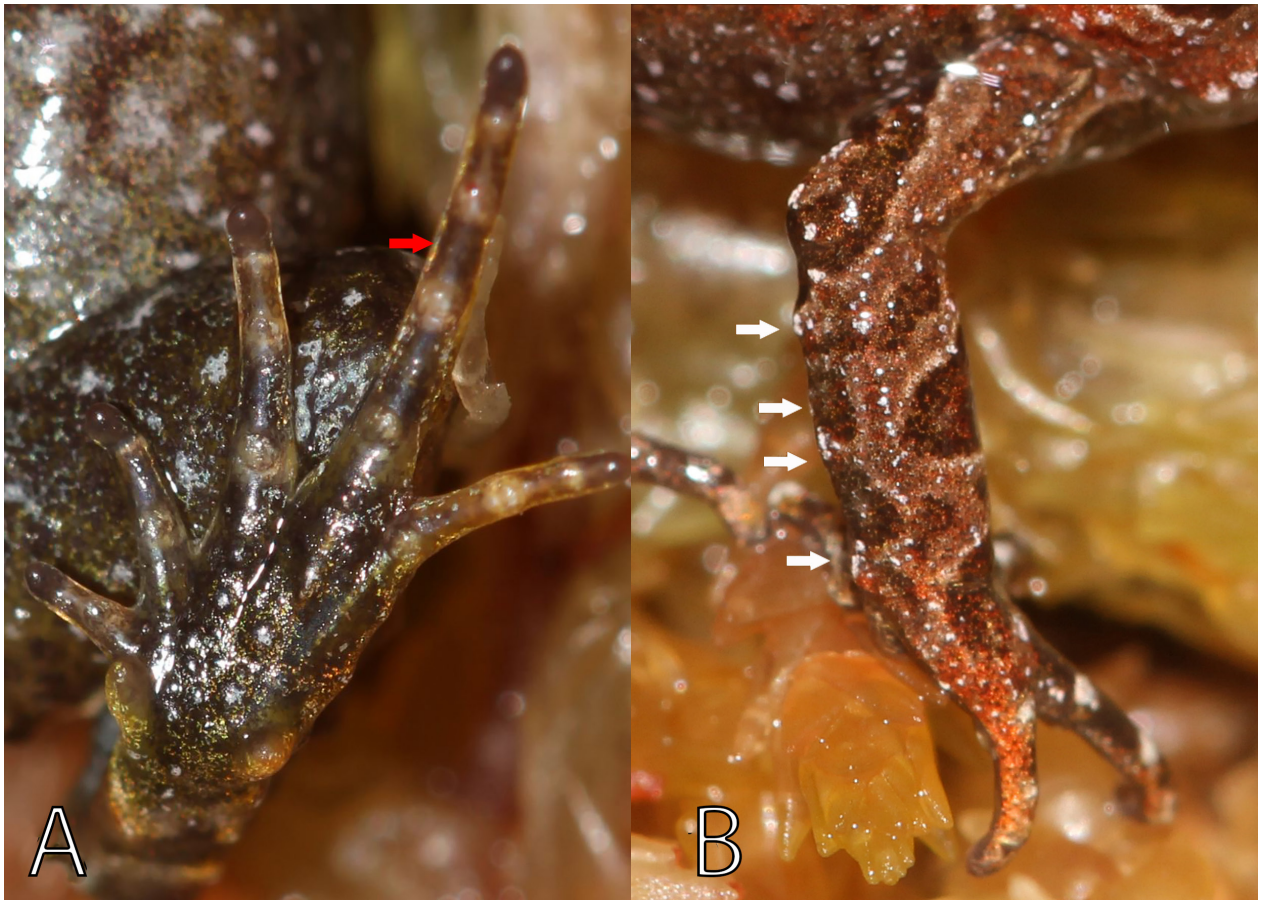


Fig. 3. Morphological details of *Psychrophrynella vilcabambensis* sp. nov. (A) Plantar surface with a red arrow indicating the presence of lateral fringes (MUBI 13484); (B) lateral view of forelimb with white arrows indicating the presence of ulnar tubercles (MUBI 13485).

upper part of pupil. Throat, chest, and anterior part of belly dark brown, fading into light brown posteriorly, with moderate small pale gray flecks and abundant tiny dots of the same color. Ventral parts of limbs brown with moderate small pale gray flecks. Dorsal surfaces of hind limbs with a dark transverse bar. Posterior surfaces of thighs and groin grayish brown; plantar and palmar surfaces brown (Fig. 3).

Coloration in preservative. Similar to coloration in life, but dorsal coloration varies from brown to light brown. The parts that were reddish brown lose the red coloration, and ventral surfaces become more gray than brown.

Variation. Dorsum coloration varies from dark reddish brown to light brown. Flecks on the back are irregular in shape. The dark brown lateral stripe extends from the tip of the snout to the insertion of the forelimb in the holotype, while in the paratypes it reaches the anterior half of the body, and the posterior half has similar coloration as the dorsum. The belly coloration in the female paratype (MUBI 13486) is light brown with abundant tiny pale gray spots. The juvenile paratype (MUBI 13484) has a creamy dorsal midline extending from the tip of the snout to the cloaca; the male holotype and female do not differ in size or general color pattern.

Etymology. The specific epithet, *vilcabambensis*, is given after the name of the mountain range “Cordillera de Vilcabamba” where the species was found.

Distribution, natural history, and threats. *Psychrophrynella vilcabambensis* sp. nov. is known only from elevations of 3,707 m asl in the type locality (Challcha, department of Cusco), near the road between Vilcabamba and Pampaconas (Fig. 4). All specimens were found in high Andean puna (Fig. 5) during the day, under mosses covering the rocks. Sympatric amphibian and reptile species include *Bryophryne flammiventris*, *Nannophryne* sp., and *Proctoporus lacertus*. The type locality suffers from anthropogenic activities, such as farming and livestock production, which might be potential threats to this species. Following the IUCN Red List criteria (IUCN 2019), in the absence of more detailed data concerning population status, extent, and occurrence, we suggest this species be placed in the Data Deficient category of the Red List.

Discussion

The highlands of the Andes of southeastern Peru (departments of Cusco and Puno) are inhabited by 23 species of small, directly developing frogs, with plump

Table 3. Measurements (in mm) of type series of *Psychrophrynella vilcabambensis* sp. nov. See text for character abbreviations.

Characters	Female (n = 1)	Male (n = 1)
SVL	16.6	16.5
TL	7.8	7.3
FL	8.5	8.5
HL	4.8	4.7
HW	5.3	5.5
ED	1.8	1.8
TY	0.9	0.9
IOD	2.3	2.2
EW	1.4	1.4
IND	2.0	1.8
E-N	1.3	1.1
TL/SVL	0.5	0.4
FL/SVL	0.5	0.5
HL/SVL	0.3	0.3
HW/SVL	0.3	0.3
HW/HL	1.1	1.2
E-N/ED	0.7	0.6
EW/OID	0.6	0.6
ED/HL	0.4	0.4

bodies and short legs, of the genera *Psychrophrynella*, *Noblella*, *Bryophryne*, *Qosqophryne*, and *Microkayla* (Frost 2020; De la Riva 2020). Likewise, the Andes of southeastern Peru are formed by six cordilleras: Apolobamba, Carabaya, Paucartambo, Urubamba, Vilcabamba, and Vilcanota (ANA 2014; Morales 2010; Lehr and Catenazzi 2008), where each Cordillera is inhabited by more than one species of frog from the subfamily Holoadeninae. Five species (*B. bakersfield*, *B. cophites*, *B. hanssaueri*, *B. nubilosus*, and *P. usurpator*) inhabit the Cordillera de Paucartambo (Chaparro et al. 2015; De la Riva et al. 2008; Lehr and Catenazzi 2008, 2009; Lynch 1975), five species (*B. tocrá*, *B. willakunka*, *M. boettgeri*, *N. thiuni*, and *P. glauca*) inhabit the Cordillera de Carabaya (Catenazzi and Ttito 2018, 2019; De la Riva et al. 2017; Lehr 2006), five species (*B. phuyuhampatu*, *B. quellokunka*, *B. zonalis*, *P. bagrecito*, and *P. chirihampatu*) inhabit the Cordillera de Vilcanota (Catenazzi and Ttito 2016; Catenazzi et al. 2017; De la Riva et al. 2017; Lehr and Catenazzi 2009; Lynch 1986), three species (*B. abramalagae*, *B. bustamantai*, and *Q. gymnotis*) inhabit the Cordillera de Urubamba (Chaparro et al. 2007; Lehr and Catenazzi 2009, 2010), three species (*P. vilcabambensis* sp. nov., *Q. flammiventris*, and *Q. mancoïnca*) inhabit the Cordillera de Vilcabamba (Lehr and Catenazzi 2010, Mamani et al. 2017), and two species (*M. chapi* and *M. chilina*) inhabit the Cordillera de Apolobamba (De la Riva et al. 2017). Eleven of these species were described during the past five years (Frost 2020) from the results of explorations of areas

with difficult access. However, there are still remote and unexplored places which could harbor additional new species or extensions of the ranges of the described species. One example is the study by Catenazzi et al. (2020), which reported three undescribed species in the genus *Psychrophrynella* (*P. sp.P.*, *P. sp.R.*, and *P. sp.*) and one in the genus *Noblella* (*N. sp.R.*). In this context, the diversity of direct-developing Andean frogs that inhabit the high Andes is underestimated, and there is a need for more expeditions to remote locations that lack records, such as much of the Cordillera de Vilcabamba.

Uncorrected *p*-distance analyses of the 16S rRNA sequences show that the new species, *Psychrophrynella vilcabambensis* sp. nov., has a narrow genetic distance from both *P. glauca* (8.3%) and *Noblella thiuni* (9.4%). The new species, *P. glauca*, and *N. thiuni* are found together in the leaf litter at the Thiuni locality, department of Puno, Peru (Catenazzi and Ttito 2018, 2019). The phylogenetic analysis obtained by Catenazzi and Ttito (2019), places *N. thiuni* as a sister group of the species of *Psychrophrynella* and *Noblella* in southern Peru. Therefore, the fact that *P. vilcabambensis* sp. nov. and *N. thiuni* have a narrow genetic distance, despite having a geographical distance of 285 km in a straight line, provides further evidence for the phylogenetic and taxonomic uncertainty between the species of the genera *Psychrophrynella* and *Noblella* (Catenazzi and Ttito 2018, 2019; De la Riva et al. 2008, 2017).

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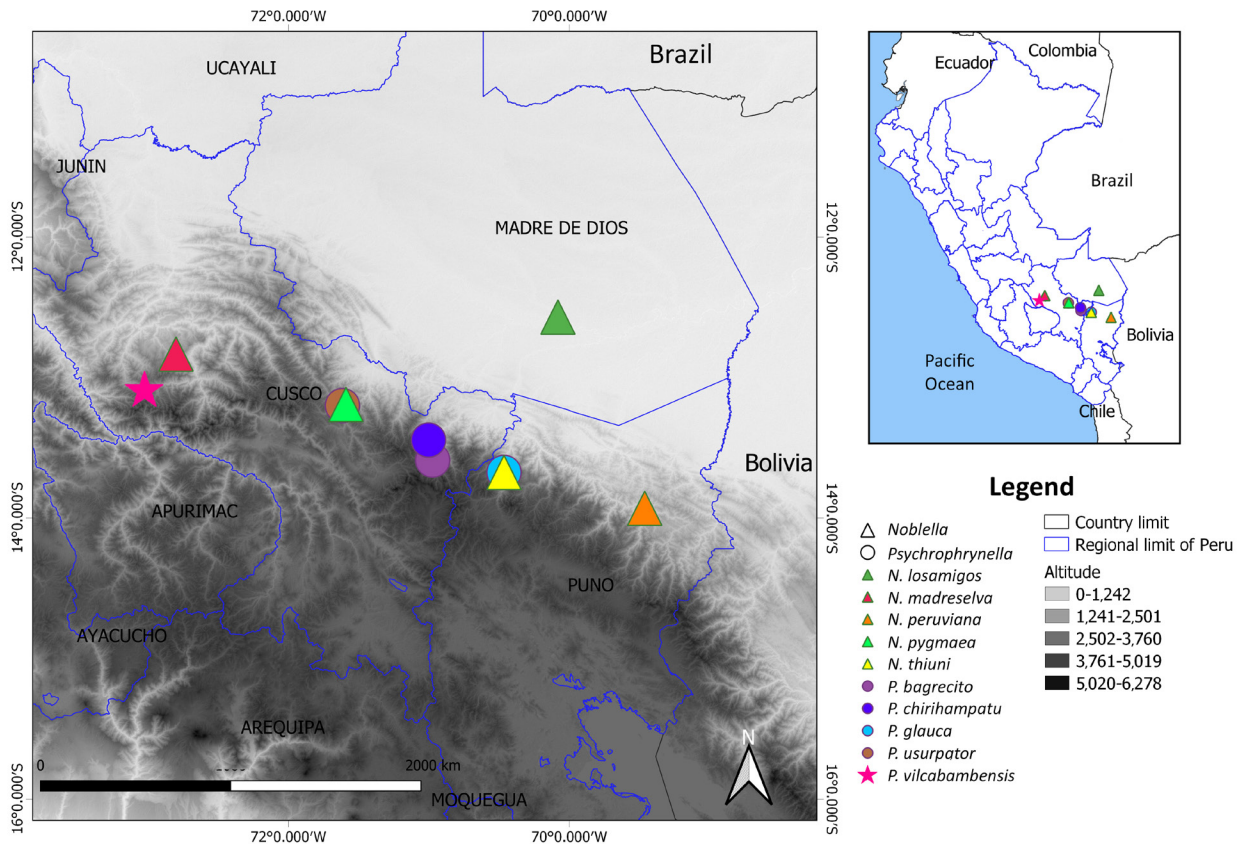


Fig. 4. Map of Peru indicating the type localities of species of *Psychrophrynella* and *Noblella* from the southeastern region of the country.

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Fig. 5. View of the type locality of *Psychrophrynella vilcabambensis* sp. nov.

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F. Peter Condori is a biologist who graduated from the Universidad Nacional de San Antonio Abad del Cusco in Peru. Peter is currently a researcher at the Museo de Biodiversidad del Peru (MUBI) and the Museo de Historia Natural de la Universidad Nacional de San Antonio Abad del Cusco (MHNC). His current research includes studies on the systematics, taxonomy, and biogeography of lizards and amphibians from the Cordillera de los Andes.



Aldemar A. Acevedo is a Colombian biologist with a Master's degree in Ecology. He has been studying ecology, conservation biology, and evolution for 12 years, generating information about butterflies, mammals, birds, and amphibians in Andean ecosystems. Aldemar has taught in the Faculty of Biological Science at the University of Pamplona in Colombia for four years. Currently, he is a Ph.D. student in Ecology in Chile, where his studies are focused on amphibian evolution, morphological variation, and the influences of climatic conditions on evolutionary processes in the Neotropics.



J. Amanda Delgado is an Associate Researcher at the Museo de Historia Natural de la Universidad Nacional de San Antonio Abad del Cusco, Peru (MHNC), as well as a curator of the amphibian and reptile collections. Amanda has a B.Sc. in Biological Sciences from the Universidad Nacional de San Antonio Abad del Cusco, Peru, and a Master's degree in Biodiversity and Conservation of Tropical Areas from the Universidad Internacional Menéndez Pelayo, Spain. Currently, she is working at the Organismo de Evaluación y Fiscalización Ambiental (OEFA), a public agency associated with the Ministry of the Environment of Peru. Amanda's research interests include the diversity, taxonomy, and ecology of amphibians and reptiles, with a particular interest in understanding how industrial and anthropogenic activities influence their environment and conservation. *Photo by Edwin Bellota.*



Juan C. Chaparro is a Peruvian biologist with extensive experience in studies on the fauna of all traditional geographic regions of Peru. He graduated from the Biological Sciences program at the Universidad Nacional Pedro Ruiz Gallo, Lambayeque, Peru, and received a Master's degree in the Biodiversity and Conservation of Tropical Areas in 2013 from an institutional consortium between the International University of Menendez Pelayo (UIMP-Spain), the Universidad Tecnológica Indoamérica (UTI-Ecuador), and the Consejo Superior de Investigaciones Científicas (CSIC-Spain). Juan is currently the president of the Herpetological Association of Peru (AHP), director of the Museo de Biodiversidad del Peru (MUBI, <https://mubi-peru.org/herpetologia/p-mubi>) and curator of the Herpetological Collection of the MUBI. He also works as a consultant in environmental studies. Juan has authored or co-authored 50 peer-reviewed scientific papers, notes, book chapters, and books on various faunal groups, especially herpetology and arachnology, in subjects such as taxonomy, biodiversity, systematics, phylogeny, conservation, and biogeography in South America. He is specifically interested in species diversity, conservation, taxonomy, systematics, phylogeny, life history, distributional patterns, and evolution using amphibians and reptiles as biological models. Juan has had four species named in his honor: *Phyllomedusa chaparroi* (Amphibia), *Phrynopus chaparroi* (Amphibia), *Hadruroides juanchaparro* (Arachnida), and *Chlorota chaparroi* (Insecta).



Luis Mamani is a Peruvian biologist and researcher at the Museo de Biodiversidad del Peru (MUBI) and the Museo de Historia Natural de la Universidad Nacional de San Antonio Abad del Cusco (MHNC). He obtained his M.Sc. degree from the Universidad de Concepción (UdeC) in Chile, and is currently a Ph.D. student in Systematics and Biodiversity at the UdeC. His current research interests include systematics, taxonomy, and biogeography of lizards from the Cordillera de los Andes.

Appendix 1. Specimens examined.

Psychrophrynella bagrecito ($n = 4$): **PERU. Cusco:** Quispicanchi: Camanti: Iskaybamba: MUBI 5255–58.

Psychrophrynella chirihampatu ($n = 12$): **PERU. Cusco:** Paucartambo: Área de Conservación Privada (ACP) Ukumari Llaqta: MUBI 14656, MUBI 14658, MUBI 14661–14662, MUBI 14664, MUBI 14666–72 (paratypes).

Psychrophrynella glauca ($n = 1$): **PERU. Puno:** Thiuni: Ollachea: MUBI 16323 (paratype).

Psychrophrynella usurpator ($n = 3$): **PERU. Cusco:** Paucartambo: Acjanacu: MUBI 4642–43 (paratypes).