Psychrophrynella glauca sp. n., a new species of terrestrial-breeding frogs (Amphibia, Anura, Strabomantidae) from the montane forests of the Amazonian Andes of Puno, Peru (#20348)

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Psychrophrynella glauca sp. n., a new species of terrestrial-breeding frogs (Amphibia, Anura, Strabomantidae) from the montane forests of the Amazonian Andes of Puno, Peru

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We describe a new species of small strabomantid frog (genus *Psychrophrynella*) from a humid montane forest in the Peruvian Department Puno. Specimens were collected at 2225 m elevation in the leaf litter of primary montane forest near Thiuni, along the Macusani–San Gabán road, in the province of Carabaya. The new species is assigned to *Psychrophrynella* on the basis of morphological similarity, including presence of a tubercle on the inner edge of the tarsus, call composed of multiple notes, and genetic data (16S rRNA partial sequences) showing similarity with *P. bagrecito*, *P. chirihampatu* and *P. usurpator*. *Psychrophrynella glauca* sp. n. is readily distinguished from three other three species of *Psychrophrynella* by its small size (snout–vent length of 11.3 mm in one male and 18.2–19.8 mm in two females), and by having belly and legs reddish-brown or red, and chest and throat brown to dark brown with a profusion of bluish-grey flecks. The new species is only known from its type locality, and has not been collected in surveyed montane forests at similar elevations in the middle Inambari valley (Puno) or in the Marcapata valley (Cusco). With the discovery of *P. glauca*, the geographic distribution of *Psychrophrynella* is again extended to Department Puno, where the genus had disappeared following the description of the genus *Microkayla*. Furthermore, the Cordillera de Carabaya is the only mountain range known to be home to species of four of the seven genera of Holoadeninae (*Bryophryne*, *Microkayla*, *Noblella*, *Psychrophrynella*), suggesting an intriguing evolutionary history for this group in southern Peru.

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Abstract

We describe a new species of small strabomantid frog (genus *Psychrophrynella*) from a humid montane forest in the Peruvian Department Puno. Specimens were collected at 2225 m a.s.l. in the leaf litter of primary montane forest near Thiuni, along the Macusani–San Gabán road, in the province of Carabaya. The new species is assigned to *Psychrophrynella* on the basis of morphological similarity, including presence of a tubercle on the inner edge of the tarsus, call composed of multiple notes, and genetic data (16S rRNA partial sequences) showing similarity with *P. bagrecito*, *P. chirihampatu* and *P. usurpator*. *Psychrophrynella glauca* sp. n. is readily distinguished from the three other species of *Psychrophrynella* by its small size (snout–vent length of 11.3 mm in one male, 18.2 and 19.8 mm in two females), and by having belly and legs reddish-brown or red, and chest and throat brown to dark brown with a profusion of bluish-grey flecks. The new species is only known from its type locality, and has not been collected in surveyed montane forests at similar elevations in the middle Inambari Valley (Puno) or in the Marcapata Valley (Cusco). With the discovery of *P. glauca*, the geographic distribution of *Psychrophrynella* is again extended to Department Puno, where the genus had disappeared following the description of the genus *Microkayla*. Furthermore, the Cordillera de Carabaya is the only mountain range known to be home to four of the seven genera of Holoadeninae (*Bryophryne*, *Microkayla*, *Noblella*, *Psychrophrynella*), suggesting an intriguing evolutionary history for this group in southern Peru.

Key words: 16S rRNA, bioacoustics, Carabaya, cloud forest, frog, leaf litter amphibian, Ollachea, taxonomy

Introduction

Frogs in the genus *Psychrophrynella* are small, terrestrial-breeding terraranas that had originally been placed ~~with~~ the genus *Phrynopus* (Lynch 1986). These high-Andean terraranas are very difficult to characterize morphologically ~~(Hedges et al. 2008). Molecular~~ analyses later revealed that these species ~~were~~ closely related to *Barycholos*, *Bryophryne* and *Holoaden* of the subfamily Holoadeninae, and not *Phrynopus* and related forms within Strabomantinae (Hedges et al. 2008). Thus, these forms were assigned to the new genus *Psychrophrynella*, with *P. bagrecito* as the type species (Hedges et al. 2008). Until recently, the genus contained 23 species, four species from the Peruvian Departments Cusco and Puno (Catenazzi & Ttito 2016), and 19 from Bolivia. Following the description of the genus *Microkayla*, which contains all Bolivian species (and the southern Peruvian species *M. boettgeri*) ~~formerly assigned to *Psychrophrynella* (De La Riva et al. 2017)~~, the genus *Psychrophrynella* presently contains three species: *P. bagrecito*, *P. chirihampatu* and *P. usurpator*.

These three species of *Psychrophrynella* are Peruvian endemics restricted to the Amazonian slopes of the Andes in the upper Kosñipata, Quespillomayo, Japumayo and Marcapata valleys in the Department Cusco, where they inhabit humid grasslands and montane forests from 1770 to 3600 m.a.s.l. (Catenazzi & Ttito 2016; Duellman & Lehr 2009; von May et al. 2017). These small frogs inhabit the leaf litter and the layer of terrestrial mosses, and thus require considerable effort to be detected, for example through intensive search within leaf litter plots (Catenazzi et al. 2011). Most of the eastern valleys of the Andes in the southern Peruvian Departments Cusco and Puno have been poorly explored, with few locations surveyed by using leaf litter plots, and are likely to contain many undescribed species of *Psychrophrynella* and other Holoadeninae (Catenazzi & von May 2014).

The taxonomy of Holadeninae has undergone frequent changes over the past decade (reviewed in De La Riva et al. 2017), in part because of our limited understanding of phylogenetic relationships among its members. As new species are discovered, and our understanding is accrued, it is likely that phylogenetic relationships will be revised again. Furthermore, the type species *P. bagrecito* shares several morphological traits with the type species of *Noblella, N. peruviana* (De la Riva et al. 2008; Lehr 2006), suggesting that the two species might be closely related. Future molecular analyses of these two species will help resolve relationships among species of *Noblella* and *Psychrophrynella*. Until DNA sequences of these two type species become available, new species can be assigned to either genus on the basis of overall morphological similarity and genetic distance with species whose genes have been sequenced.

During a rapid survey of the amphibian fauna of several tributaries of the Inambari River in Department Puno, we surveyed a humid montane forest in the Ollachea Valley. As a result of opportunistic, intensive search of the leaf litter, we found four specimens and recorded the call of a new species of Holoadeninae. Because the advertisement call sounded similar to the calls of *P. chirihampatu* and *P. usurpator* (Catenazzi & Ttito 2016), we suspected that the new species was a *Psychrophrynella*. Here we describe this new species, and provide morphological and molecular evidence for its generic allocation.

Methods

We follow Duellman & Lehr (2009) and Lynch & Duellman (1997) for the format of the diagnosis and description, except that the term dentigerous processes of vomers is used instead of vomerine odontophores (Duellman et al. 2006). We follow Heinicke et al. (2017) for taxonomic arrangement of genera within subfamilies. We derived meristic traits of similar species from the specimens we examined, from species descriptions, and from published photographs of live or preserved specimens.

We fixed and preserved specimens in 70% ethanol. We determined sex and maturity of specimens by observing sexual characters and gonads through dissections. We measured the following variables (Table 1) to the nearest 0.1 mm with digital calipers under a stereomicroscope: snout–vent length (SVL), tibia length (TL), 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), 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 anterior corner of orbit and posterior margin of external nares). Fingers and toes are numbered preaxially to postaxially from I–IV and I–V respectively. We determined comparative lengths of toes III and V by adpressing both toes against Toe IV; lengths of fingers I and II were determined by adpressing these fingers against each other. We describe variation in coloration on the basis of field notes and photographs of live frogs. We deposited photographs of live specimens (taken by A. Catenazzi) at the Calphoto online database (http://calphotos.berkeley.edu).

We recorded advertisement calls of an unvouchered male of *Psychrophrynella glauca* sp. n. at the type locality on 14 August 2017, and recorded air temperature with a quick reading thermometer (recording ##### deposited at the Fonoteca Zoológica, Museo Nacional de Ciencias Naturales, Madrid, www.fonozoo.com). We used a digital recorder (Zoom H2, recording at 48 kHz, 24-bit, WAV format) for field recording, and Raven Pro version 1.4 (Cornell Laboratory of Ornithology, Ithaca, NY) to analyze call variables. We analyzed a single call. We measured the following variables from the oscillogram: note ~~and~~ duration and rate, interval between notes or calls, number of pulses, and presence of amplitude modulation. ~~We measured these variables~~ from the spectrogram~~;~~ dominant frequency~~,~~ and presence of frequency modulation or harmonics. Spectral parameters were calculated through ~~fast Fourier transform~~ (FFT) set at a length of 512 points (Hann window, 50% overlap). Averages are reported ± SE.

We estimated genetic distances to confirm generic placement of the new species within *Psychrophrynella* through analysis of the non-coding 16S rRNA mitochondrial fragment. We used liver tissue from all type specimens (Table 1) to obtain DNA sequences for the new species (Appendix 1 with Genbak accession codes; FASTA supplementary file). We compared our sequences with those of other species of *Psychrophrynella*, and with those of Holoadeninae species in related genera (*Barycholos*, *Bryophryne*, *Holoaden*, *Microkayla* and *Noblella*) from GenBank (Appendix 1). We extracted DNA with a commercial extraction kit (IBI Scientific, Peosta, USA). We followed standard protocols for DNA amplification and sequencing (Hedges et al. 2008). We used the 16Sar (forward) primer (5'-3' sequence: CGCCTGTTTATCAAAAACAT) and the 16Sbr (reverse) primer (5'-3' sequence: CCGGTCTGAACTCAGATCACGT) (Palumbi et al. 2002). For the polymerase chain reaction (PCR) we used these thermocycling conditions: 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. We used a Veriti thermal cycler (Applied Biosystems). We purified PCR products with Exosap-IT (Affymetrix, Santa Clara, CA), and shipped purified samples to MCLAB (San Francisco, CA) for sequencing. We aligned sequences using Geneious R8, version 8.1.6 (Biomatters, http://www.geneious.com/) with the MAFFT v7.017 alignment program (Katoh & Standley 2013). We estimated uncorrected p-distances (i.e., the proportion of nucleotide sites at which any two sequences are different) with the R package "ape" (Paradis et al. 2004).

We list specimens examined in Appendix 2. For codes of collections we used the following acronyms: CORBIDI = Herpetology Collection, Centro de Ornitología y Biodiversidad, Lima, Peru; KU = Natural History Museum, University of Kansas, Lawrence, Kansas, USA; MHNC = Museo de Historia Natural del Cusco, Peru; MUBI = Museo de Biodiversidad del Perú, Cusco, Peru; MHNG = Muséum d'Histoire Naturelle, Genève, Switzerland; and MUSM = Museo de Historia Natural Universidad Nacional Mayor de San Marcos, Lima, Peru.

Our research was approved by the Institutional Animal Care and Use Committee of Southern Illinois University Carbondale (protocol #13-027). The Peruvian Ministry of Agriculture issued the permit authorizing this research (permit #292-2014-MINAGRI-DGFFS-DGEFFS).

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Results

***Psychrophrynella glauca* sp. n.** lsid:zoobank.org:act:E815EC45-81B4-46BF-A9A7-3E359DEBDB73

http://zoobank.org/NomenclaturalActs/E815EC45-81B4-46BF-A9A7-3E359DEBDB73

Holotype (Figs. 1–3, Table 1). CORBIDI 18729, an adult female (Figs. 2, 3) from 13.67603S; 70.46588W (WGS84), 2225 m a.s.l., near Thiuni, Distrito Ollachea, Provincia Carabaya, Departamento Puno, Peru, collected by A. Catenazzi and A. Ttito on 14 August 2017.

Paratypes (Fig. 4, Table 1). Three total: one adult male, CORBIDI 18730, one adult female, MUBI 16322, and one juvenile, MUBI 16323 collected at the type locality by A. Catenazzi and A. Ttito on 14 August 2017.

Generic placement. A new species of *Psychrophrynella* as defined by De ~~La~~ Riva et al. (2017). Frogs of the genus *Psychrophrynella* are morphologically similar and closely related to *Barycholos*, *Bryophryne*, *Holoaden*, *Microkayla* and *Noblella* (De ~~La~~ Riva et al. 2017; Duellman & Lehr 2009; Hedges et al. 2008; Heinicke et al. 2007; Padial et al. 2014). The new species is assigned to *Psychrophrynella* rather than any of the other genera on the basis of molecular data (Table 2), call composed of multiple notes, and overall morphological resemblance with the type species *P. bagrecito*, including presence of a short fold-like tubercle on the inner edge of tarsus. Our genetic distance data (Table 2) support the generic placement of the new species within *Psychrophrynella*. The most closely related species is *P. usurpator* (16S rRNA uncorrected p-distance: 12.3–12.5%), followed by *P. chirihampatu* (12.5–12.7%). Species from other genera ~~had~~ genetic distances above 18%, with the exception of *N. heyeri* (16.7%) and *N. myrmecoides* (16.8%).

Diagnosis. A species of *Psychrophrynella* characterized by (1) skin on dorsum smooth to finely shagreen; skin on venter smooth, discoidal fold present; (2) tympanic membrane not differentiated, anteroventral part of tympanic annulus visible below skin; (3) snout very short, bluntly rounded in dorsal view and in profile; (4) upper eyelid lacking tubercles, narrower than IOD; cranial crests absent; (5) dentigerous process of vomers absent; (6) vocal slits present; nuptial pads absent; (7) Finger I slightly shorter than Finger II; tips of digits bulbous, not expanded laterally; (8) fingers lacking lateral fringes; (9) ulnar tubercles absent; (10) heel lacking tubercles; inner edge of tarsus bearing a short, obliquous fold-like tubercle; (11) inner metatarsal tubercle elliptical, of similar relief and length of prominent, ovoid, outer metatarsal tubercle; supernumerary plantar tubercles absent; (12) toes lacking lateral fringes; webbing absent; Toe V slightly shorter, or about the same length as Toe III; tips of digits not expanded, weakly pointed; (13) dorsum reddish-brown to tan, with dark brown markings; ~~one juvenile with an orange middorsal line extending from tip of snout to cloaca and to posterior surface of thighs;~~ interorbital bar present; flanks brown with dark markings or entirely dark; chest dark brown with bluish-grey flecks; throat and palmar and plantar surfaces grayish-brown with small, bluish-grey flecks; belly and legs red or reddish-brown with bluish-grey flecks; (14) ~~SVL 11.3 mm in one male, 18.2 and 19.8 mm in two females~~.

Comparisons. The new species differs from the three known species of *Psychrophrynella* by its unique combination of red coloration on legs and belly, and profusion of bluish-grey flecks on ventral surfaces of head, body and legs. Morphologically, it is most similar to *P. bagrecito* in having a short fold-like tubercle on the inner edge of tarsus, a prominent ovoid outer metatarsal tubercle, discoidal fold present, an elliptical pupil, small size reaching ~19 mm, and dark brown flanks in at least some specimens. It can be distinguished from *P. bagrecito* (characters in parenthesis) by having smooth skin on venter (areolate), dorsal coloration with broad markings (longitudinal stripes), snout short and bluntly rounded (snout moderately long, rounded in dorsal view and in profile), and ventral coloration in preservative brown with light grey flecks (white to cream with brown mottling). *Psychrophrynella glauca* sp. n. can be distinguished from *P. chirihampatu* by having reddish-brown to dark brown coloration and bluish-grey flecks on ventral parts (yellow coloration with reddish-brown or grey flecks), Finger I slightly shorter or same length as Finger II (Finger I shorter than Finger II), inner metatarsal tubercle the same length of outer metatarsal tubercle (inner metatarsal tubercle at least three times the size of outer metatarsal tubercle), more bluntly rounded head (slender and longer head), smaller size reaching 19.8 mm in females (27.7 mm), and ~~by the~~ advertisement call having 26 notes and a fundamental frequency of 3027 Hz (up to 68 notes, 2712 Hz). The new species differs from *P. usurpator* by its reddish-brown ventral coloration (dull brown, gray or black with cream flecks), smaller SVL reaching 19.8 mm in females (SVL up to 30.5 mm), and by the fold-like tubercle on the inner edge of tarsus being short (long and prominent tubercle).

Description of holotype. Adult female (18.2 mm SVL); head narrower than body, its length 34.6% of SVL; head slightly longer than wide, head length 108.6% of head width; head width 31.9% of SVL; snout very short, bluntly rounded in dorsal and lateral views (Fig. 2), eye diameter 31.7% of head length, its diameter 1.3 times as large as its distance from the nostril; nostrils not protuberant, close to snout, directed laterally; canthus rostralis slightly concave in dorsal view, convex in profile; loreal region flat; lips rounded; upper eyelids lacking tubercles; upper eyelid width 65.0% of interorbital distance; interorbital region flat, lacking cranial crests; eye-nostril distance 75.0% of eye diameter; supratympanic fold weak; tympanic membrane not differentiated, anteroventral part of tympanic annulus visible below skin; postrictal tubercles absent. Choanae round, very small, positioned far anterior and laterally, widely separated from each other; dentigerous processes of vomers and vomerine teeth absent; tongue large, ovoid, not notched.

Skin on dorsum smooth to finely shagreen; ~~barely visible~~ dorsolateral folds anteriorly; skin on flanks and venter smooth; no pectoral ~~fold, no~~ discoidal fold; cloaca not protuberant, cloacal region without tubercles. Ulnar tubercles and folds absent; palmar tubercle flat and oval, approximately same length but twice the width of elongate, thenar tubercle; supernumerary palmar tubercles absent; subarticular tubercles prominent, ovoid in ventral view, rounded in lateral view, largest at base of fingers; fingers lacking lateral fringes; relative lengths of fingers 3 > 4 > 2 ≥ 1 (Fig. 3); tips of digits bulbous, not expanded laterally (Fig. 3); forearm lacking tubercles.

Hindlimbs moderately long, tibia length 46.2% of SVL; foot length 45.1% of SVL; upper and posterior surfaces of hindlimbs smooth; heel without tubercles; inner edge of tarsus bearing a short, oblique fold-like tubercle, outer edge of tarsus lacking tubercles; inner metatarsal tubercle elliptical, of similar relief and length of prominent, ovoid, outer metatarsal tubercle; minute plantar supernumerary tubercles weakly defined; subarticular tubercles rounded, ovoid in ventral view; toes lacking lateral fringes, not webbed; toe tips weakly pointed, not expanded laterally; relative lengths of toes 4 > 3 > 5 > 2 > 1 (Fig. 3); foot length 45.1% of SVL.

Measurements of holotype (in mm): SVL 18.2, TL 8.4, FL 8.2, HL 6.3, HW 5.8, ED 2.0, IOD 2.0, EW 1.3, IND 1.8, E–N 1.5.

Coloration of holotype in alcohol. Dorsal surfaces of head, body, and limbs grayish tan, with a dark brown X-shaped middorsal mark bordered laterally by a cream line. The interorbital bar is a narrow dark stripe and is bordered anteriorly by a poorly defined cream stripe. There is a dark brown stripe extending from the tip of the snout to above the tympanum and the insertion of forelimb; furthermore, there are two longitudinal dark markings along the line separating the dorsum from the flanks, and dark markings on each side of dorsum near the point of hind limb insertion. The iris is dark gray. The throat is brown coloration anteriorly, fading into pale brown with light grey flecks posteriorly. Chest and belly brown with light grey mottling and large flecks. Ventral parts of limbs reddish-brown with cream mottling and flecks on brachium and thighs, and tan with light grey flecks on antebrachium, crus and pes. The dorsal surfaces of hind limbs have transverse dark bars. The posterior surfaces of thighs are reddish-brown with a large, dark tan marking surrounding the cloaca and reaching one third the length of thigh, bordered anteriorly by a narrow, cream stripe; the plantar and palmar surfaces are tan, fading into light grey along fingers and toes.

Coloration of holotype in life. Similar to coloration in alcohol, but the dorsal coloration varies from beige to brown, and the thighs are reddish-brown with brown mottling. Ventrally, flecks are bluish-grey, largest and most noticeable on chest, and the belly and ventral surfaces of limbs are red or reddish-brown. The iris is dark tan with golden flecks, forming a ring around the pupil.

Variation. Coloration in life is based on field notes and photographs taken by A. Catenazzi of the three types (Fig. 4). These three types have two subocular dark brown spots, which are not visible in the holotype. Furthermore, all three have more extensive dark coloration on flanks, either forming a nearly continuous dorsolateral line connected to the supratympanic marking as in MUBI 16322, or having several dark markings as in CORBIDI 18730, or the entire flank dark as in MUBI 16323. The latter ~~specimen, juvenile MUBI 16323~~ also has much darker dorsum than the other type specimens, as well as an orange middorsal line extending from the tip of snout to the cloaca and to the posterior surface of thighs. The ventral coloration of thighs varies from reddish-brown with brown mottling in the two females (the holotype and MUBI 16322), to bright red with little mottling in the male, and orange with little brown mottling in the juvenile.

Advertisement call. A single call of an unvouchered specimen was recorded at 19h45 on 14 August 2017 (Fig. 5). At a T_{air} = 13.7°C, the advertisement calls lasted 2188 ms, and consisted of 26 single-pulsed notes, produced at a rate of 11.88 notes/s. Low amplitude and poor recording quality prevented analysis of the first five notes. In the remaining 21 notes, peak frequency averaged 3027 ± 22 Hz (range 2756–3100 Hz) and increased during calls ($F_{1,19} = 21.3$, $p < 0.001$); peak frequency averaged 2900 ± 29 Hz for the sixth to 11th note, and 3078 ± 16 Hz for the last three notes of each call. Amplitude also increased during each call ($F_{1,19} = 6.7$, $p < 0.017$), reaching peak amplitude for notes that had highest frequency and longest duration (notes 19 through 21). Average note duration was 15.6 ± 2.4 ms (range 9–53 ms), and the 19th, 20th and 21st notes had longer duration (38.3 ± 7.9 ms) than all other notes (11.9 ± 1.0 ms).

Etymology. The specific name *glauca* is the feminine form of the Latin adjective *glaucus*, from the ancient Greek noun *glaûkos*, meaning “bluish-grey”, in reference to the bluish-grey flecks on the ventral parts of body and limbs.

Distribution, natural history and threats. The four specimens were found in the leaf litter along a descending ridge separating two creeks in the humid montane forest along the road from Thiuni to Ollachea. Sympatric species detected during our quick survey included *Gastrotheca testudinea*, *Pristimantis platydactylus*, and an undescribed *Pristimantis* sp. Much of the original forest vegetation has been replaced by cultivated fields and pasture along the road, but this remnant forest extended from nearly the side of road to the upper ridge of the mountain. Further advance of agriculture, or clearing of forest might threaten this species if its distribution is restricted to the Ollachea Valley. In absence of more detailed data regarding its extent of occurrence, and according to the IUCN Red List criteria and categories (IUCN 2013), we suggest this species to be in the "Data Deficient" category of the Red List.

Discussion

The diversity of small, terrestrial-breeding frogs in the humid grasslands and montane forests of the Tropical Andes has until recently been grossly underestimated (De La Riva et al. 2017). A similar pattern has occurred in the Atlantic forest of Brazil, where the diversity and micro-endemism of the minute terrestrial-breeding *Bracychephalus* was long unappreciated (Pie et al. 2017; Pie & Ribeiro 2015; Ribeiro et al. 2017). In Peru, most species of Holoadeninae have been described since 2008 (Catenazzi & Ttito 2016; Catenazzi et al. 2017b; Catenazzi et al. 2015; De La Riva et al. 2017; De la Riva et al. 2008; Lehr & Catenazzi 2008; Lehr & Catenazzi 2009a; Lehr & Catenazzi 2009b; Lehr & Catenazzi 2010). Additional, undescribed species of *Psychrophrynella* have already been identified (Catenazzi et al. 2013; von May et al. 2017), and museum material indicates several more species might exist among misidentified specimens, such as in the type series of *P. bagrecito* (De la Riva et al. 2008; Duellman & Lehr 2009).

Therefore, we can expect that additional field work, specimen comparisons, bioacoustics and genetic or genomic analyses will reveal many more species of *Psychrophrynella* and related Holoadeninae genera from the Tropical Andes.

De ~~La~~ Riva et al. (2017) recently allocated all Bolivian species previously assigned to *Psychrophrynella*, and the Peruvian species *boettgeri* from Department Puno, to the new genus *Microkayla*. Accordingly, the genus *Psychrophrynella* was left with only three species, *P. bagrecito*, *P. chirihampatu* and *P. usurpator* all distributed around the Vilcanota massif and its associated cordilleras in the Peruvian Department Cusco. Using phylogenomic approaches, Heinicke et al. (2017) validated the allocation of *Barycholos*, *Bryophryne*, *Euparkerella*, *Holoaden*, *Microkayla*, *Noblella* and *Psychrophrynella* (but not of *Niceforonia*, *Lynchius*, *Oreobates* and *Phrynopus*) within Holoadeninae; we follow their proposed taxonomic arrangement here. With the description of *P. glauca*, the geographic distribution of *Psychrophrynella* is ~~again~~ extended to Department Puno~~, and to a tributary of the Inambari River in the Cordillera de Carabaya.~~ The Cordillera de Carabaya also contains the type localities of *Noblella peruviana*, the type species of *Noblella*, at Santo Domingo in the upper reaches of a small tributary of the Inambari River, of *Microkayla boettgeri* at Phara in Province Sandia, and of *Bryophryne tocra* and *B. wilakunka* in Province Carabaya. The Cordillera de Carabaya is thus unique in being home to four of the seven genera of Holoadeninae: *Bryophryne*, *Microkayla*, *Noblella*, and *Psychrophrynella*. Only three genera are known to occur in the northern Cordillera de Vilcanota and associated cordilleras (*Bryophryne*, *Noblella* and *Psychrophrynella*), and only two genera in the northern Cordillera de Urubamba (*Bryophryne* and *Noblella*), and in the southern Cordillera de Apolobamba (*Microkayla* and *Noblella*). Therefore, the Cordillera de

Carabaya appears to host substantial beta diversity of Holadeninae, suggesting an intriguing evolutionary history for this group in southern Peru.

Our generic allocation remain tentative in light of an unresolved taxonomic situation regarding *Noblella* and *Psychrophrynella*, as previously reviewed (Catenazzi & Ttito 2016; De ~~La~~ Riva et al. 2017; De la Riva et al. 2008). In short, the type species of both genera, ~~which~~ have not been included in phylogenetic analyses ~~for~~ lack of DNA sequences, share several morphological traits ~~such that the hypothetical scenario of them forming~~ part of the same clade ~~is a plausible hypothesis~~. Here we have assigned the new species to *Psychrophrynella* on the basis of general body shape and appearance, overall similarity with the type species *P. bagrecito*, and relatively small uncorrected p-distances of 16S rRNA and similarities in advertisement call with *P. chirihampatu* and *P. usurpator*. As a priority, future work should sample tissues and record advertisement calls of *P. bagrecito* and *N. peruviana*, so that multiples approaches can be pursued to determine the phylogenetic relationships of species of *Noblella* and *Psychrophrynella*.

Anuran communities in the humid montane forests of southern Peru have undergone sharp reductions in species richness and abundance following epizootics of chytridiomycosis (Catenazzi et al. 2011; Catenazzi et al. 2014). The disease/host dynamics now seem to be enzootic (Catenazzi et al. 2017a), and although experimental infection trials have shown that terrestrial-breeding frogs can be highly susceptible to chytridiomycosis (Catenazzi et al. 2017a), populations of Strabomantidae generally have not declined as sharply as those of aquatic-breeding sympatric frogs. Thus, chytridiomycosis might not directly threaten *P. glauca*. A more immediate threat to *P. glauca* is embodied by hydroelectric projects that are planned or under construction along the San Gabán River. The new dams might directly flood montane forest, or intercept water from streams and rivers that drain the forest, thus reducing habitat quality. These projects are part of many planned dams in the Inambari watershed that threaten to alter fish migrations, biodiversity and geochemical cycles locally and downstream throughout the Amazon basin (Forsberg et al. 2017; Latrubesse et al. 2017). These consequences that might not have been properly taken into consideration during the decision-making progress evaluating financial interests and the findings of the Environmental Impact Assessment (Rode et al. 2015). We hope that the timely description of new species such as *P. glauca* will contribute to the conservation of the humid montane forest, and promote mitigating solutions including restoration of degraded forest habitat.

Conclusions

We describe a new species of terrestrial-breeding frog of the family Strabomantidae, and provide evidence for its allocation within the genus *Psychrophrynella*. The new species *P. glauca* is only known from its type locality, similarly to most other small Holoadeninae known to occur at high elevations in the Andes of southern Peru and Bolivia. With our description we contribute to a better knowledge of the diversity of this group, and reveal the presence of four genera of Holoadeninae in the Cordillera de Carabaya of southern Peru, suggesting that phylogeographic studies of the Holoadeninae species of this mountain range may shed insights into radiation in this group.

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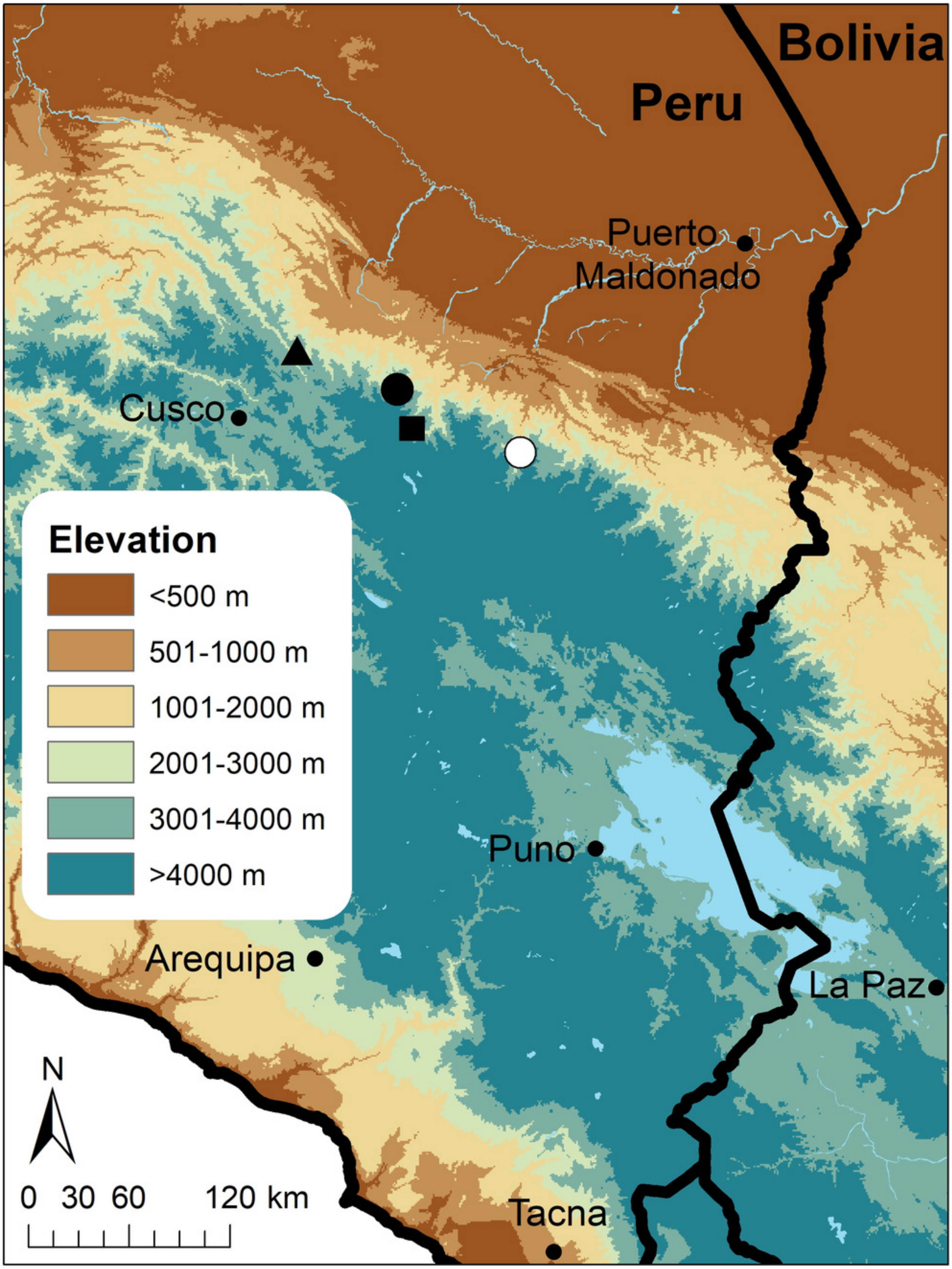
Figure 1

Map of Peru indicating the type localities of Peruvian species of *Psychrophrynella*.

Psychrophrynella bagrecito (black square), *P. chirihampatu* (black circle), *P. glauca* sp. n. (white circle), and *P. usurpator* (triangle).



Bolivia
Peru
Puerto
Maldonado
Cusco
Elevation
<500 m
501-1000 m
1001-2000 m
2001-3000 m
3001-4000 m
>4000 m
Puno
Arequipa
La Paz
N
0 30 60 120 km
Tacna

Figure 2

Photographs of live and preserved specimen of the holotype of *Psychrophrynella glauca* sp. n.

Live (**A, C, E**) and preserved (**B, D, F**) specimen of the holotype, female CORBIDI 18729 (SVL 18.2 mm) in dorsolateral (**A, B**), dorsal (**C, D**) and ventral (**E, F**) views. Photographs by A. Catenazzi.

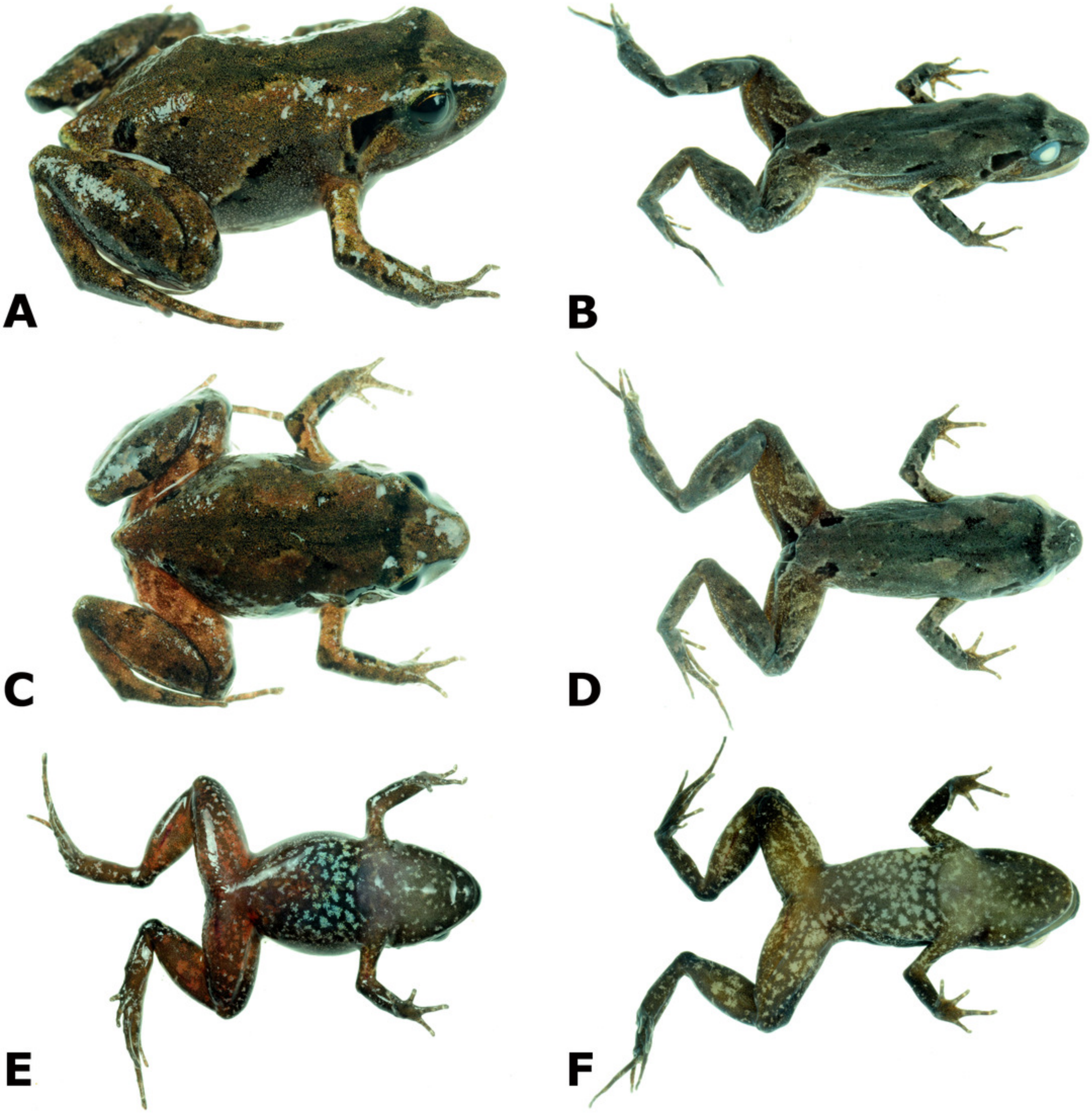

A
B
C
D
E
F

Figure 3

Palmar and plantar surfaces of the holotype of *Psychrophrynella glauca* sp. n.

Ventral views of hand (**A**) and foot (**B**) of holotype, CORBIDI 18729 (hand length 3.8 mm, foot length 8.2 mm). Photographs by A. Catenazzi.

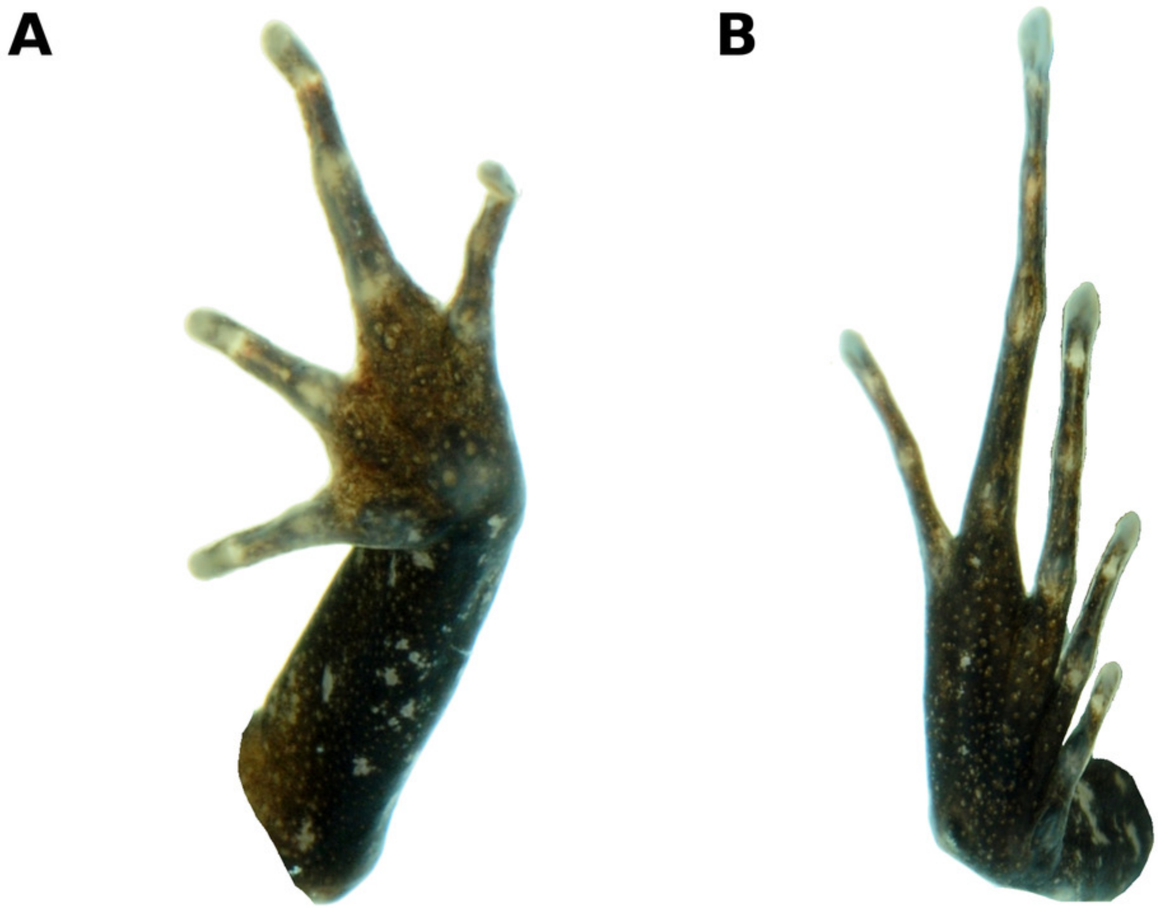


Figure 4

Dorsolateral and ventral views of four paratypes of *Psychrophrynella glauca* sp. n. showing variation in dorsal and ventral coloration.

Female MUBI 16322 (**A, B**). Male CORBIDI 18730 (**E, F**). Juvenile MUBI 16323 (**G, H**).

Photographs by A. Catenazzi.

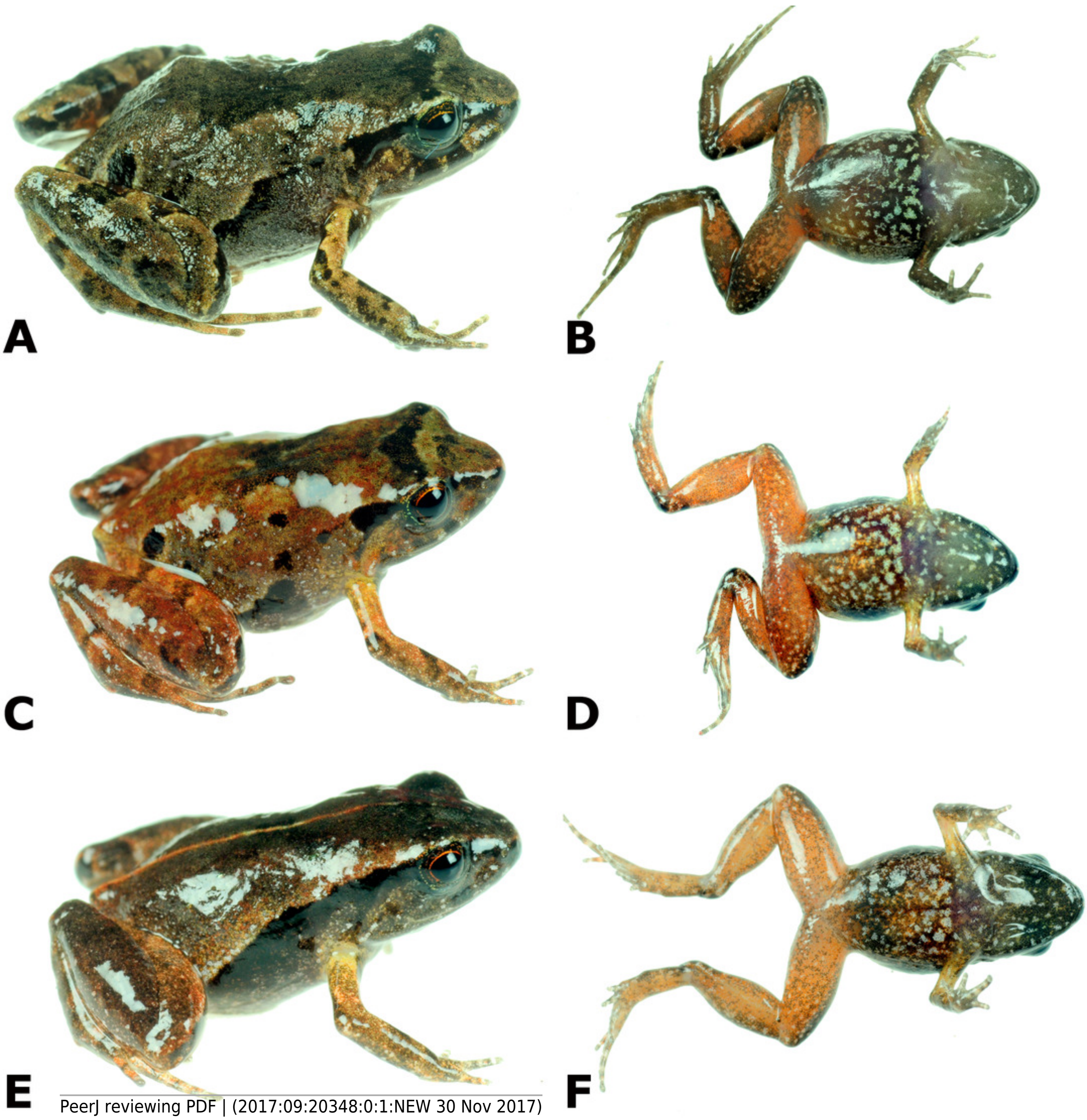


Figure 5

Advertisement call of *Psychrophrynella glauca* sp. n.

Advertisement call of an unvouchered male, recorded at the type locality on 14 August 2017 (T_{air} = 13.7°C).

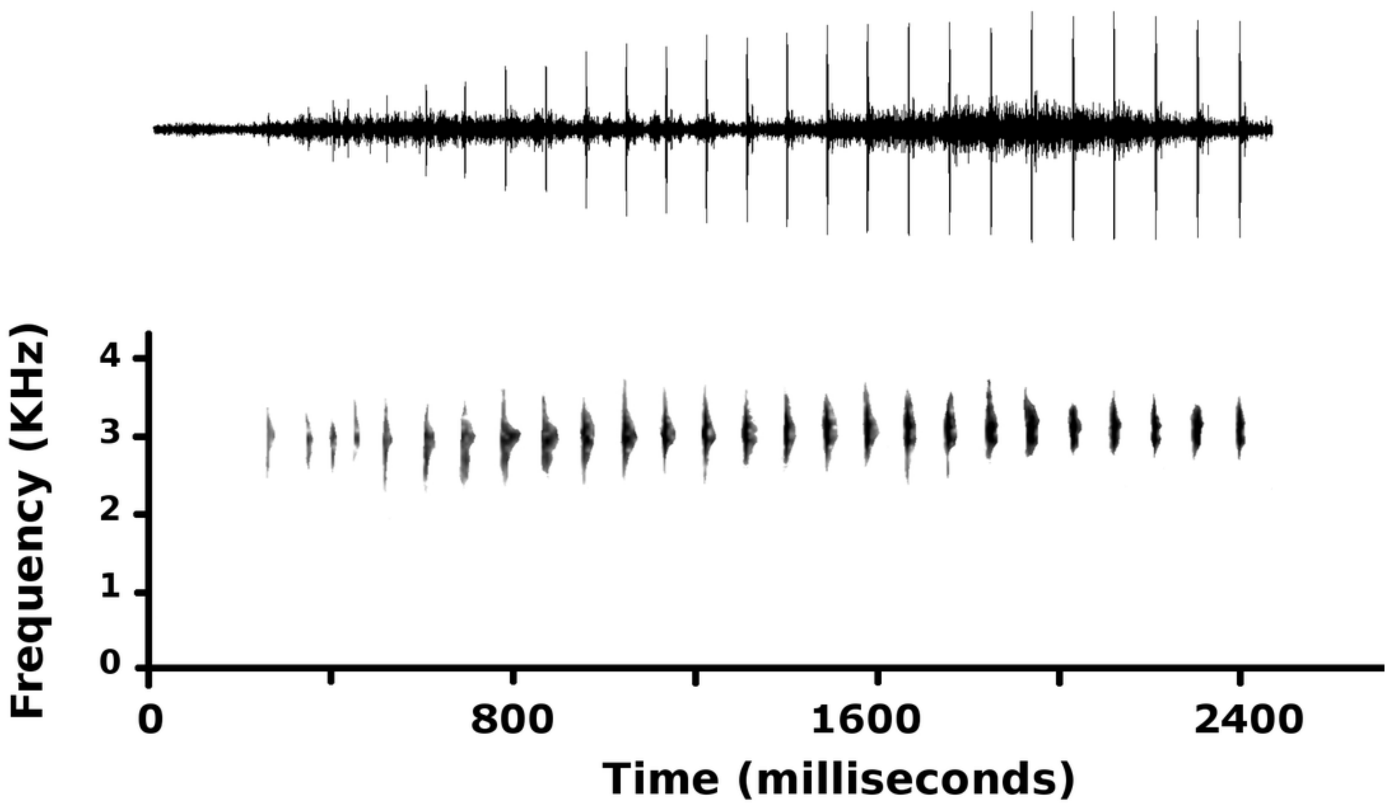


Table 1 (on next page)

Measurements of the type series of *Psychrophrynella glauca* sp. n.

Range and average (± standard deviation) measurements (in mm) of males and females of the type series of *Psychrophrynella glauca* sp. n.

Characters	Females (*n* =*2*)	Males (*n* = *1*)
SVL	18.2–19.8	11.3
TL	8.41–9.5	6.3
FL	8.2–9.4	5.5
HL	6.3–6.6	4.3
HW	5.8–6.3	3.9
ED	2.0–2.1	1.5
IOD	2.0–2.1	1.6
EW	1.3–1.5	1.1
IND	1.8–2.0	1.0
E–N	1.5–1.5	1.1
TL/SVL	0.46–0.48	0.56
FL/SVL	0.45–0.47	0.49
HL/SVL	0.33–0.35	0.38
HW/SVL	0.32	0.35
HW/HL	0.92–0.95	0.91
E–N/ED	0.75–0.76	0.73
EW/IOD	0.65–0.71	0.69

1

Table 2(on next page)

Uncorrected p-distance for 16S rRNA between *Psychrophrynella glauca* sp. n. and related taxa in the subfamily Holadeninae.

Percent genetic distances estimated from the non-coding 16S rRNA mitochondrial fragment (highlighted in grey the most closely related species).

	Barycholos pulcher	*Bryophryne bakersfield*	*Bryophryne bustamantei*	*Bryophryne cophites*	*Bryophryne phuyuhampatu*	*Bryophryne quellokunka*	*Bryophryne tocra*	*Holoaden luederwaldti*	*Microkayla boettgeri*	*Microkayla chaupi*	*Microkayla chilina*	*Microkayla guillei*	*Microkayla wettseteini*	*Noblella heyeri*	*Noblella lochites*	*Noblella myrmecoides*	*Noblella sp. (SanMartin)*	*Psychrophrynella chirihampatu*	*Psychrophrynella glauca MUBI16322*	*Psychrophrynella glauca (holotype)*	*Psychrophrynella glauca MUBI16323*	*Psychrophrynella glauca CORBIDI 18730*	*Psychrophrynella usurpator*	*Strabomantis sulcatus*
Barycholos pulcher																								
Bryophryne bakersfield	25.3																							
Bryophryne bustamantei	25.1	5.4																						
Bryophryne cophites	25.5	7.2	10.1																					
Bryophryne phuyuhampatu	25.9	7.5	9.6	7.1																				
Bryophryne quellokunka	26.4	5.4	7.6	6.3	6.0																			
Bryophryne tocra	27.1	7.2	10.0	10.1	10.9	8.3																		
Holoaden luederwaldti	26.6	20.6	21.0	22.0	21.4	21.7	20.6																	
Microkayla boettgeri	24.2	19.9	20.0	21.7	20.3	20.7	20.9	21.8																
Microkayla chaupi	25.0	18.8	19.6	21.4	20.1	20.0	19.9	22.1	4.7															
Microkayla chilina	25.3	19.7	20.3	21.4	20.1	20.7	20.4	22.0	2.6	4.5														
Microkayla guillei	25.0	19.1	20.1	22.5	21.3	21.3	19.6	20.8	9.3	9.7	9.5													
Microkayla wettseteini	27.8	21.4	22.6	21.3	21.0	20.7	20.8	20.8	14.8	14.5	14.5	12.7												
Noblella heyeri	14.5	14.4	13.9	14.8	13.7	15.4	16.2	18.2	13.9	14.4	13.9	13.9	15.0											
Noblella lochites	23.5	22.6	22.9	23.9	22.4	24.4	24.1	23.1	23.3	23.6	23.3	23.0	25.0	8.9										
Noblella myrmecoides	11.8	13.9	12.3	14.4	12.7	14.9	16.9	15.6	11.2	11.7	11.2	11.2	12.8	11.8	10.6									
Noblella sp. (SanMartin)	25.8	22.2	23.6	24.2	21.2	22.8	22.5	24.0	25.8	26.3	25.7	25.6	25.4	11.7	19.2	6.7								
Psychrophrynella chirihampatu	24.0	22.3	22.0	21.0	22.6	22.2	22.0	22.1	21.5	21.0	21.0	21.4	21.2	19.3	23.1	17.2	25.3							
Psychrophrynella glauca MUBI16322	23.8	18.4	18.3	20.8	20.7	20.2	19.9	20.5	19.0	19.0	19.0	19.3	19.2	16.7	23.4	16.8	24.9	12.5						
Psychrophrynella glauca (holotype)	23.8	18.6	18.5	21.0	20.7	20.3	20.1	20.6	19.1	19.2	19.1	19.4	19.4	16.7	23.3	16.8	24.8	12.7	0.2					
Psychrophrynella glauca MUBI16323	23.8	18.4	18.3	20.8	20.7	20.2	19.9	20.5	19.0	19.0	19.0	19.3	19.2	16.7	23.4	16.8	24.9	12.5	0.0	0.2				
Psychrophrynella glauca CORBIDI 18730	23.8	18.4	18.3	20.8	20.7	20.2	19.9	20.5	19.0	19.0	19.0	19.3	19.2	16.7	23.4	16.8	24.9	12.5	0.0	0.2	0.0			
Psychrophrynella usurpator	24.0	21.6	21.1	22.8	23.0	22.6	21.9	21.3	21.7	21.5	21.3	20.8	20.4	18.9	22.6	16.8	26.3	7.9	12.3	12.5	12.3	12.3		
Strabomantis sulcatus	24.3	19.3	20.8	17.6	17.5	19.3	20.4	20.1	21.4	21.4	20.8	21.9	21.7	17.0	24.2	13.8	24.8	19.8	18.4	18.6	18.4	18.4	20.7	

1