

Distribution, ecology, and conservation of the critically endangered frog *Psychrophrynella illimani* (Anura, Craugastoridae) with the description of its call

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Abstract. Amphibian populations have been declining worldwide for decades with a multitude of causes having been identified. Conservationists try to reverse the situation, but for many species, important information on distribution, habitat and ecology are missing, which makes the assessment of conservation priorities problematic. Although South America holds the largest number of extant amphibian species in the world, many of them are poorly studied. This is also the case for most species of *Psychrophrynella*, a genus of cold-adapted frogs occurring in the high Andes, the majority of which having only recently been described. We organized an extensive field survey to study the ecology of *Psychrophrynella illimani*, a critically endangered species endemic to a single valley in Bolivia that has not been reported on again since its discovery in 2002. We found *P. illimani* to be locally common and here report new localities, extending its known distribution. Furthermore, we provide new information on its morphology, ecology, and reproductive behaviour and describe for the first time its call. We also identify and discuss several threats that might affect this species' survival.

Key words. Amphibia, Anura, Andes, Bolivia, endemism, grazing, mining.

Introduction

The global amphibian crisis is a hot topic. Populations have been declining worldwide for decades with a multitude of causes having been identified (HOF et al. 2011, STUART et al. 2004, WAKE & VREDENBURG 2008). At the same time, more than a hundred new amphibian species are described yearly (Amphibiaweb 2014). Taxonomists, conservationists, and other researchers are working hard trying to catalogue amphibian diversity on earth, as well as identifying and understanding causes of population declines with the hope of eventually counteracting these threats. The recent rediscovery of several amphibian species that were believed to be extinct is a positive sign (BITON et al. 2013,

LYNCH et al. 2014, MIN et al. 2011), but the decline of many more urges the need for conservation plans.

Currently, 266 amphibian species are known to occur in Bolivia, which is about 25% more than 15 years ago, and even more than twice the number of species that were reported from the country 25 years ago (DE LA RIVA 1990, DE LA RIVA et al. 2000, DE LA RIVA & REICHLÉ 2014). This demonstrates the relatively recent start of intensified herpetological research in this mega-diverse country, where 66 (24.8%) of the amphibian species are endemic. Based on potential new country records and new species awaiting description, it is estimated that a total of 300 to 350 amphibian species are present in Bolivia (DE LA RIVA & REICHLÉ 2014). From amongst the currently known spe-

cies, AGUAYO (2009) regarded 54 as threatened (i.e., assigned to the categories “Vulnerable”, “Endangered” or “Critically endangered”) in the Bolivian red list, while 35 are considered “Threatened” in the IUCN red list (IUCN 2014). A recent study by DE LA RIVA & REICHLER (2014) identified deforestation, general habitat destruction, water pollution, and chytridiomycosis as the main causes of amphibian declines in Bolivia and suggests that 57 species be classified as “Threatened” and 18 as “Data deficient”.

To date, 21 species are recognized in the genus *Psychrophrynella* (FROST 2014), all of which have very restricted ranges in wet areas of the high Andes (1,830–4,190 m above sea level [a.s.l.]) of Peru and Bolivia (DE LA RIVA 2007). It is however assumed that more are to be discovered as expeditions to poorly known areas are undertaken (DE LA RIVA 2007, DE LA RIVA & BURROWES 2014, DE LA RIVA & REICHLER 2014). The genus was erected in 2008 after phylogenetic analyses had demonstrated that several species previously assigned to the genus *Phrynopus* form a distinct “basal” clade of the Holoadeninae, while *Phrynopus* sensu stricto clusters with *Oreobates* and *Lynchius* in the Strabomantiinae (HEDGES et al. 2008). However, an even more recent study places *Phrynopus*, *Oreobates*, and *Lynchius* back in the Holoadeninae, with that clade being sister to Holoadeninae sensu HEDGES et al. (2008) (PADIAL et al. 2014). *Psychrophrynella* species are known to be direct developers with terrestrial egg clutches being deposited under moss or stones, but further information on reproductive behaviour in this genus is scarce (DE LA RIVA 2007). In *P. wettsteini*, a brief inguinal amplexus – even if not followed by oviposition – has been observed in captivity, and some aspects of egg clutches have been published (ERGUETA 1993, TERCEROS 2010). The 18 species that are currently known from Bolivia are all endemic to that country and have extremely small ranges. They are therefore vulnerable to human alteration of their habitat and the impact of climate change (DE LA RIVA 2007, DE LA RIVA & REICHLER 2014).

Psychrophrynella illimani was discovered in 2002 and described in 2007 (DE LA RIVA & PADIAL 2007), along with 11 other new *Psychrophrynella* species from Bolivia (DE LA RIVA 2007). Four individuals were found at a single location on the north side of the Nevado Illimani, along the road from Totoral to Cooperativa 15 de Agosto (DE LA RIVA & PADIAL 2007). To our knowledge, *P. illimani* has not been reported since its discovery, probably because nobody has searched for it again. The species is classified as “Critically endangered” by the IUCN and “Endangered” in the Bolivian Red List, as it is only known from a single locality not lying in a protected area. It is believed to be rare, and mining activities in the area may have a negative impact on its habitat (AGUAYO 2009, DE LA RIVA & ANGULO 2009). As for most other *Psychrophrynella* species, little is known about its ecology. Its call has never been described, and there is no published information about its reproduction.

In order to gather data on the distribution, ecology, and life history of *Psychrophrynella illimani*, we organized an extensive field campaign. In this paper we show that the species has a much wider distribution and is more com-

mon than previously thought. Furthermore, we describe for the first time its call and provide information on its morphology, ecology and possible threats.

Material and methods

We conducted visual and auditory surveys, both by day and night, during a two-month period (October–November of 2013). Surveys were conducted in the region between Tres Ríos, Cooperativa 15 de Agosto, Lambate, and Chuñawi (see Fig. 1 for a detailed map). Coordinates of all individuals encountered were saved in a GPS (Garmin 60CSx). Occurrence maps were created using QGIS 2.4.0 (Quantum GIS Development Team 2014) and Google Earth (Google Earth 2013). Individuals from different localities were collected by hand, placed in Ziploc bags, and immediately carried to our field laboratory where they were photographed and subsequently euthanised in a MS222 solution before being fixed in a plastic box with paper tissue saturated with 70% ethanol. After 24 hours, specimens were moved to a container containing a 70% ethanol solution for permanent storage. The following measurements were taken from all collected adults two weeks after preservation: snout–vent length (SVL), head length (HL), head width (HW), internarial distance (IND), eye–nostril distance (END), eye diameter (ED), tibia length (TL), and foot length (FL) as described by DE LA RIVA (2007). All measurements were taken by the same person (BW) with a vernier calliper with a precision of 0.02 mm and rounded to the nearest 0.1 mm. All measurements were taken twice and the average was used.

Evaluations of density and habitat use were carried out using quadrant counts in locations where the species was detected. After finding a random individual, we defined a square of 10 × 10 m around its spot with a long rope. In that square, all stones were overturned and patches of moist vegetation were carefully examined. For every quadrant, we wrote down the percentage of stone cover (subdivided in smaller [< 30 cm] and larger [> 30 cm] rocks), plant cover (subdivided in mosses, short grazed vegetation, and untouched, natural vegetation) and the area covered with mud. We also noted the presence of trees and larger shrubs and estimated the size of the part of the quadrant that was shaded. Snout–vent length was taken in situ from every animal encountered, except from males tending to egg clutches. We performed correlation tests in R version 3.0.1 (R Core Team 2013) to see if the abundance of egg clutches and frogs could be explained by any of the recorded parameters.

Advertisement calls were recorded using a Zoom H4n Handy Mobile 4-Track Recorder using the built-in microphone and analysed at a sampling rate of 44.1 kHz using Raven Pro 1.3 software (CHARIF et al. 2008). The following call characteristics were measured as defined by KOK & KALAMANDEEN (2008): call duration, inter-call interval, call rate, note duration, inter-note interval, and note repetition rate. The dominant frequency was measured by tak-

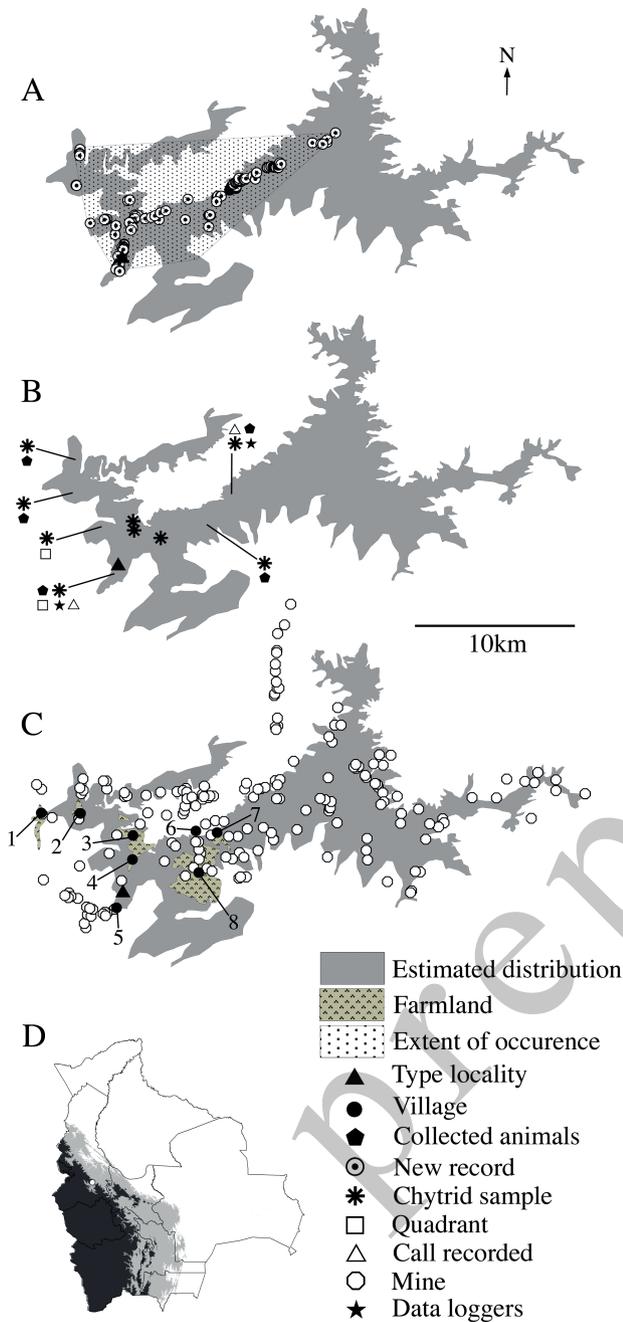


Figure 1. A–C) Maps with estimated distribution (as described in the running text), extent of occurrence as defined by the IUCN (2001) of *Psychrophrynella illimani* and other parameters (see legend in figure); D) Map of Bolivia with white areas corresponding to altitudes of 0–1,000 m, light grey 1,000–3,500 m and dark grey > 3,500 m [data available from WorldClim version 1 (HIJMAN et al. 2005) and edited in QGIS 2.4.0 (Quantum GIS Development Team 2014)]. Location of maps A–C is indicated with a white circle in D. Numbers in map C correspond to the following villages: 1 – Tres Ríos; 2 – Cañojuma; 3 – Iquico; 4 – Totoral; 5 – Cooperativa 15 de Agosto; 6 – Santa Rosa; 7 – Chuñawi; and 8 – Lambate.

ing a spectral slice with the Blackman window function at a 3-dB filter bandwidth of 120 Hz (KOK & KALAMANDEEN 2008).

Because *Psychrophrynella illimani* is classified as “Critically endangered”, we refrained from touching viable egg clutches and only counted the number of eggs in clutches that had already hatched or were covered with fungus and/or obviously not properly developing. This proved to be a good approach as the eggs could still easily be separated and counted even after hatching. To obtain information on temperature and humidity requirements for egg development, we placed two data loggers (HOBO U23-001) under stones with egg clutches and two others (HOBO U23-001 and HOBO U22-001) nearby, but not under the stone cover. Temperature and relative humidity were logged every hour and data taken during the day when the data loggers were set up were omitted to exclude measuring data that might have been influenced by the investigator. Data were read with the HOBO base-U-4 and analysed with HOBOware 3.5.0.

To test for the presence of *Batrachochytrium dendrobatidis*, frogs were swabbed according to KRIGER et al. (2006). A Copan dry swab (160 C) and a pair of powder-free nitril gloves were used for each individual. The swab was firmly rubbed against the ventrum, hands, and feet five times each and stored at -20°C until analysis. DNA extraction and subsequent Real-time Taqman PCR were performed following BOYLE et al. (2004), using swabs instead of toe-clips.

Results and discussion

Distribution and habitat

Individuals of *Psychrophrynella illimani* were detected at several new locations between the villages of Tres Ríos, Cooperativa 15 de Agosto, Chuñawi, and Lambate. Specimens were found between 3,321 and 4,044 m a.s.l., both during the day and at night. According to our observations, the extent of the species’ occurrence as defined by the IUCN is 81.38 km² (IUCN 2001), which is a large area compared to the previously known single locality (DE LA RIVA 2007), but possibly still remains an underestimation of the real distribution range as our search efforts were limited in time and space. In Fig. 1A, we mapped all localities where specimens were encountered, the currently known extent of occurrence, and a rough distribution estimate based on our current knowledge of habitat suitability (wet páramo above the tree line) as seen on Google Earth (Google Earth 2013), as well as the known altitudinal distribution. This estimate results in an area of 150.2 km². It must however be noted that the distribution is probably not continuous as it strongly depends on habitat. We could not achieve a more realistic estimate of the area of occupancy (IUCN 2001) due to the limited time available and the difficulties in accessing some parts within the expected

extent of occurrence. The species seems to be quite tolerant to habitat alteration, however, as we encountered individuals in a wide variety of landscapes: under stones on rocky mountain slopes, in patches with thick mosses, under stones in short grazed vegetation, and even in stone walls in the centre of Totoral (see Figs 2A–C). It is therefore likely that more localities will be discovered during future surveys. EMBERT et al. (2011) already suggested that *Psychrophrynella* species are able to persist in disturbed areas. The only habitats where we never encountered individuals are dense elfin forest at slightly lower altitudes, short grazed altiplano meadows without cover (e.g., stones, shrubs, patches of remnant elfin forest) and large stretches of cultivated land without cover. Seemingly appropriate habitat was not a guarantee for the presence of the species, however, as individuals were only found in certain parts in some locations with an apparent continuous vegetation cover, even during repeated visits. This absence in parts of seemingly continuous habitat is also seen in other species of the genus (DE LA RIVA 2007). Other anuran species encountered in the same area were *Pleurodema marmoratum*, *Gastrotheca marsupiata*, and *Pristimantis platydactylus*,

but all of these were less abundant than *Psychrophrynella illimani*. *Pristimantis platydactylus* was only encountered at the lower limits of the vertical distribution of *Psychrophrynella illimani*. Most *Psychrophrynella* species are currently known from very restricted ranges only (DE LA RIVA 2007). Like possibly in other *Psychrophrynella* species, too, extensive surveys might lead to the discovery of more populations and therefore expand the known distributions of this and other species.

Morphology

A total of 19 individuals were collected and deposited in the collections of the Museo de Historia Natural Alcide d'Orbigny in Cochabamba (numbers MHNC-A 2125 to MHNC-A 2143). SVL varied from 8.3 to 24.6 mm, thus exceeding the maximum of 22.3 mm reported in the original species description (DE LA RIVA & PADIAL 2007). This is not surprising, as the authors could only rely on four specimens for the species description, a number that is likely insufficient to obtain a good estimate of its size. Other

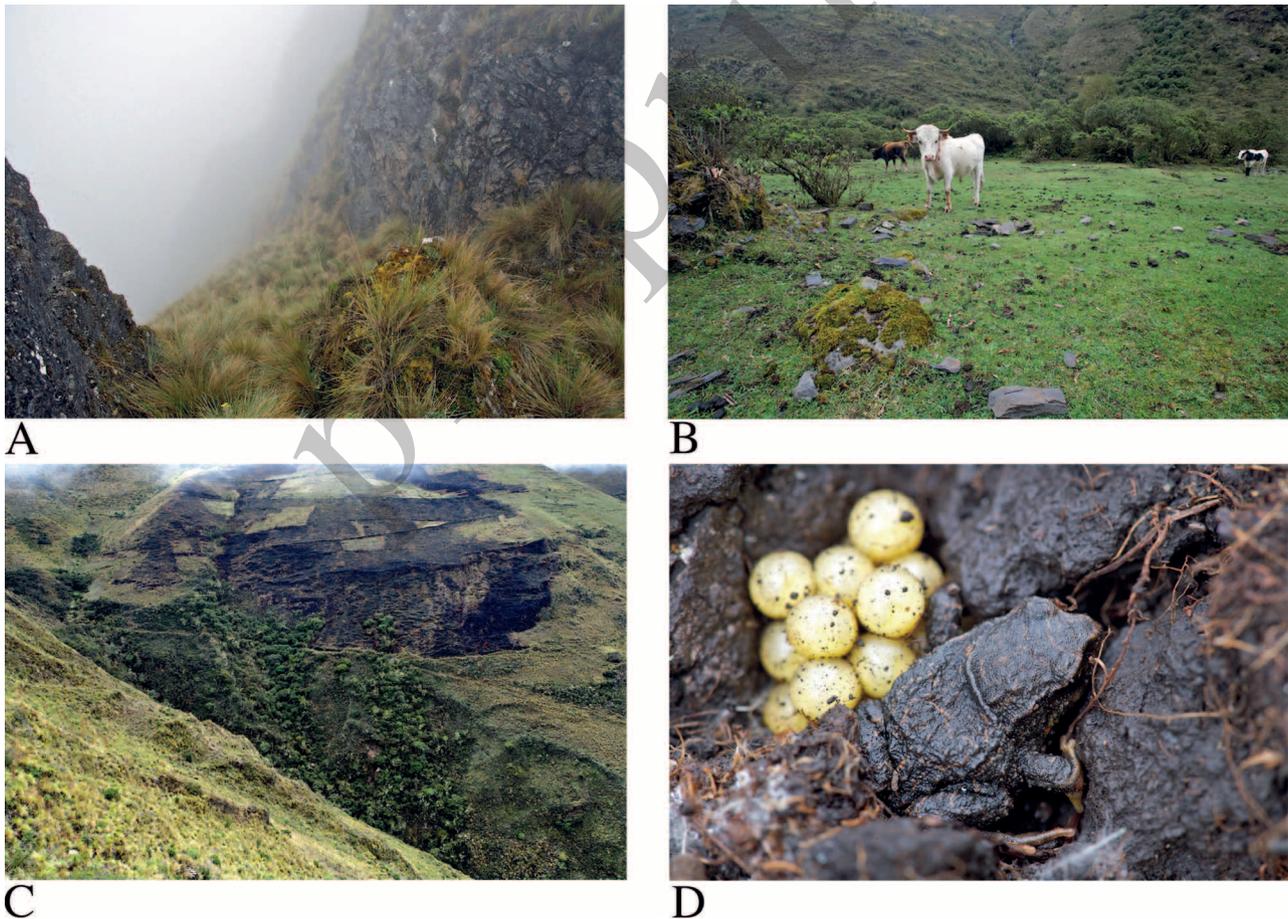


Figure 2. A) A rare patch of undisturbed *Psychrophrynella illimani* habitat on a steep mountain slope near Totoral at 4,044 m a.s.l.; B) Short-grazed vegetation near the type locality, where *P. illimani* can be found under stones; C) Habitat of *P. illimani* near Chuñawi, with recently burnt pastures and elfin forest remnants; D) Male *P. illimani* tending to an egg clutch under a stone near the type locality. All photos by B. WILLAERT.

Table 1. Temperature (T) and relative humidity (RH) collected over 28 consecutive days at two different locations near sheltered egg clutches and in the immediate surroundings.

	Type locality		Chuñawi	
	In nest	Surroundings	In nest	Surroundings
Overall	10.0±3.1 (4.2–19.0)	9.2±3.0 (3.3–18.3)	12.1±4.2 (5.5–24.2)	9.3±5.4 (-0.9–27.4)
T (°C) MIDDAY	12.7±3.5 (8.4–18.2)	11.5±3.5 (6.6–18.1)	17.7±4.5 (9.0–24.2)	15.6±4.4 (6.1–21.4)
MIDNIGHT	8.8±1.4 (6.4–11.1)	8.3±1.7 (5.5–11.8)	9.4±1.1 (6.9–11.5)	5.6±1.5 (1.5–8.3)
Overall	99.6±1.7 (88.4–100)	83.5±15.6 (35.9–100)	99.95±0.3 (97.0–100)	–
RH (%) MIDDAY	98.2±3.2 (91.2–100)	84.2± 17.6 (44.2–100)	99.8±0.6 (97.6–100)	–
MIDNIGHT	100 ±0	81.7±16.2 (49.7–98.9)	100±0	–

measurements were only taken from adults ($n = 15$) and are consolidated in Supplementary table 1. For a diagnosis, we refer to the original description (DE LA RIVA & PADIAL 2007), while we focus on the variation in morphometric measurements and coloration here. Measurements concur with those provided in the original description, but with a slightly higher variation. The head is wider than long, with the width corresponding to 33% (28–36%) of the SVL and the length to 29% (24–33%) of the SVL. The legs are moderately short with TL+FL reaching 73% (59–79%) of the SVL, with a tibia length of 35% (29–38%) and a foot length of 38% (30–41%) of the SVL, respectively. Only the proportion between the eye–nostril distance and the eye length presented here is markedly smaller than in the original description, being 61% (51–71%) versus 83% (65–94%) (DE LA RIVA & PADIAL 2007). This is possibly due to the bias of different measurers, but a part of this deviation could also be explained by differences in preservation periods before measurements were taken (LEE 1982). The extent of colour variation is illustrated in Fig. 3 and surpasses earlier observations (DE LA RIVA & PADIAL 2007). Colour varies greatly between individuals from the same location, with the dorsum being mostly brownish, blackish or greenish, sometimes with dark or orange blotches. In some individuals, a light vertebral stripe is present (see Figs 3A and 3H). The venter is mostly cream to yellow with a variable number of irregularly shaped black and sometimes also blue blotches. At one location, we encountered two individuals that were yellowish throughout (see Fig. 3G). All observed individuals had orange (or rarely yellow) blotches in the groin and axilla regions, a character that is diagnostic for this and some other *Psychrophrynella* species (DE LA RIVA 2007).

Density

We carried out six quadrant counts – three in two different valleys each (see Fig. 1B for exact locations) – to obtain an idea on population densities of *Psychrophrynella illimani*. These habitats consisted of mosaics of short grazed vegetation, rocks, mosses, and shrubs for which the respective percentage of cover varied between quadrants (see Supplementary table 2). On average, seven individuals were found per 100 m² (range: 4–13, see Supplementary table 2 for an

overview). None of the recorded parameters were significantly correlated with the number of frogs or egg clutches found, but we did find a positive correlation between the number of frogs and the number of egg clutches ($r = 0.97$, $n = 6$, $p < 0.001$). These results should however be treated with caution due to the low number of samples ($n = 6$). We did not analyse quadrants in other, less disturbed habitat types (e.g., thickly packed mosses), as this would have required destroying the habitat to find all animals inhabiting the quadrant. Additionally, individuals are extremely difficult to locate within this type of vegetation, possibly leading to underestimates anyway. Natural habitat is also hard to find, as livestock animals have significantly reduced the natural vegetation in most places. The figures shown here thus only refer to a degraded – but widespread – habitat type, where cattle roam freely. In other habitat types, densities may be higher or lower, but further research is needed to see if there are large differences in density between distinct habitat types. Few quantitative data are available on densities of other *Psychrophrynella* species, but maximum densities of 2.1 and 1.2 individuals per 100 m² were calculated for *P. wettsteini* and *P. iatamasi*, respectively, in two other surveys (CAMACHO 2008, TERCEROS 2010). These numbers are based on a different census method though, whereby mainly auditory cues were used, probably resulting in an underestimate of the factual density. *Psychrophrynella illimani* was originally believed to be rare after considerable search efforts in November of 2002 had yielded only four specimens (DE LA RIVA & PADIAL 2007), but this might be due to the variable weather in the region and the secretive lifestyle of this species. Indeed, we also observed large variation in abundance during repeated visits to the same locations, often due to distinctly different weather conditions. Like for *P. iatamasi* and *P. chacaltaya*, variations in abundance throughout the year were observed, with limited observations during dry periods (CAMACHO 2008, CORTEZ 2009).

Reproduction

The average number of eggs in the counted clutches was 17.8 ± 3.5 ($n = 6$, range = 14–24). All clutches were found under stones or in thick tufts of mosses and tend to be by a male

frog (see Fig. 2D). On a single occasion, a male was found tending to two egg clutches. The two egg masses were in close proximity to one another, but clearly separated and at different stages of development. The role of this paternal care is unknown, but egg-tending is known to benefit the embryos and larvae by protecting them against certain predators and pathogens and keeping the eggs hydrated in several other amphibian species (e.g., TAIGEN et al. 1984, CRUMP 1995). Previous research described a single egg clutch containing 8 eggs in *P. illampu*, and for *P. wettsteini* clutches with an average of 18 eggs were found, with pa-

ternal clutch-tending being known from the latter (DE LA RIVA et al. 2007, TERCEROS 2010). In the newly described *P. teqta*, two egg clutches have been observed holding 28 and 41 eggs, both being tended to by the male (DE LA RIVA & BURROWES 2014). We obtained temperature and relative humidity data for two egg clutches for 28 consecutive days between mid-October and mid-November of 2013. Both clutches had not yet hatched after 28 days, but tiny froglets were clearly visible in all eggs by then. An overview of these data is presented in Table 1. For the first egg clutch, located at the type locality (see Fig. 1B), the average tempera-

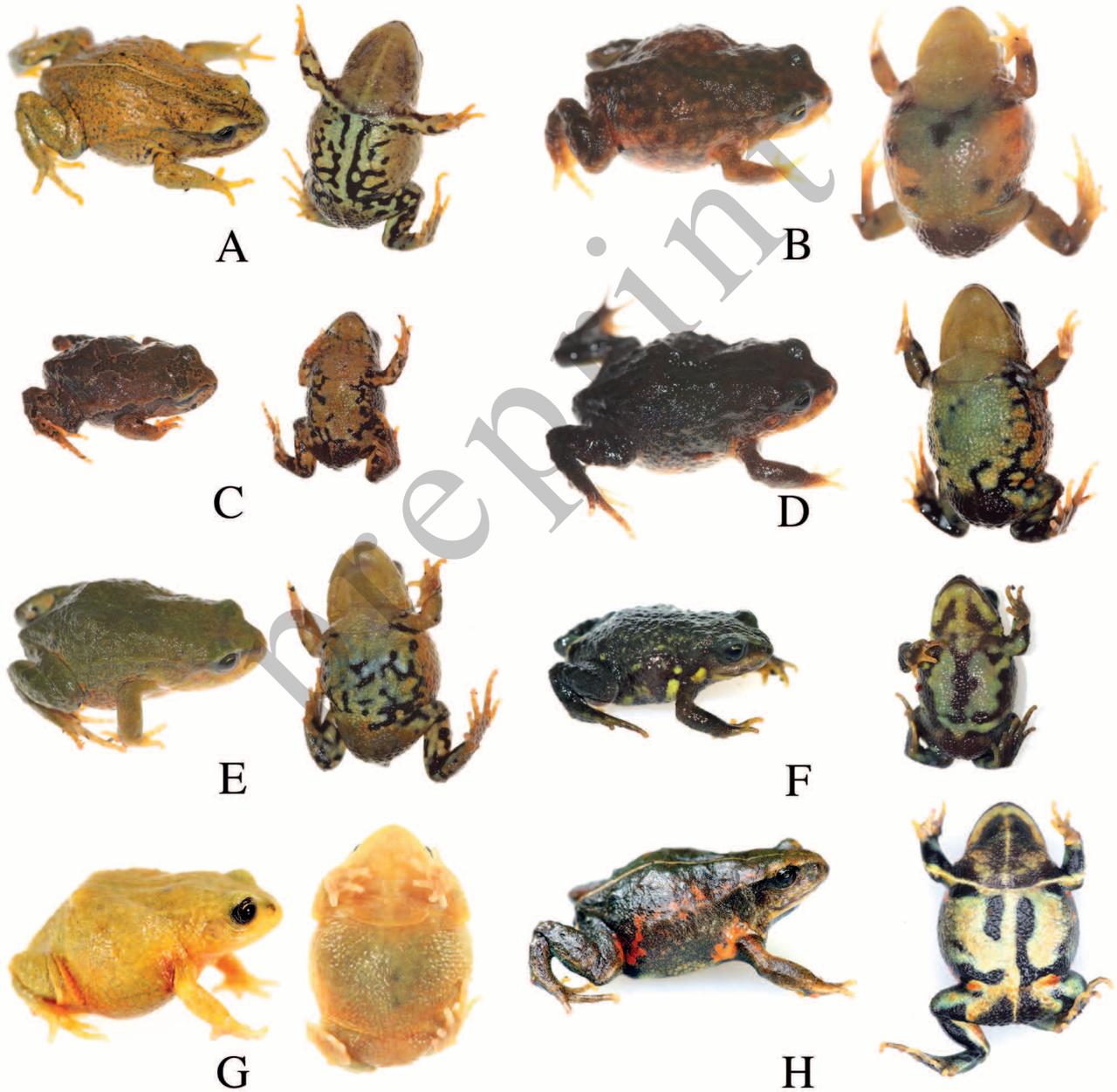


Figure 3. Dorsolateral and ventral views of *Psychrophrynella illimani* in life (images not to scale), showing its variation in colour pattern: A) MHNC-A 2133, Chuñawi; B) MHNC-A 2128, type locality; C) MHNC-A 2138, road between Chuñawi and Lambate; D) MHNC-A 2125, type locality; E) MHNC-A 2132, Cañojuma; F) MHNC-A 2136, Cañojuma; G) uncollected specimen, Totoral (30/10/2013); H) uncollected specimen, Chuñawi (19/10/2013). All photos by B. WILLAERT.

ture was $10.0 \pm 3.1^{\circ}\text{C}$ ($4.2\text{--}19.0^{\circ}\text{C}$) and the relative humidity $99.6 \pm 1.7\%$ ($88.4\text{--}100\%$). While ambient temperatures were similar, relative humidity was much more variable (see Table 1). For the other egg clutch, located at Chuñawi (see Fig. 1B), the average temperature was $12.1 \pm 4.2^{\circ}\text{C}$ ($5.5\text{--}24.2^{\circ}\text{C}$) and the relative humidity $99.9 \pm 0.3\%$ ($97.0\text{--}100\%$). Ambient temperature was more extreme here, with a recorded minimum of -0.9°C and a lower average (see Table 1). No data are available on relative humidity in the immediate surroundings for this location. The sheltered location of the egg clutches shows an advantage for both temperature and relative humidity, with relative humidity never dropping below 88.4% and temperatures being less extreme (especially in Chuñawi). A high level of relative humidity is of critical importance for the development of terrestrial eggs, as low humidity could lead to desiccation and result in mortality or smaller hatchlings (TAIGEN et al. 1984). The optimal temperature range for amphibian egg development is species-specific, with extremely low or high temperatures being lethal (MOORE 1939, DUELLMAN & TRUEB 1986, WELLS 2007). The higher average temperature near the egg clutches is beneficial, as there is a negative correlation between temperature and development duration (DUELLMAN & TRUEB 1986, WELLS 2007). Both clutches were still viable after 28 days but had not yet hatched, so that the exact incubation period remains unknown. There is no published data on egg development in other *Psychrophrynella* species, but previous research on other anuran species with direct development reported 15 to 49 days before hatching takes place (DUELLMAN & TRUEB 1986).

Advertisement call

Males were heard calling both during the day and night, but always from spots concealed under stones or in moss. They quickly stopped producing calls when disturbed and only on a single occasion, at night, a male was found calling from an open spot at the type locality (see Supplementary video 1). The call characteristics described below are

based on recordings of three different males, all uncollected. Two males were recorded at the type locality, from which one recording had too much background noise to obtain all the relevant data. The third male was recorded at Chuñawi, 7.5 km airline distant from the type locality. Characteristics of the individual calls are presented in Table 2 and Fig. 4. The advertisement call consists of 13.9 ± 1.9 notes (11–18) with a mean note duration of 14 ± 5 ms (3–25) and a mean inter-note interval of 41 ± 22 ms (8–86). The inter-note interval is not uniform, but decreases in duration from the beginning to the end of the call. Calls are repeated at a rate of 5.44–7.86 calls per minute. Mean call duration is 0.73 ± 0.38 s (0.24–1.12) with an inter-call interval of 9.20 ± 4.38 s (4.97–20.86s). Four to five harmonics are developed with a dominant frequency of $2,840 \pm 148$ Hz (2,701–3,086) and located in the fundamental harmonic. The call recorded from the male at Chuñawi is very similar to the ones from the type locality, differing only in a much higher note repetition rate with shorter notes and shorter inter-note intervals (see Table 2). The advertisement call of *Psychrophrynella illimani* is most similar to the calls of *P. saltator* and *P. wettsteini* whose calls also consist of groups of 7–36 and 7–18 notes, respectively (DE LA RIVA 2007). The call of *P. illimani* differs from the call of *P. wettsteini* by having a higher dominant frequency (1,962 Hz in *P. wettsteini*), a higher call rate (1.5 calls per minute in *P. wettsteini*), and a shorter note duration (191 ms in *P. wettsteini*) (DE LA RIVA 2007). The call of *P. illimani* differs from that of *P. saltator* by having a slightly lower dominant frequency (3,126 Hz in *P. saltator*), a higher call rate (1.5 calls per minute in *P. saltator*), and a shorter note duration (47 ms in *P. saltator*) (DE LA RIVA 2007).

Threats

To test for the presence of *Batrachochytrium dendrobatidis* (*Bd*), a total of 63 individuals were swabbed at nine different locations between 3,350 and 3,996 m a.s.l. (see Fig. 1B for locations). Additionally, three individuals of *Gastro-*

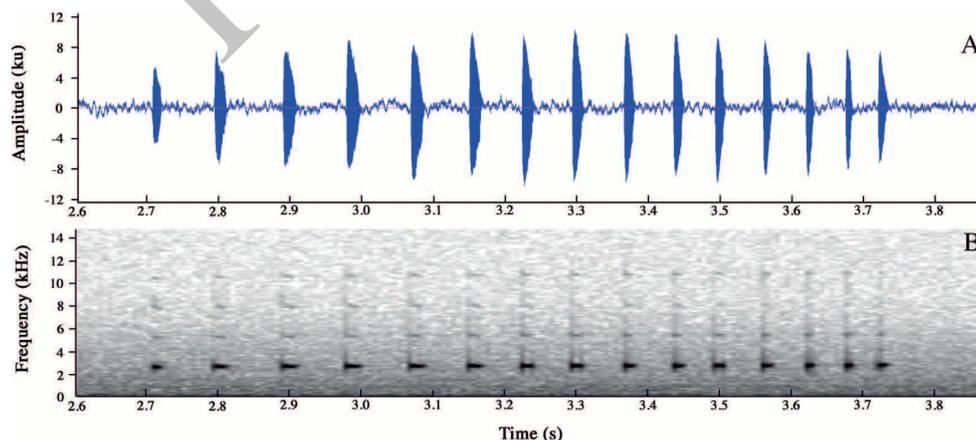


Figure 4. A) Oscillogram and B) spectrogram showing a single advertisement call of an uncollected male of *Psychrophrynella illimani* recorded at the type locality (air temperature 16.4°C). See Table 2 (call 1) for the corresponding parameters.

Table 2. Advertisement call parameters of three recorded males of *Psychrophrynella illimani*.

	note repetition rate	inter-note interval		note duration	dominant frequency	inter-call interval		call duration	notes/call	call rate	SVL	RH	T
		ms	ms			s	s						
Call 1	14.13±0.34	54±14	16±4	2707±11	7.27±2.47	1.03±0.06	15.27±0.90	7.86	16.7	89.3	16.8		
(type locality)	range	13.56–14.65	25–86	3–25	2701–2725	4.97–12.80	14–17	NA	NA	NA	NA	NA	
Call 2	–	–	–	3026±32	–	–	13.27±2.28	5.44	16.2	90.7	15.3		
(type locality)	range	–	–	2983–3086	–	–	11–18	NA	NA	NA	NA	NA	
Call 3	44.23±0.92	15±4	8±2	2765±13	11.97±5.18	0.28±0.03	12.75±1.28	5.71	17.1	89.6	15.9		
(Chuñawi)	range	43.01–45.83	8–26	2741–2789	6.17–20.99	0.24–0.33	11–15	NA	NA	NA	NA	NA	

theca marsupiata, two *Pleurodema marmoratum*, and a single individual of *Pristimantis platydactylus* were swabbed during the surveys. All swabs tested negative for the presence of *Bd*, suggesting that this chytrid hitherto poses no threat to this species. Previous studies have already shown the infection of other *Psychrophrynella* species in both Bolivia and Peru (CATENAZZI et al. 2010, DE LA RIVA & BURROWES 2011), but since there is no historical data on population sizes available, the effect of this infection is unknown. DE LA RIVA & BURROWES (2014) reported on two individuals of *P. teqta* with moderate to high loads of *Bd* zoospores without showing any signs of chytridiomycosis. It remains however unknown how *P. illimani* would respond to *Bd* exposure and how threatening this infection is for *Psychrophrynella* species in general.

Several villages are located within the distribution area of *P. illimani* (see Fig. 1C), and locals mainly live from the mining industry and small-scale agriculture. On Fig. 1C, plotted patches of farmland are visible in satellite images (Google Earth 2013). Livestock animals roam freely within the entire distribution area of *P. illimani* with the exclusion of some very steep mountain slopes and patches with dense vegetation. Livestock commonly bred in the region are sheep, pigs, horses, cows, and llamas. Previous research on the influence of cattle grazing on amphibian communities has mainly focussed on wetland ponds used by aquatically reproducing amphibians and yielded variable results (ADAMS et al. 2009, SCHMUTZER et al. 2008, ROCHE et al. 2012, VERGA et al. 2012). Some papers suggest an increased diversity and abundance of amphibians in cattle-grazed habitat (VERGA et al. 2012), while other studies find the opposite (SCHMUTZER et al. 2008). The positive or negative effects can be species-specific (BURTON et al. 2009) and the historical presence of wild grazers may also have an influence on the response shown by amphibian communities to the presence of domestic cattle that have replaced native grazers in many areas (VERGA et al. 2012). For *Psychrophrynella* species it is believed that they have a certain tolerance to the presence of cattle (EMBERT et al. 2011) and we indeed encountered individuals of *P. illimani* in heavily grazed habitats (see Fig. 2B). As pristine habitat within the range of *P. illimani* is now extremely rare, we are unable to compare densities with more natural habitat types, however. To study the effect of cattle on this particular species, establishing grazing-exclusion zones would be necessary.

The area around the Nevado Illimani is rich in minerals and gold mining is particularly common. Bolivia has a long mining history and it is still an important aspect of its economy (MAURICE-BOURGOIN et al. 1999, SALMAN et al. 2013). As seen in Fig. 1C, mines are abundant throughout the region, varying from small, single-person operations to larger cooperatives. Mercury is used for the extraction process, and the toxic waste is commonly dumped in rivers. It is expected that most of the streams in the region will be contaminated with high levels of mercury, an assumption that was confirmed by the villagers of Totoral. Mercury is known to bioaccumulate in several taxa (e.g.,

fish) and pose a human health hazard (LODENIUS & MALM 1998). Since *P. illimani* is a terrestrially breeding species, it is probably not directly affected by mercury pollution in streams. Pollution might however also disturb terrestrial ecosystems in the long run (LODENIUS & MALM 1998) and the frogs could be indirectly affected by bioaccumulating mercury when consuming contaminated invertebrates. We therefore recommend an in-depth study of mercury pollution in this region. Mining is also directly responsible for habitat degradation, as erosion and the construction of roads and mining camps have dramatically changed the landscape. Previous studies have suggested the establishment of local micro-size conservation areas (ranging from a few hectares to a few square kilometres) to protect species with restricted ranges (EMBERT et al. 2011, DE LA RIVA & REICHLÉ 2014). In the case of *P. illimani*, this could easily be arranged by excluding cattle- and mining-related activities from fenced-in parts of its habitat in a concerted effort involving local communities.

Climate change is of particular concern for montane taxa (MCCAIN & COLWELL 2011) and it is therefore predicted that global warming will be a serious threat to *P. illimani* (AGUAYO 2009, EMBERT et al. 2011, DE LA RIVA & REICHLÉ 2014). Our data show that the relative humidity near egg clutches never dropped below 88%, so that changes in precipitation and prolonged periods of drought might have profound effects on the survival of this and other *Psychrophrynella* species, even if large tracts of their habitat were protected.

Conclusion

We obtained new data on the distribution, ecology, reproduction, and call of *Psychrophrynella illimani*. The species turns out to be more widespread and common than previously thought, with this result probably being due to a lack of monitoring in the past and its secretive lifestyle. It is likely that more populations of this and other *Psychrophrynella* species are overlooked for the same reasons. With more resources for field research we would be able to obtain a better understanding of the distribution, ecology, and behaviour of this remarkable taxon. The habitat throughout the entire range is heavily impacted by human activities such as mining and agriculture, and future research should focus on how this impacts this species' survival. Establishing micro-sized protected areas within its distribution range might be beneficial to reducing further habitat degradation. Our screening for *Batrachochytrium dendrobatidis* suggests that populations are currently unaffected by this chytrid fungus. Climate change is likely to be a major concern for the species' survival, though, as the development of egg clutches seems to be highly dependant on constantly high levels of relative humidity. We recommend that *P. illimani* remains listed as "Critically endangered", since the increased extent of its known occurrence is still smaller than 100 km² and the quality of its habitats continues to decline.

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Supplementary material

Additional information is available in the online version of this article at <http://www.salamandra-journal.com>

2 Supplementary tables and link to video.

Supplementary table 1. Measurements of collected specimens in millimetres. Abbreviations are explained in Material and methods.

Specimen	SVL	HL	HW	IND	ED	END	TL	FL
MHNC-A 2143	8.3	–	–	–	–	–	–	–
MHNC-A 2133	19.1	6.0	6.8	1.7	2.3	1.4	7.3	7.7
MHNC-A 2128	17.5	5.8	6.3	1.4	2.2	1.2	6.4	6.6
MHNC-A 2141	11.8	–	–	–	–	–	–	–
MHNC-A 2126	16.5	4.9	6.0	1.4	2.1	1.1	6.0	6.6
MHNC-A 2138	14.5	–	–	–	–	–	–	–
MHNC-A 2125	22.3	6.6	7.6	1.8	2.6	1.5	7.5	8.3
MHNC-A 2131	21.6	6.2	7.2	1.8	2.2	1.3	7.7	8.2
MHNC-A 2134	17.6	5.3	5.9	1.6	1.9	1.4	6.5	7.2
MHNC-A 2135	21.0	5.6	7.1	1.7	2.1	1.5	7.8	8.4
MHNC-A 2132	21.4	5.9	7.1	1.8	2.3	1.5	7.3	7.6
MHNC-A 2140	17.0	5.1	5.5	1.5	2.0	1.2	6.3	6.8
MHNC-A 2136	18.2	5.2	6.0	1.7	2.0	1.2	6.3	6.9
MHNC-A 2142	12.5	–	–	–	–	–	–	–
MHNC-A 2137	22.4	5.4	6.2	1.6	2.2	1.2	6.5	6.8
MHNC-A 2139	18.4	5.0	6.1	1.5	1.9	1.4	6.5	6.6
MHNC-A 2127	22.4	6.1	7.2	1.8	2.4	1.5	7.9	8.0
MHNC-A 2129	19.2	5.7	6.7	1.6	2.2	1.1	6.4	7.8
MHNC-A 2130	24.6	6.4	8.2	1.9	2.6	1.5	7.6	8.6

Supplementary table 2. Soil cover, vegetation parameters, and number of frogs and egg clutches encountered in 6 quadrants (see Fig. 1B for locations).

Quad.	% stone cover		% mud	% vegetation cover			#shrubs	% shadow	#frogs	#clutches
	<30 cm	>30 cm		grazed	mosses	natural				
1	40	50	0	3	7	0	0	0	5	0
2	30	40	0	15	13	2	6	1	4	0
3	5	40	2	40	10	3	6	2	13	3
4	4	4	0	86	4	2	20	50	5	0
5	5	1	0	87	5	2	15	12	6	1
6	5	25	0	63	4	3	17	30	9	2

Supplementary video 1. An uncollected male of *Psychrophrynella illimani* calling (see Table 2 [call 1] and Fig. 4) at the type locality on 17 October 2013. The movie can be watched here (in Adobe Acrobat or Reader Version 6 or higher, click on the link to watch the video):

http://www.salamandra-journal.com/images/stories/Willaert-o818-supplementary_movie.m4v