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# Climatic Heterogeneity in the Bolivian Andes: Are Frogs Trapped?

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**Abstract.** Short-legged, small, robust frogs of the family Craugastoridae present a remarkable beta-diversity in the high Andes, their distributions being characterized by a very high degree of micro-endemism to specific valleys. We used dataloggers to study the temperature and humidity conditions of microhabitats of several species of the genus *Microkayla* at three elevation belts: below, within, and above the altitudinal range of their distribution in Bolivia. We also conducted thermal physiology experiments on a limited number of individuals of one of these species. Our aim was to infer on factors that may limit the distribution of anurans in a biological hotspot that is threatened by climate warming. We found an unexpected thermal heterogeneity within the slopes at three different Andean valleys that explained the specific distribution of species of *Microkayla* at each site. Species distribution was associated to elevation belts with the highest ambient relative humidity, and there was high variability in thermal preference when individuals were experimentally exposed to a thermal gradient. Critical thermal maxima compared to the temperatures that frogs confront in nature, as well as thermal performance trials, revealed that the studied species has a broad physiological tolerance to temperature. These results point to moisture, and not temperature, as the limiting climatic factor determining the occurrence of these species in high Andean slopes, but further experimental work on water balance is needed. The predicted desertification of the Andes in future climate change scenarios poses a potentially serious threat to this highly diverse group of amphibians.

**Keywords.** Amphibians; climate change; distributions; elevational gradient; relative humidity; thermal physiology.

**Resumen.** Las pequeñas ranas robustas y de patas cortas de la familia Craugastoridae presentan una alta diversidad beta en la región altoandina, donde la distribución de las distintas especies se caracteriza por un alto grado de endemismo, restringiéndose a valles concretos. Utilizamos “dataloggers” para estudiar las condiciones de temperatura y humedad en los microhábitats de algunas especies de *Microkayla* en tres franjas a distinta elevación: por debajo de su rango altitudinal de distribución, dentro de dicho rango, y por encima. Además, realizamos experimentos de fisiología térmica con un limitado número de ejemplares de una especie de *Microkayla*. Nuestro objetivo era averiguar los factores que pueden limitar la distribución de los anuros en un punto caliente de biodiversidad amenazado por el calentamiento climático. Encontramos una sorprendente heterogeneidad térmica en las laderas de tres valles andinos diferentes, que explican la distribución de las especies de *Microkayla* en cada sitio. La distribución de las especies está asociada a las franjas altitudinales de humedad ambiental más alta, y se observa una alta variabilidad en las preferencias térmicas cuando los individuos son sometidos experimentalmente a gradientes de temperatura. Las temperaturas críticas máximas, comparadas con las temperaturas que las ranas confrontan en la naturaleza, así como los experimentos de desempeño térmico realizados, revelan que la especie estudiada tiene una amplia tolerancia térmica. Estos resultados apuntan a la humedad, y no la temperatura, como el factor climático limitante que determina la existencia de estas especies en las laderas altoandinas, aunque se necesita más trabajo experimental en balance hídrico para comprobar esto. La previsible desertificación de los Andes bajo escenarios de cambio climático futuros, supone por tanto una seria amenaza potencial para este grupo tan diverso de anfibios.

## INTRODUCTION

In a classic and seminal article, Daniel Janzen enlightened us with the realization that topographic barriers in tropical mountains are critical in determining the distribution of their biotas, mostly because of the altitudinal differences in temperature and humidity (Janzen, 1967). The Andes are an important biodiversity hotspot (Myers et al., 2000) and a significant region in the radiation of direct-developing frogs of the family Craugastoridae Hedges et al., 2008 (De la Riva, 2007, 2020; De la Riva

et al., 2017). From southern Peru to central Bolivia, there is a speciose genus of small craugastorid frogs within the subfamily Holoadeninae Hedges et al., 2008, *Microkayla* De la Riva et al., 2017, characterized by robust bodies and short extremities with unexpanded digital tips (De la Riva, 2007; De la Riva et al., 2017; Fig. 1). These frogs have a high degree of micro-endemism in the Andes, being historically restricted to particular cool, wet valleys, either occupying wet grasslands above the tree line, or elfin and cloud forests, between ca. 2,500–4,200 m (De la Riva, 2007). As such, *Microkayla* species are expected

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**Figure 1.** (A) *Microkayla* cf. *quimsacrucis*, (B) *Microkayla illimani*, and (C) *Microkayla illampu*. Photos: I. De la Riva.

to be adapted to specific environmental conditions and, hence, might be ecologically trapped in narrow distributional ranges.

The microhabitat used or selected by ectothermic animals determines the thermal environment they confront, the thermal range they are exposed to, and the effects of such range on their physiological performance, in some contexts with a toll on individual fitness (Huey, 1991; Adolph and Porter, 1993; Storlie et al., 2014). Because high Andean *craugastorid* frogs are small and have low vagility (De la Riva, 2007; Duellman and Lehr, 2009; Catenazzi et al., 2014), we expect these frogs to experience body temperatures matching those of their thermal environments, being thermoconformers (versus thermoregulators) and active at low body temperatures in their retreat sites and the immediate surroundings, as described for other small, terrestrial, high-elevation *craugastorids* such as members of the genus *Pristimantis* Jiménez de la Espada, 1870 (Navas, 1996a,b). Under these circumstances, operative environmental temperatures ( $= T_e$ , equilibrium body temperatures that one animal experiments in its habitat; Bakken and Gates, 1975) are appropriate to construct the thermal map of the habitat of that these frogs occupy at different sites. Determining the thermal maps at different elevations in the Andes can provide a framework to identify not only possible temperature barriers to species dispersal, but also exceptional contexts of thermal biology and other issues (such as disease) generally relevant to understanding how Andean amphibians may be threatened by climate warming (Rohr et al., 2008; Navas et al., 2013; Catenazzi et al., 2014; Burrowes and De la Riva, 2017; von May et al., 2017).

The South American Andes are showing clear signs of climate warming, with increases in temperature up to 0.34°C per decade in the last 25 years of the twentieth century, which represents a three-fold increase in the warming rate observed for the first part of the century (Vuille and Bradley, 2000). And in Bolivia, temperature has increased at a rate greater than 0.10°C per decade in the Andean region from 1960–2009, with a decrease in precipitation evident after 1984 (Seiler et al., 2013). Two of the indicators of the effect of modern climate change (sensu IPCC 2014) in the Andes are the increase of deglaciation rates in the last 56 years (Vuille et al., 2003; Seimon et al.,

2007), and in the frequency and intensity of droughts and floods brought about by severe El Niño Southern Oscillation (ENSO) events (Timmermann et al., 1999; Seiler et al., 2013). Climate change projections for the 21st century predict a significant warming trend for the tropical Andes that intensifies at higher elevations, compromising water availability (Bradley et al., 2006; Vergara et al., 2007; Urrutia and Vuille, 2009). For example, in Bolivia, the Andean region has experienced severe droughts in the present century (Rangwala and Miller, 2012; Seiler et al., 2013), but in the months of November–February of 2016–2017, it underwent what journalists referred to as the worst drought in 25 years (The Guardian, 2017; see also Bolivian Meteorology and Hydrology Service, 2018).

In this work, we evaluate the temperature and moisture characteristics of three different sites in the Amazonian slopes of the Andes of central Bolivia, where species of *Microkayla* occur. The objective is to identify the potential role of these factors at limiting the distribution of these highly endemic frogs, while also considering the thermal physiological limitations that they may have. An obvious caveat of our data is that they were collected during a short period in February 2016 and 2017 and do not reflect yearly weather fluctuations (Storlie et al., 2014). However, since fieldwork was done during the Bolivian wet-season, when moisture is least likely to be a limiting factor, our findings might reflect a conservative underestimation of the importance of moisture conditions for these frogs. The data we present provide a snapshot of the climatic variation by elevation in high Andean slopes at present times, providing a baseline for future comparisons. Results will advance our understanding of the threat of climate warming to this group of frogs and, potentially, to other high-Andean organisms adapted to specific environmental conditions circumscribed by valleys, ridges, canyons, or plateaus constituting the complex topography of this mountain range.

## MATERIALS AND METHODS

### Fieldwork

Data collection was conducted in the Bolivian Andes in February 2016 and 2017. According to the known eleva-

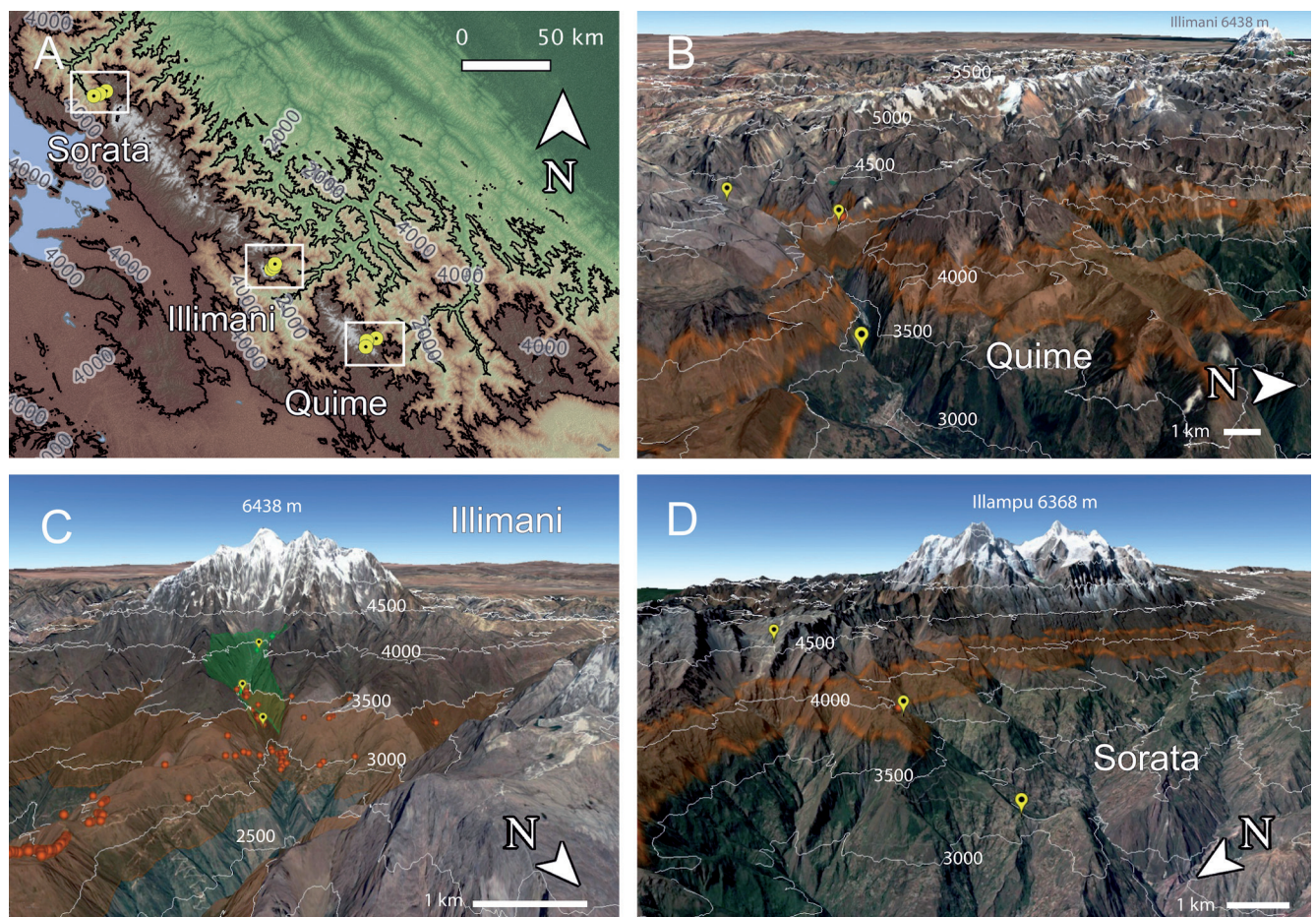


tional range of our target *Microkayla* species in Bolivia (De la Riva, 2007), we studied the thermal conditions of three different valleys in the Amazonian slopes of the Andes, at three sites per valley: low elevation (3,160–3,573 m), mid-elevation (3,895–3,917 m), and high elevation (between 4,252–4,359 m). Mid-elevation sites correspond to the elevational belt where our target species occur, while those at low and high elevation are mostly outside their range.

We studied nine sites in which we placed Onset Hobo® digital thermal couplers (dataloggers) inserted into frog models in typical *Microkayla* microhabitat for 48–72 h to measure frog operative temperatures ( $T_o$ ) every 15 min throughout the day (Navas and Araujo, 2000). Microclimate data taken at microhabitats are better suited for studies of vulnerability because they are consistent with actual species occurrences and physiological constraints (Storlie et al., 2014). Thus, we placed four dataloggers at each site separated by 5–8 m in a variety of microhabitats (rocks, moss, or vegetation roots) 10–20 cm below ground surface as usually occupied by *Microkayla* (De la Riva, 2007). Frog models mimicking thermal and

hydric frog characteristics were built previously in the laboratory to the size and shape of *Microkayla* frogs using either agar (Navas and Araujo, 2000; Rowley and Alford, 2010) or similarly scaled blocks of dark red gelatin (in this specific case, alternative concentrations were tested until we obtained models similar in texture to those made with agar). We did not calibrate our models against real frogs, but agar models built to scale have been validated to provide comparable measures of amphibian temperature and hydric regimes (Rowley and Alford 2010). Within each site, we always placed the amphibian models connected to dataloggers on the same mountain slope so that the local differences corresponded to elevational variation while minimizing variation by topo-climate effects such as air mass movements and solar radiation affecting different orientations (Dobrowski, 2011).

The three valleys studied are located, from southeast to northwest, near the villages of Quime (16°58'53"S/67°13'00"W, 3,030 m), Totoral (= Illimani, 16°35'42"S/67°44'30"W, 3,400 m), and Sorata (15°46'22"S/68°38'58"W, 2,700 m), within the provinces of



**Figure 2.** Map showing the three localities studied in the Bolivian Andes (A) and 3D images of our sampling sites (yellow tags) for temperature and relative humidity within the slopes at (B) Quime, (C) Illimani, and (D) Sorata. The orange shaded area at each site represents the estimated elevational range at which local species of *Microkayla* have been encountered; orange dots represent actual records. In the case of Illimani (C), the green shaded area corresponds to the area where the new (yet undescribed) species of *Microkayla* sp. occurs.

**Table 1.** Description of study sites at each elevation sampling station in the Bolivian Andes. Localities correspond to the map in Figure 1.  $T_{air}$  is air temperature, and  $T_e$  is operative temperature (measured by frog models in their microhabitats). RH is the ambient relative humidity. Data from four data-loggers per site for  $T_e$ , and one each for  $T_{air}$  and RH at Quime and Sorata. \* Indicate level of significance of t-test results for comparison of air versus operative temperature means at each elevation.

Elevation Site	Elevation (m)	GIS Coordinates	Mean Temperature °C (SD)		Mean % RH (SD)
			Tair	Te	
Quime					
Low	3160	16°59'18"S, 67°13'58"W	11.68 (2.76)***	14.06 (2.11)	88.82 (12.34)
Median	3895	17°00'03.5"S, 67°16'54"W	6.94 (1.87)***	9.57 (1.60)	97.02 (6.36)
High	4252	17°01'54.5"S, 67°17'07.5"W	6.42 (3.46)***	10.51 (3.78)	96.08 (8.06)
Illimani					
Low	3573	16°36'24.5"S, 67°44'45"W	---	11.59 (2.34)	---
Median	3917	16°37'20"S, 67°45'02"W	---	11.85 (4.15)	---
High	4359	16°38'00"S, 67°45'53"W	---	12.17 (3.74)	---
Sorata					
Low	3240	15°44'43.5"S, 68°39'56.5"W	12.24 (2.54)	12.61 (1.79)	97.34 (6.43)
Median	3840	15°44'10.8"S, 68°38'14.1"W	7.48 (1.69)***	9.02 (1.70)	99.15 (1.45)
High	4552	15°43'13.9"S, 68°36'10.0"W	5.15 (2.35)**	6.31 (2.37)	92.48 (5.24)

Inquisivi, Sudyungas and Larecaja respectively, in the Department of La Paz, Bolivia (Table 1 and Fig. 2A). We chose these particular mountain valleys because the *Microkayla* species known to occur there exhibit a high degree of micro-endemism related to elevation range and associated vegetation (De la Riva et al., 2017) and the three valleys show different orientations and landscape characteristics, thus offering a broad spectrum of the conditions to which these frogs are subjected in the Bolivian Andes. To get an idea of the narrowness of each valley, we used Google Earth to calculate the distance between the 4,000 m elevation contour lines on opposite sides of each valley right above our mid-elevation site (see below). The estimation of the spatial distribution of each species is based on data collected over 30 years of field experience of I. De la Riva working with *Microkayla* in Bolivia (De la Riva, 2007; De la Riva et al., 2017).

In the Cordillera Quimsa Cruz, where the Quime valley is located, one species has been identified, *Microkayla* cf. *quimsacrusis* (similar to *M. quimsacrusis* De la Riva et al., 2007a), which occurs in the adjacent valley to the northwest of our study site; Fig. 1A). Based on our past fieldwork with *Microkayla* in Quime, we estimate that *M. cf. quimsacrusis* occurs between ca. 3,500–4,100 m. The Quime valley is a moderately closed basin oriented mostly to the east (Fig. 2B). The distribution of frogs is limited to the west by high elevation steep slopes with unsuitable habitat. At 4,000 m, slightly above our mid-elevation site (3,895 m), the amplitude of the Quime valley is approximately 1.9 km.

Throughout this paper we refer to our second valley, near Totoral, as “Illimani” because it is at the foot of the Illimani peak (6,438 m), which represents the southeastern limit of the Cordillera Real (Fig. 2A). Our target species here was *Microkayla illimani* (De la Riva and Pádal, 2007) (Fig. 1B), but during this study it was found that these slopes are actually inhabited by two species,

one of them undescribed; the two species together span 3,300–4,700 m (Willaert et al., 2016; De la Riva and Burrowes, unpublished). While *M. illimani* has a moderately broad distribution, being found between 3,321–4,044 m (Willaert et al., 2016), that of the undescribed species seems restricted to higher elevations, although they overlap slightly around the 4,000 m contour line (Fig. 2B). The mid- and high-elevation Illimani study sites lie in the very narrow glacial, U-shaped valley of the Caballuni river (see De la Riva, 2007), mostly oriented to the northeast, with an amplitude of approximately 0.4 km just above our mid-elevation study site at 3,917 m (Fig. 2B).

Finally, the Sorata Valley represents the northwestern end of the Cordillera Real (Fig. 2A). One species of *Microkayla*, *M. illampu* (De la Riva et al., 2007b) (Fig. 1C), is found within our study area along an estimated elevation range of 3,700–4,200 m that presumably extends south and north for several kilometers where suitable habitat exists. Our sampling sites near Sorata are on the right bank (facing west) of a wide valley that extends from southeast to northwest in the Bolivian central Andes (Fig. 2A, D). The valley is flanked on the right by the massif formed by the Illampu (6,368 m) and the Ankohuma (6,427 m) peaks and on the left by the eastern slopes of the Cordillera de Apolobamba, which are lower (Fig. 2D). Above our mid-elevation site, the amplitude of this valley is approximately 22 km, which is much wider than the other two sites (Fig. 2B–D).

In Quime and Sorata we simultaneously placed one external air temperature and relative humidity digital datalogger approximately 2 m above one of the microhabitats where we measured frog operative temperatures (Table 1). We did not measure the relationship between ambient relative humidity and that present in frog retreat sites, so these data represent a proxy of the humidity available in their microhabitats at the time. We did not



**Table 2.** Descriptive parameters of individual frogs (*Microkayla cf. quimsacrucis*) tested for thermal preference. Body size is given by snout-to-vent length (SVL), Preferred temperature ( $T_{pref}$ ) is given as the mean ( $\pm$  95% Confidence Interval) of three trials per individual, and the Critical Thermal Maximum ( $CT_{max}$ ) as the body temperature at which an individual lost the ability to right itself.

Individual	Age/Sex	SVL (mm)	Weight (g)	$T_{pref}$ °C	$CT_{max}$ °C
Frog 1	Adult Male	22.8	1.05	23.96 ( $\pm$ 1.62)	32.5
Frog 2	Juvenile	17.4	0.49	16.2 ( $\pm$ 1.24)	35.2
Frog 3	Adult Female	24.7	1.2	17.88 ( $\pm$ 6.37)	36.0
Frog 4	Juvenile	17.6	0.51	18.68 ( $\pm$ 5.03)	37.4

have dataloggers for air temperature and humidity when we worked at the Illimani slopes in 2016.

### Experimental work

We chose to run experiments to measure thermal parameters of *Microkayla cf. quimsacrucis* frogs from the surroundings of Quime because in previous field trips one of the authors (I. De la Riva) had collected this species without difficulty and found it to be abundant. Unfortunately, in February 2017, after 64 person-hours of search time by three experienced field herpetologists sampling appropriate habitat, we only found four frogs. The most plausible explanation for this is the severe drought that affected Bolivia from November 2016 to March 2017 (The Guardian, 2017; Bolivian National Meteorology and Hydrology Service, 2018), a time of year normally corresponding to the rainy season (Köhler, 2000). Further searches for other *Microkayla* species in the following days in other areas of the Bolivian Andes rendered identical low results. Thus, we ran the experiments on this limited number of specimens realizing it would be valuable because there are very few data on the thermal biology of small, cold-adapted, direct-developing frogs confronting climate change and disease risk in the Andes (Catenazzi et al., 2014; von May et al., 2017). We measured three thermal parameters for these individuals (Table 2): Temperature preference ( $T_{pref}$ ), Performance at different target temperatures, and Critical Thermal Maximum ( $CT_{max}$ ). We did not measure the Critical Thermal Minimum ( $CT_{min}$ ) because it became obvious from  $T_{pref}$  experiments that it was very low, and thus, not a limiting factor to the performance of this species within their distributional range.

### Temperature preference

Frogs were placed in a 40 cm  $\times$  6 cm rectangular chamber built with wooden walls, a metal bottom, and an open top. We maintained a temperature gradient of 6.5–40°C throughout the bottom of the chamber by using ice blocks on the cold extreme and a water-bag with boiling water on

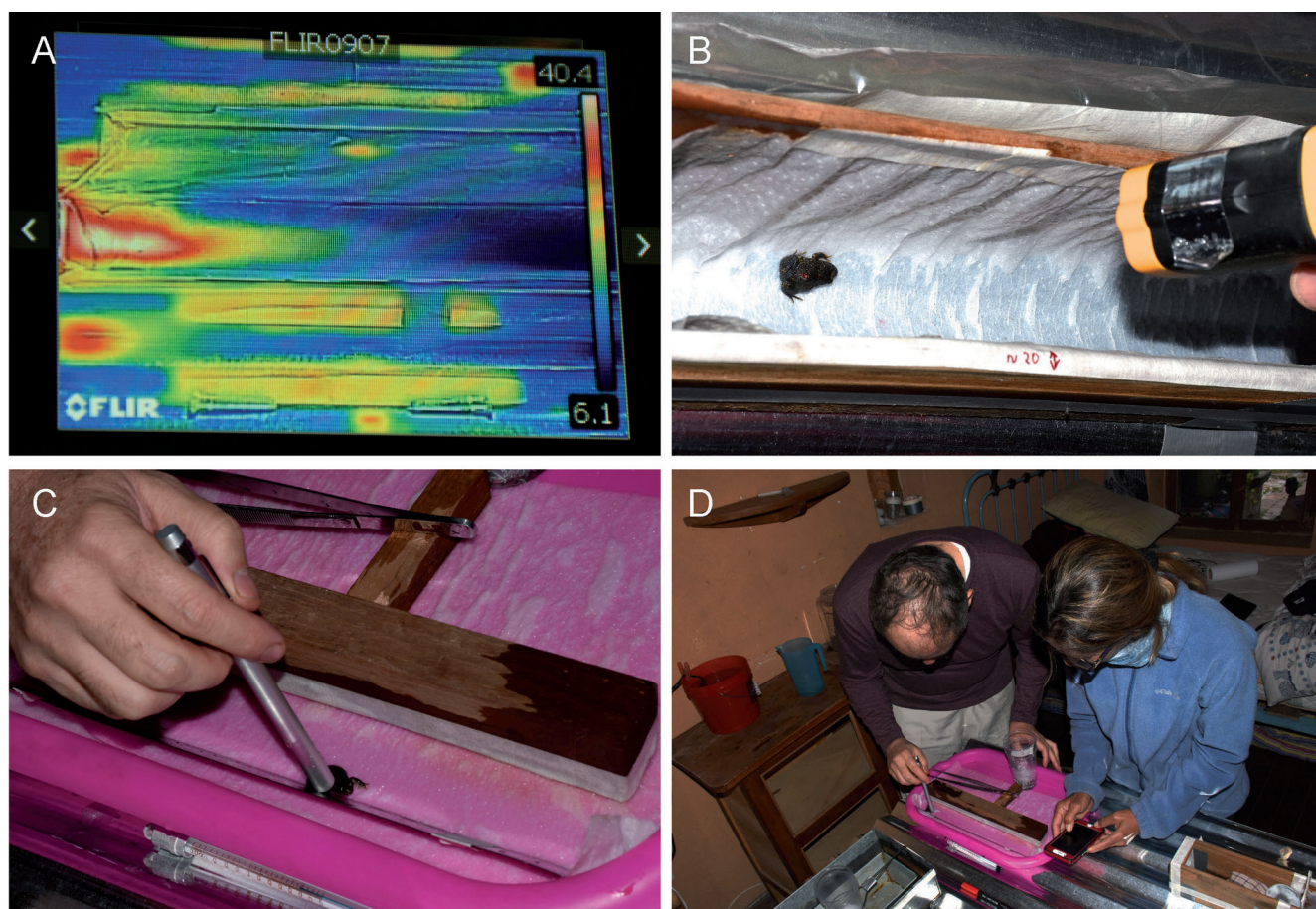
the opposite end (Fig. 3A). The temperature gradient was checked between trials with an infrared thermometer (temperature gun - General® IRT850K) and visualized in its full range using a FLIR C2 camera calibrated to pertinent relative humidity, short distances and paper emissivity (0.90). More ice cubes or hot water were added at the corresponding ends when adjustments were needed to retain the desired temperature gradient between 6.5–40°C. We used wet paper towels to line the sides and bottom of the chamber and periodically dripped water to keep humidity constant throughout the temperature gradient (Fig. 3B). Each trial began by placing two frogs opposite to each other, one at 20°C and the other at 15°C, within the gradient. Frogs were allowed to move freely throughout the bottom of the gradient, but we pushed them down if they attempted to climb the walls. The temperature at which they positioned themselves was recorded with an infrared thermometer every 10 min over 180 min for the first two frogs, but we stopped at 90 min for the second pair of individuals because the temperature preference was constant after 60 min.

### Thermal performance

We assessed frog performance at five experimental temperatures separated by intervals of 5°C (5, 10, 15, 20, 25°C). Given the characteristics of *Microkayla*, the target performance trait was speed, a variable assessed as the time it took an individual to walk a 20 cm transect under constant humidity and maintained as described for  $T_{pref}$  trials. We led frogs to desired (target) body temperatures ( $T_b$ ) by placing them in a plastic cup that was submerged in a water bath thermally regulated with either ice cubes or hot water. We used an infrared thermometer to measure the frog's body temperature and determine when individuals were ready for a specific trial. Once placed at the start of the transect, we stimulated frogs to walk forward by gently tapping their backs with a glass rod (Fig. 3C). The time it took to walk the 20 cm transect was recorded in seconds using a stopwatch (Fig. 3D). This exercise was repeated three times per individual at each target temperature. We calculated mean performance (walking time) per target temperature for all frogs and then considered the temperature at which they walked the fastest as the body temperature at which frogs performed best ( $T_{opt}$ ; i.e. 100% performance). Performances at other temperatures were calculated as a percent of the best performance and used to build a temperature-dependent curve by joining points and ending at the mean  $CT_{max}$  value at which 0% performance is assumed (Fig. 4).

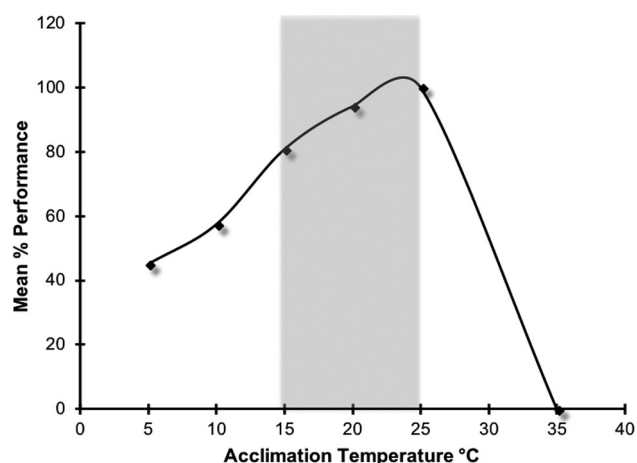
### Critical thermal maximum

We started the experiment after bringing the frog's body temperature to 25°C, which took approximately



**Figure 3.** Thermal physiology experiments with *Microkayla* cf. *quimsacrus* at our field site in Quime, Bolivia. **(A)** Thermograph showing temperature range for  $T_{pref}$  tests. **(B)** Photograph showing a frog in  $T_{pref}$  arena and temperature gun to monitor body temperatures. **(C–D)** Photograph showing the setup and how we encouraged frogs to move in thermal performance trials.

3 min using the water bath described above. Then, the plastic cup containing the experimental frog was placed into a stable water bath kept at 50°C. We realize that this system does not allow for precise heating rate regulation,



**Figure 4.** Performance curve for *Microkayla* cf. *quimsacrus* showing that 100% performance is achieved at about 24°C. The shaded area indicates the range of temperatures where frogs positioned themselves 80% of the time during temperature preference trials.

but it does produce a heating rate compatible with those of typical acute tests, between 1–2°C per minute, and it is a practical way to do this work at field sites. Periodically, we turned the frogs over on their backs and measured their body temperature with an infrared thermometer. The body temperature at which they could no longer right themselves was considered the  $CT_{max}$  (Navas et al., 2007; Catenazzi et al., 2014) (Table 2).

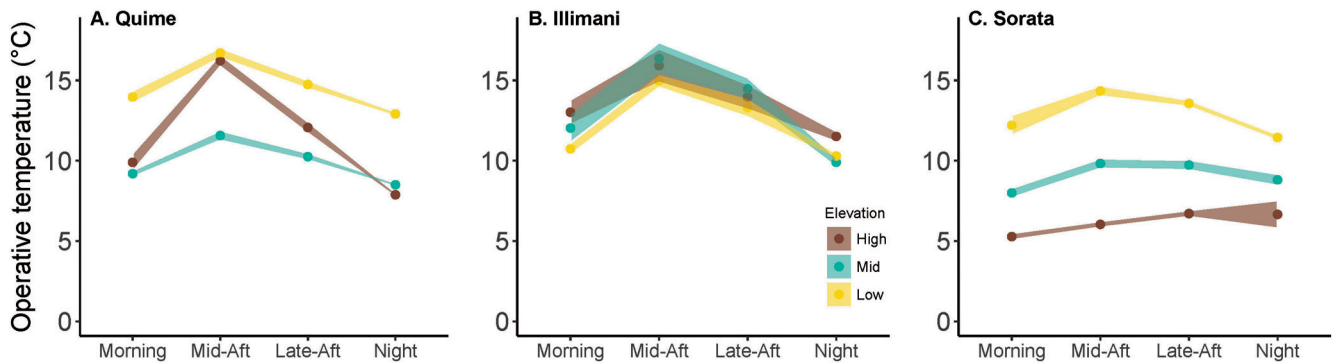
### Data analysis

Because daily temperature is known to condition ectotherm activity patterns (Angilleta et al., 2002), we were interested in assessing if the temperature frogs confront ( $T_e$ ) at each valley was affected by the time of day and elevation. We divided the climate data obtained from dataloggers categorically among four time slots: morning (6:30–12:00), mid-afternoon (12:15–16:00), late afternoon (16:15 to 19:00), and night (19:15–6:00). We used two-way ANOVA to test for an effect of the two categorical variables time of day (morning, mid-afternoon, late-afternoon, and night) and elevation (low, medium,

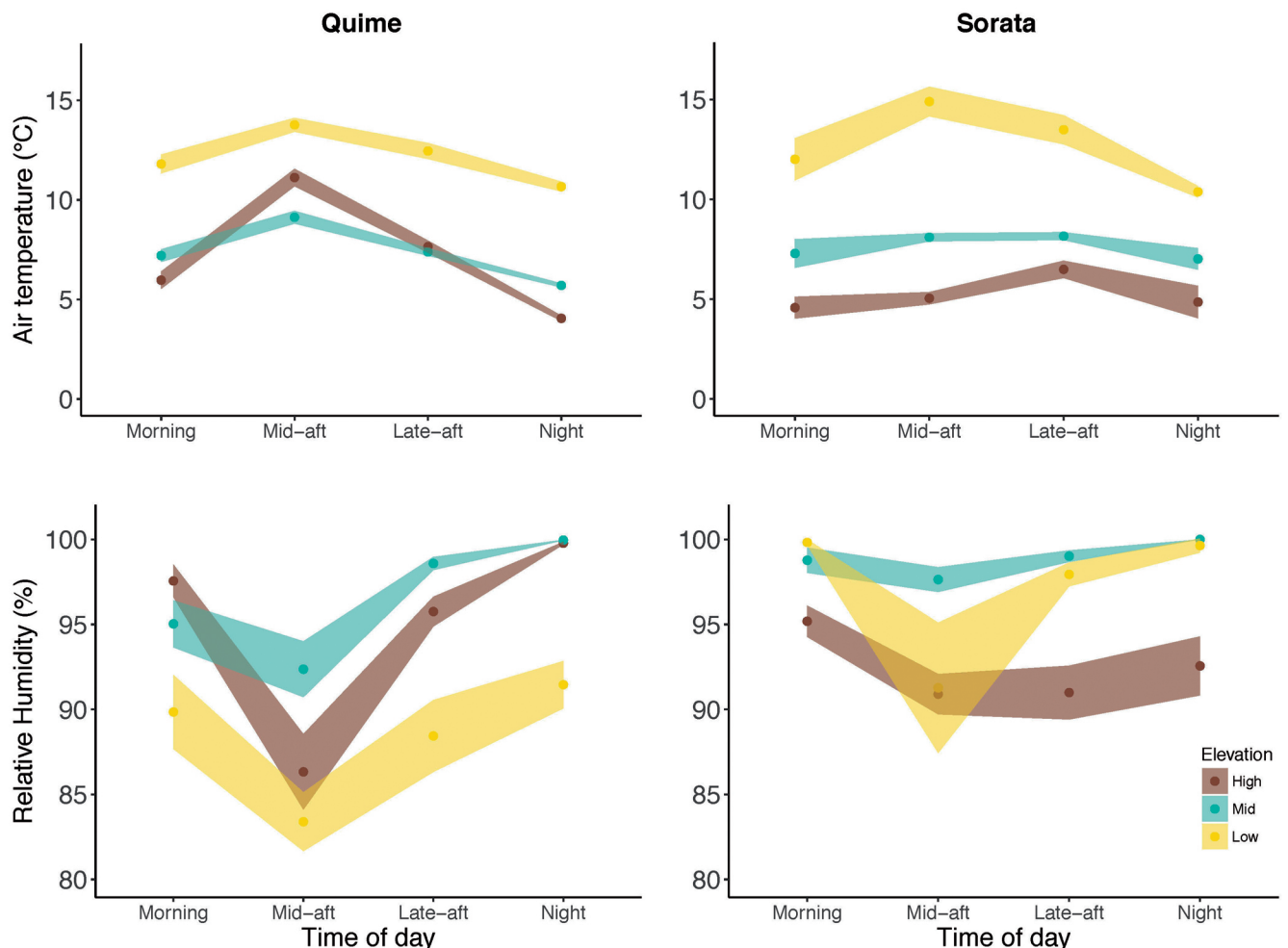


and high; see Table 1) on operative temperatures at each valley, as well as a potential interaction between these two variables. We did not use a linear model because, although time of day covaries with daily temperature, it does not vary linearly, and thus would invalidate one of

the primary assumptions of this type of model. We also ran one-way ANOVAs to determine if the operative temperatures at late afternoon, when *Microkayla* frogs usually begin calling activity (De la Riva, 2007; De la Riva and Burrowes, 2014), were associated with elevation at each



**Figure 5.** Operative temperatures throughout the day taken with dataloggers attached to frog models and placed in typical *Microkayla* retreat sites in the field at three elevations in (A) Quime, (B) Illimani, and (C) Sorata. Elevations and geographic coordinates (GIS) for each site are provided in Table 1. For each graph, the dots represent the mean temperature for that time period within a day, and the breadth of the colored bands is the 95% confidence interval for the means. Elevation ranges for each site are given in Table 1.



**Figure 6.** Air temperatures and ambient relative humidity throughout the day taken with dataloggers placed in vegetation above *Microkayla* retreat sites at three elevations in Quime and Sorata. Elevations, and geographic coordinates (GIS) for each site are provided in Table 1. For each graph the point represents the mean for that time period, and the breadth of the colored bands is the 95% confidence interval for the means.



valley. We compared ambient air ( $T_{\text{air}}$ ) versus operative ( $T_e$  at microhabitats) temperatures at Quime and Sorata graphically according to time of day (Figs. 5–6) and tested for mean differences at each elevation sampling site using T-tests (Table 1). Statistical analyses were performed with Minitab Release 1.5.1 (Minitab Inc., 2017) and R statistical package (R Core Team, 2018).

## RESULTS

### Temperature and humidity on different Andean slopes

Operative temperatures varied significantly with elevation and with time of day at every valley, and there was a significant interaction indicating that the elevation effect at each valley varied differently depending on the time of day (Table 3). The two-way ANOVA model explained more than 70% of the variation in operative temperatures in Quime and Sorata but only 32% in Illimani (Table 3). In fact, the effect of elevation and time of day on operative temperatures showed a different pattern at each valley that was mostly non-monotonic (Fig. 5). In Quime, if frogs were to occur at both higher and lower elevations they would experience significantly warmer temperatures than at mid-elevation at any time of day (Fig. 5A). At Illimani, the temperatures that frogs confront from morning to late afternoon in the mid-elevation belt overlap considerably with those they would experience at higher elevations (Fig. 5B). And finally, in Sorata, frogs at mid-elevations (where they occur) experience a thermal map that seems intermediate with respect to cooler or lower

temperatures available at correspondingly higher and lower elevations. (Fig. 5C).

We specifically assessed the effect of elevation on operative temperatures during late afternoon, because this is the time when *Microkayla* frogs usually begin calling (De la Riva, 2007). Results revealed that elevation had a significant effect on operative temperatures at mid-afternoon in Quime ( $F_{2, 263} = 314.64$ ,  $P < 0.0001$ ) and Sorata ( $F_{2, 119} = 526.93$ ,  $P < 0.0001$ ) slopes, but not in Illimani ( $F_{2, 161} = 1.91$ ,  $P = 0.152$ ), providing further evidence for less heterogeneity by elevation at this site, especially between high- and mid-elevations (Fig. 5).

In Quime and Sorata, for which air temperatures were available, we found that mean air temperatures ( $T_{\text{air}}$ ) are generally cooler than those at microhabitats ( $T_e$ ) at each elevation site, but this difference was not significant at low elevations in Sorata (Figs. 5–6, Table 1). Ambient relative humidity was higher during the later hours of the day, when temperature drops, and was higher and less variable (lower SD) in the mid-elevation belt at all times at both sites (Fig. 6, Table 1).

### Thermal physiology of *Microkayla cf. quimsacrucis*

Results from our temperature preference experiment revealed that, given a range of 6.5–40°C, *Microkayla cf. quimsacrucis* chose to position itself on average at 19.46°C, a temperature at which individuals performed slightly above 80% of their optimum level (Fig. 4). However, there is evident variation in temperature choice, with individual confidence intervals of 1.2–6.4°C (Table 2). This result indicates loose thermal choice in the gradient

**Table 3.** Source table and descriptive statistics from a two-way ANOVA to assess the effects of two categorical values, Elevation and Time of Day, on operative temperatures at the three valleys studied.

Source	DF	Sum of Squares	Mean Sum of Squares	R <sup>2</sup>	F value	P value
<b>Quime</b>						
Elevation	3	9820.4	3273.47	0.29	1267.94	< 0.05
Time of Day	2	12200.1	6100.04	0.37	2362.77	< 0.05
Elevation*Time of day	6	1711.4	285.23	0.05	110.48	< 0.0001
Error	3326	8586.8	2.58			
Total	3337	33299		0.74		
<b>Illimani</b>						
Elevation	3	4794.8	1598.28	0.27	174.3	< 0.0001
Time of Day	2	239.9	119.95	0.01	13.08	< 0.0001
Elevation*Time of day	6	266.1	44.34	0.68	4.84	< 0.0001
Error	1316	12067.6	9.17			
Total	1327	17679.1		0.32		
<b>Sorata</b>						
Elevation	3	208.34	69.45	0.03	25.38	< 0.0001
Time of Day	2	3683.45	1841.73	0.60	673.07	< 0.0001
Elevation*Time of day	6	183.45	30.57	0.03	11.17	< 0.0001
Error	649	1775.86	2.74			
Total	660	6124.49		0.71		

and perhaps avoidances, rather than well-defined thermal preferences. The full extent of temperatures explored by the species was large, stretching from 8.2–33.2°C, with 80% of the data ranging from 15–25°C (Fig. 4). The frogs achieved their critical thermal maxima on average at 35.28°C (95% CI = 1.60°C).

## DISCUSSION

Results revealed that Andean slopes offer distinct microclimates over an elevation gradient. Orientation and topography of the valleys strongly condition temperature and humidity, which in turn most likely determine the distribution of frogs. Similar microclimatic effects of topographic depressions have been observed to be critical for explaining the distribution of other cold-adapted ectotherms in the eastern Andes of Bolivia (Jiménez-Robles and De la Riva, 2019). The mechanistic factors involved in the origin and maintenance of those microclimate refugia are beyond the scope of this paper, but we have three interesting cases: Quime shows the unexpected non-monotonic outcome of higher temperatures above mid elevations (Fig. 5A). This may be an effect of the shape of the valley and the fact that our mid-elevation sampling site in Quime lies at the end of a cul-de-sac in its upper part, that may favor episodic thermal inversions as cold air gets trapped at mid elevations by subsiding warmer air. Lower temperatures at mid compared to higher elevations have also been reported for some Andean slopes in Colombia, suggesting that micro-endemic frogs at a given site may be more cold-adapted than those at higher elevations within the same slope (Navas et al., 2013). In Illimani we found the most remarkable results. Surely, the orientation and the extreme narrowness of this valley, with a steep end forming a circus, influences the thermal homogeneity at microhabitats observed throughout the elevation range sampled (Figs. 2C, 5B). Coincidentally, although the distribution of *M. illimani* falls within the mid-elevation belt, this valley also harbors the undescribed (and unexpected) species of *Microkayla* mentioned above, which holds the absolute record of elevation, ca. 4,700 m. No other species of *Microkayla*, nor other similar high-Andean Craugastoridae from Bolivia and Peru (i.e. *Bryophryne* Hedges et al., 2008, *Phrynopus* Peters, 1873, *Psychrophrynella* Hedges et al., 2008, and *Qosqoprhyne* Catenazzi et al., 2020), are known to reach such elevations, being the highest records so far 4,100 m in *B. abramalagae* Lehr and Catenazzi, 2010; 4,390 m in *Ph. pesantesi* Lehr et al., 2005; 3,613 m in *Ps. usurpator* De la Riva et al., 2008 (von May et al., 2017); 3,850 m in *Q. flammiventris* (Lehr and Catenazzi 2010); and 4,192 m in *M. iatamasi* (Aguayo-Vedia and Harvey, 2001) (De la Riva, 2020). At the wider Sorata valley, daily temperatures follow the expected pattern in which higher elevations are cooler, and the highest humidity is found at

intermediate elevations (Figs. 5C and 6) where *M. illampu* occurs (Fig. 2D).

Our data on the thermal biology of *Microkayla* were informative in spite of our small sample size. We found that these small frogs can tolerate a very wide temperature range (from < 8.2°C to a mean  $CT_{max}$  of 35.28°C) and performed best at the warmer end of this range at approximately 24°C (Fig. 4). These results reveal that optimal performance temperatures for *Microkayla* cf. *quimsacruensis* are 8–13°C above the operative temperatures they experience at the mid-elevation belt at Quime, where they occur (Fig. 5A), and suggest that, in the wild, frogs function at the 45–60% level of their performance capacity (Fig. 3). These findings might explain the slow-moving nature of these frogs (De la Riva et al., 2017) and point to other climatic factors, such as moisture, as key drivers of the time of activity (nocturnal) and distributional range of these species.

In addition, our results raise the question of whether small, terrestrial, high Andean frogs are truly “cold-adapted” or are constantly thermoregulating behaviorally or by some other means to meet their physiological needs (Navas, 1996a). Temperature preference trials were inconclusive because frogs explored a wide range of temperatures with considerable individual variation on choice (Table 2). Anurans in gradients can choose temperatures that seem particularly warmer relative to field conditions, as shown for *Dendropsophus molitor* (Schmidt, 1857) (Angilletta et al., 2002), or do not reach preferred temperatures when it would seem possible that they could if they modified their activity patterns (Noronha-De Souza et al., 2015). Given the broad range of possible responses, we interpret the  $T_{pref}$  values recorded for *Microkayla*, even if few, as suggesting that these frogs do not have well-defined thermophilic behavior and that temperature does not drive their preference in a thermal gradient. In contrast, the critical thermal maxima ( $\bar{x}$  = 35.2°C), was consistent for all individuals and uncommonly high compared to other grassland, high-Andean craugastorid anurans at similar elevations in the Peruvian Andes, which have an average  $CT_{max}$  of 28.1°C (Catenazzi et al., 2014; von May et al., 2017).

To compensate for being active during cold nocturnal temperatures in the high Andes of Colombia, Navas (1996a) observed that frogs in the genus *Pristimantis* thermoregulate by moving to the edge of their rocky retreat sites during sunny days. Although it is plausible that *Microkayla* utilizing rock piles as diurnal refugia might thermoregulate by moving vertically towards (and away from) the warmer surface, this is unlikely because our laboratory trials revealed that thermophilia does not characterize their behavior within a temperature gradient. Activity below optimal performance temperatures has also been reported for the large-bodied, nocturnal toad *Rhinella diptycha* (Cope, 1862) (Noronha-De Souza et al., 2015), suggesting that the compromise between thermo-

regulation and nocturnal activity might be phylogenetically widespread in anurans, due to similar physiological constraints that favor activity at a time when the risk of dehydration is lower.

As found for other small, nocturnal, high Andean frogs in Peru (Catenazzi et al., 2014; von May et al., 2017) and Colombia (Navas et al., 1996a,b; 1997), *Microkayla* cf. *quimsacruensis* in Bolivia has a broad thermal physiological tolerance. Furthermore, the warming tolerance ( $CT_{max}$  – average  $T_c$ ) for this species was 25°C, approximately 10°C higher than that found for upland craugastorid frogs during the same time of the year in Peru (Catenazzi et al., 2014). Thus, we may ask why these frogs exhibit such narrow elevational distribution ranges, and the answer may lie in their hydric requirements. Both in Quime and in Sorata, *Microkayla* species occur only at localities within the mid-elevation belts of the mountain slopes, which, coincidentally, harbor the highest ambient relative humidity (Fig. 6, Table S1). Frogs in this genus often begin calling around dusk (De la Riva, 2007; De la Riva and Burrowes, 2014) when temperatures are approximately 10°C lower than their optimum but relative humidity is significantly higher than at earlier times of the day (Table S1, Fig. 6). It is possible that *Microkayla* requires relative humidity levels above 95% to afford activity and prevent risk of dehydration (Fig. 6). Thus, it might be humidity (and not directly temperature) that traps these cold-adapted frogs within specific elevational belts in high Andean valleys. Unfortunately, we do not have ambient relative humidity readings from Illimani to compare across the more homogeneous temperature landscape that expands the broad elevational distribution of *Microkayla* in this valley (Fig. 2C, 5B).

An interesting consequence of these results, already outlined by other authors (Catenazzi et al., 2014), is that global warming per se will not pose a threat for high Andean anurans, unless this warming has a direct effect on ambient relative humidity. However, this is very likely because as temperature rises the saturation water vapor pressure increases, resulting in low relative humidity. Amphibians exposed to high temperatures risk an increase in cutaneous evapotranspiration that is lethal if not balanced by water intake (Tracy, 1976). The fact that these cold-adapted, high-mountain frogs are terrestrial direct-developers makes them highly dependent on ambient relative humidity because they do not move to water bodies to reproduce or rehydrate and need high humidity to prevent desiccation of their egg clutches. Furthermore, their small body size results in a high surface-volume relation that is unfavorable for water conservation. Hence, for these high Andean terrestrial frogs with low vagility, dew and precipitation are their only means of recuperating water loss.

A consequence of a decrease in humidity in high Andean slopes is the change in substrate vegetation from bunch grasses and mosses that provide humid shelters,

to barren soil or rock beds unlikely to support anurans (Köhler, 2000; Forero-Medina et al., 2010). As such, elevation belts that maintain high relative humidity represent traps, as well as windows of survival opportunity, for frogs with this particular ecomorph in the high Andes. If the global warming trend and consequent desertification reported for the high Andes continue (Urrutia and Vuille, 2009; Vergara, 2009), and droughts like the one experienced in the Bolivian Andes during the expected “rainy season” of 2016–2017 become recurrent, we expect a range contraction for the already micro-endemic *Microkayla* species and/or a reduction in the periods in which relative humidity is suitable for their activity, which might result in the extinction of many known and undescribed species of small craugastorid frogs. Considering that other stressors like the pathogenic chytrid fungus are already affecting many highland amphibians in Bolivia, including *Microkayla* (Burrowes and De la Riva, 2017; De la Riva et al., 2017), prioritizing high-Andean habitats for conservation actions is recommended.

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## ONLINE SUPPORTING INFORMATION

The following Supporting Information is available for this article online:

**Table S1.** Comparison of mean relative ambient humidity at the different elevational belts sampled for two of the three localities studied in the Bolivian Andes.