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Upward range extension of Andean anurans and chytridiomycosis to extreme elevations in response to tropical deglaciation

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Abstract

High-alpine life forms and ecosystems exist at the limits of habitable environments, and thus, are especially sensitive to environmental change. Here we report a recent increase in the elevational limit of anurans following glacial retreat in the tropical Peruvian Andes. Three species have colonized ponds in recently deglaciated terrain at new record elevations for amphibians worldwide (5244–5400 m). Two of these species were also found to be infected with *Batrachochytrium dendrobatidis* (*Bd*), an emerging fungal pathogen causally associated with global amphibian declines, including the disappearance of several Latin American species. The presence of this pathogen was associated with elevated mortality rates of at least one species. These results represent the first evidence of upward expansion of anurans to newly available habitat brought about by recent deglaciation. Furthermore, the large increase in the upper limit of known *Bd* infections, previously reported as 4112 m in Ecuador, to 5348 m in this study, also expands the spatial domain of potential *Bd* pathogenicity to encompass virtually all high elevation anuran habitats in the tropical Andes.

Keywords: alpine biodiversity, amphibians, amphibian decline, chytridiomycosis, climate change, deglaciation, ecological succession, *Pleurodema*, *Telmatobius*, tropical andes

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Introduction

Global environmental changes such as climatic warming and emerging infectious diseases are impacting animal biodiversity in the Neotropics, and are having particularly adverse consequences for amphibian populations. Climate warming has been shown to influence species distribution and abundance, and cause upward migration of species in tropical mountains (Parmesan, 1996; Pounds *et al*., 1999). Investigators in Costa Rica have demonstrated upward migration of a range of bird populations in response to an elevational increase in the adiabatically produced cloud base (and resulting cloud forest fauna), a phenomenon that is also associated with declines in anuran and lizard populations (Pounds *et al*., 1999). Infectious diseases have increasingly been reported to be associated with population declines of amphibian species (Daszak *et al*., 2000), and climate-induced ecological changes can alter host–parasite dynamics so that infectious diseases exert a greater impact on certain species (Pounds *et al*., 1999). Chytridiomycosis, an emerging fungal disease of

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amphibians caused by *Batrachochytrium dendrobatidis* (*Bd*), is causally linked to a loss of amphibian biodiversity in tropical montane amphibian species (Lips *et al.*, 2006). Recently, Pounds *et al.* (2006) introduced the chytrid-thermal-optimum hypothesis and proposed that large-scale warming is likely a key factor in driving epidemic disease and the disappearance of anuran species in Central and South America. Their data show that extinctions in the Neotropics occur where climatic conditions may be more favorable for disease development, and that elevations below 1000 m, and above 3500 m, would likely fall outside of the optimum thermal growth conditions to support disease outbreaks.

As most amphibian surveys have been conducted at elevations below 4000 m, relatively little is known about survival of amphibians above this elevation, the impact that climatic warming is having upon these populations, or whether chytridiomycosis may also be affecting these communities. Climatic warming is also implicated in ongoing tropical deglaciation (i.e. the reduction in high-altitude glacial ice volume over recent decades in low latitude mountains), the impact of which has yet to be ascertained for anurans inhabiting high alpine zones.

The principal goal of this study was to investigate if anurans in high Andean environments are being impacted by recent environmental changes. Our field study site is in the Cordillera Vilcanota, a heavily glacierized range in southern Peru within a region of the tropical Andes where significant changes in temperature and environmental responses have been recorded in recent decades. Documented changes include significant tropospheric warming, averaging 0.33 °C per decade (Vuille *et al.*, 2003), a corresponding rise in freezing level (Diaz & Graham, 1996), and a rapid melting of glaciers since the Little Ice Age (LIA: ca. 1520–1880; Thompson, 1992; Francou *et al.*, 2003). We conducted eight multidisciplinary field investigations in the Cordillera Vilcanota between 2000 and 2005, including surveys of anuran populations at six sites from 2003–2005, to study rapid ecological successions by high-Andean flora and fauna as ice retreats. Our study area offers an unusual opportunity to evaluate ecological observations in contexts of 75 years of landscape change. The upper reaches of the watershed are heavily glacierized, but over recent decades have experienced marked retreat of its ice margins concurrent with region wide climatic warming. An air photo series from 1931 and subsequent aerial and satellite imagery from 1962, 1980, and 2005 provide a temporally resolved portrayal of ice margins during this period of glacial retreat, and when combined with field surveys, allows for the monitoring of species migration into newly available habitat exposed by deglaciation.

Materials and methods

Geographic referencing

Topographic map quads 28t-I-SE and 28t-II-NE [1 : 25 000 scale, 25 m elevation contour interval; Instituto Geográfico Militar (IGM), Lima, 1972] served as the reference base for this study. All GPS field measurements were made with a Garmin XL12 set to the Provisional South America 1956 datum for consistency with the topographic map series. Elevation observations specified in the text are from GPS and are consistent, though slightly more elevated, than interpolated values indicated on the topographic maps (mean difference +24.7 m, standard deviation 7.95 m, $N = 18$ points); only points from within areas that were unglaciated in 1962, when data for topographic mapping was originally obtained, were compared to eliminate elevation differences related to changes in ice thickness.

Ice recession analysis

The temporal evolution of glacial recession was examined by pairing ground-based observations with aerial photography (available since 1931) and satellite imagery (since 1966). Only images exhibiting clearly defined glacial margins (unobscured by recent snowfall) were considered for analysis. Due to the variety of image types, view angles and resolutions, the isoline placements may have positional errors of up to 50 m. The exception is the 1962 isoline, which was precision-mapped by the IGM, and therefore, considered an accurate representation of glacial extent.

LIA. The position of the LIA ice extent maximum was inferred by the visual discontinuity in substrate tone related to the abrupt change in abundance of lichens across this former glacial trimline. This ecotone is clearly evident in all imagery in the vicinity of site D, where its position was locally confirmed with multiple GPS ground measurements.

1931. The 1931 isoline was determined by analysis of oblique aerial photographs taken during an overflight of the Cordillera Vilcanota by the Shippee–Johnson expedition on approximately 1 June 1931 (date inferred from Shippee, 1933). Examination of the series identified 11 large-format ($17 \times 23\,\mathrm{cm}^2$) glass plate negatives (nos. O86–O93 and O101–O103) displaying the pass region containing Sites D–F. These negatives were scanned by personnel at the American Museum of Natural History (AMNH) and then photogrammetrically analyzed to determine aircraft position and altitude for each image. Ice margin positions were then established through photogrammetric referencing of image content to the topographic base map.

1962. The 1962 glacial margin isoline is reproduced from the 1 : 25 000 topographic maps. We confirmed the absence of seasonal snow by examining vertical image stereopairs used by the IGM to generate the maps.

1980. A declassified CORONA satellite black and white positive transparency (R0744 232 009) taken on August 3, 1980 was used to derive the 1980 isoline. The Sibinacocha watershed is located in the far upper left corner of the scene, and thus contains some geometric distortion of landscape features owing to the obliquity of the view angle. The image was cropped, scanned into Photoshop® and rescaled to the base map projection. The brightness gradient of the ice margin was then used as a first-guess for the isoline location. Manual corrections were then applied where geometric distortion required local realignment of image content with geo-referenced landscape features.

2005. Numerous aerial images, including repeated scenes from the 1931 Shippee–Johnson series, were taken during an overflight of the Cordillera Vilcanota on March 15, 2005. This image series was utilized to locate the 2005 ice margin position; ground-truth GPS measurements made during fieldwork on March 20, 2005 were used to refine ice margin positions near sampling sites in the pass region.

Field surveys

Multiday field surveys of amphibian populations in the Cordillera Vilcanota were conducted during two dry seasons (August 6–17, 2003; July 13–25, 2004) and two wet seasons (March 3–7, 2003; March 14–21, 2005). Visual encounter surveys by two to four researchers identified habitats supporting anuran populations. Researchers surveyed each site for 1–5 h, overturning rocks and sifting ponds with nets to locate hidden specimens, and collecting environmental data [water temperature, pH, vegetation types (aquatic and terrestrial), GPS coordinates and elevation, and photographs]. Soil temperatures were measured by Hobo data loggers (Onset Computer Corporation, Bourne, MA, USA). Water temperature was measured using a digital thermometer (Oregon Scientific, Tualatin, OR, USA). As a proxy for population density we recorded the number of specimens observed and express these as the number of individuals/the number of surveyors × the number of hours of survey (survey hours). Captured specimens were photographed and measured for snout-vent length (SVL), then released. Voucher specimens were collected in July 2002, August 2003, and March 2005 for species identification and *Bd* analysis. All voucher specimens are housed at the Museo de Historia Natural de la Universidad Nacional San Antonio Abad de Cusco.

Botanical diversity was assessed to characterize habitat occupied by amphibians and other fauna. Plant species and richness are based on observations during two expeditions, July 2002 and August 2003, in which plants, reptiles, amphibians, and some invertebrates were collected. The main sampling area is 300 m from site D at a monitoring site for the Global Research Initiative in Alpine Areas (GLORIA), but also includes general area sampling outside the LIA boundary. Although these months are mid-winter (dry season) there was considerable snow cover most of the time in 2002 and over parts of the area (particularly south slopes) in 2003. Many plants are dormant at this time of the year but some were flowering. Five days in 2002 and 8 days in 2003 were spent in the Sibinacocha watershed above 4880 m. In 2004, two days were spent sampling the GLORIA site at 5250 m, while 3 days were spent there in 2003 (GLORIA methodology is described in Pauli *et al*., 2004). Further sampling in 2005 has not been analyzed for this account. The detailed description of this GLORIA site will be made available on the GLORIA website (http://www.gloria.ac.at/res/gloria_home/) once it is completed.

Plants were identified to the highest level possible in the field. Vouchers collected were deposited at the Herbario Vargas of the Universidad Nacional San Antonio Abad del Cusco.

Tissue preservation, histology, and polymerase chain reaction (PCR) analysis

Live frogs were collected from the above referenced sites A–D, quickly euthanized and preserved in a 70% ethanol solution. Larger dead frogs were subsequently injected with 5 cm^3 of 70% ethanol. Ventral hind limb skin was excised, stored in 70% ethanol, and transferred to neutral-buffered 10% formalin before embedding in paraffin. Tissues were then sectioned at 6 μm thickness for histological staining with hematoxylin and eosin (H and E), and imaged using an Olympus IX-70 inverted light microscope (New Jersey Scientific, Middlebush, NJ, USA) equipped with an Olympus LCPlanF1 ×20 objective, Imaging software (Roper Scientific, Tucson, AZ, USA), and a Cool Snap CCD camera (RS Photometrics, Tucson, AZ, USA). Ten fields of view (×20) were examined on two slides from different planes of section for each individual. Presence of a single *Bd* sporangium denoted a positive individual.

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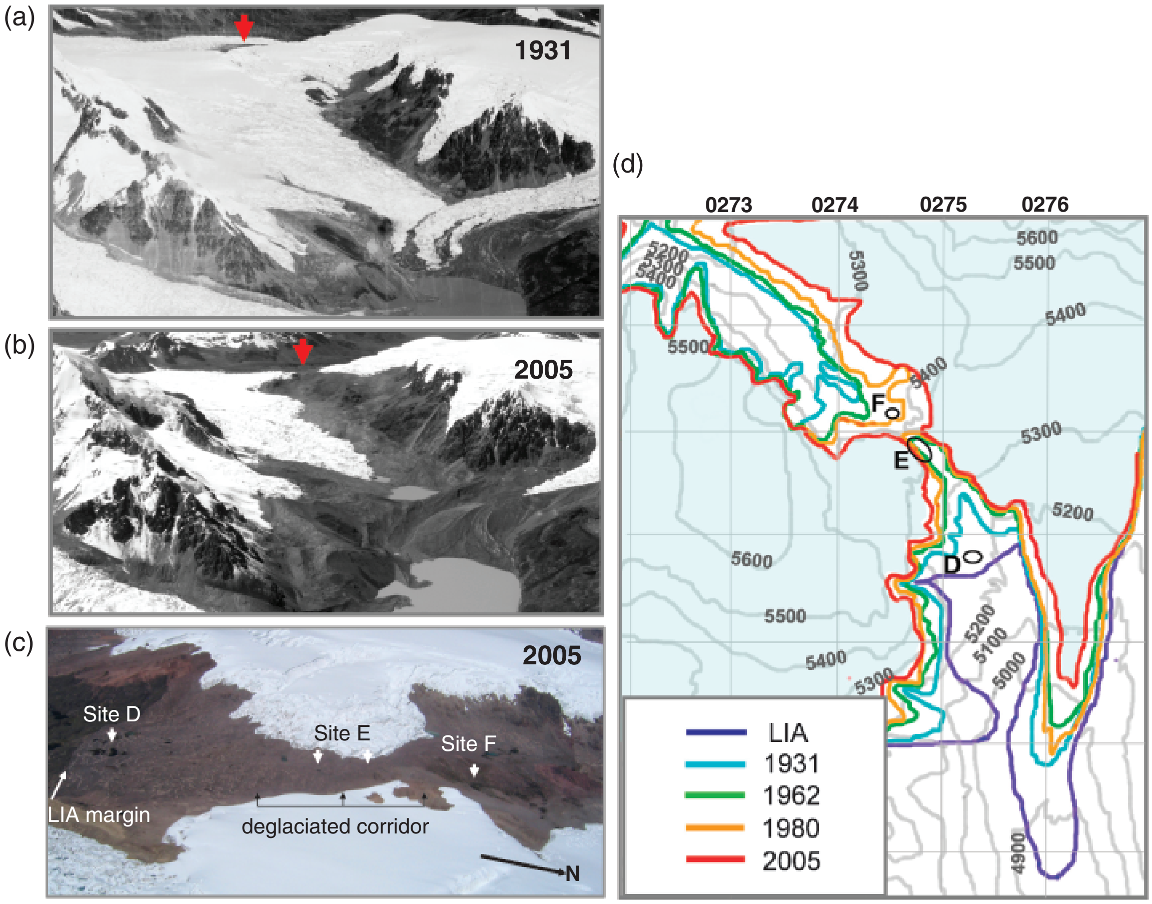

Fig. 1 Glacial recession since the Little Ice Age. (a) Aerial perspective from the north at 6600 m from June 1931 showing a 5400 m pass at upper-center of image completely icebound (red arrow). (b) Repeat image of the same scene on March 15, 2005, revealing significant glacial recession and development of an ice-free corridor (red arrow) across the pass. (c) Aerial perspective on March 15, 2005 looking SW from 6600 m across the recently deglaciated corridor atop the pass. Annotations identify LIA margin and anuran sampling sites within deglaciated terrain; arrow on lower right points N. (d) Topographic map section (100 m contour interval) of the pass region showing glacial margin positions at the ca. 1850–1900 LIA maximum (blue), 1931 (cyan), 1962 (green), 1980 (gold) and 2005 (red). Glacial coverage in 2005 is shaded light blue. 1-km UTM grid is overlaid for reference [Photo credit for (a): Negative No. O-103 Photo: Shippee–Johnson Collection. Department of Library Services, American Museum Natural History (AMNH)].

Infectivity was scored by averaging the highest observed number of cell layers of *Bd* per field, for 10 fields. All specimens in 2005 were analyzed for chytridiomycosis using dry skin swabs (MW100–100, Medical Wire and Equipment Co. (UK), Wiltshire, UK) of collected frogs in the field. Collected frogs were swabbed on the dorsal and ventral surface 20 times, and five times between the webbing of the limbs, and then released. The swab was then placed in a sterile plastic vial and stored in a backpack for the duration of the trip. Swabs were then stored at −20 °C until analysis. The swabs were analyzed in triplicate by Taqman real-time PCR assay (Boyle *et al*., 2004), CSIRO Livestock Industries, Australian Animal Health Laboratory, Australia. All specimens were collected under the authority of Universidad Nacional San Antonio Abad de Cusco.

Results

Landscape and biotic changes are both strongly evident in the watershed of Laguna Sibinacocha (13.85°S, 71.05°W; 4900 m m.s.l., 33 km^2), the largest high-alpine lake in the Andes, and a principal source to the Urubamba tributary of the Amazon River. A 5400 m pass 5 km NNW of Lake Sibinacocha was thickly icebound when first photographed in 1931, but by 2005, deglaciation had exposed much of the rock substrate leading to the pass summit (Fig. 1a and b). The glacier change analysis reveals an ice-free corridor across the pass became established around 1980, and by 2005 had expanded to 238 m wide (measured in the field by Global Positioning System, GPS), an average rate of 9.52 $m\,yr^{-1}$ (Fig. 1c and d). Thus, an ecological corridor exists today between watershed habitats previously

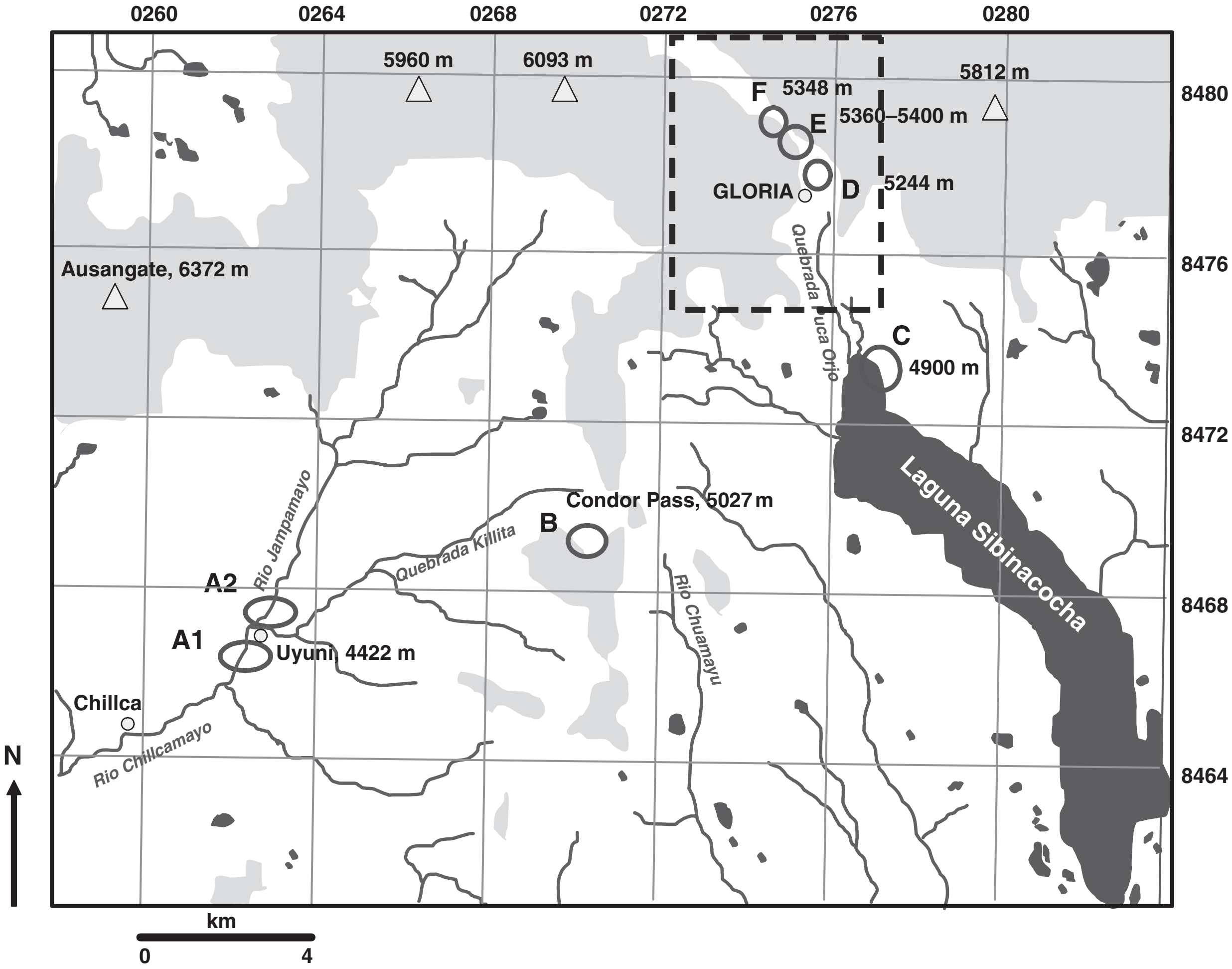

Fig. 2 Location of anuran sampling sites A–F in the Cordillera Vilcanota of southern Peru. Glaciers are shown in light grey with mountain summits marked by triangles. Sites A–F are circled. The Global Research Initiative in Alpine Areas (GLORIA) site and villages of Uyuni and Chillca are shown. The dashed box indicates the domain of Fig. 1d.

segregated by an ice barrier for centuries, perhaps millennia.

We studied anuran populations at six locations (sites A–F) above 4400 m within and close to the Sibinacocha watershed, including three (sites D–F) within the recently deglaciated terrain above 5244 m north of Lake Sibinacocha (Fig. 1c and d, and Fig. 2). Three species of three genera were identified: *Pleurodema marmorata*, *Bufo spinulosus*, and *Telmatobius marmoratus*. Site D, a cluster of tarns at 5244 m within the LIA moraine harbored all three species, but in adjacent water bodies there was a considerable variation in relative species abundance. Each species had 100% representation in one of the five ponds surveyed, while the other two ponds showed cohabitation by *P. marmorata* and *T. marmoratus* (Fig. 3a and b). The LIA moraine abruptly delimits sparsely vegetated terrain on its uphill side from more biodiverse alpine habitat just meters away. The area containing the ponds exhibits scattered lichen cover on rocks ($<5\%$) and patchy vascular plant growth ($\sim$12 species). Outside the LIA boundary, over 39 species of lichens and bryophytes cover 75–90% of available rock surfaces, and over 54 species of vascular plants have been recorded (see ‘Materials and methods’) (Halloy *et al*., 2005). The ponds at site D are evident below the retreating ice margin in 1931 aerial photographs, so presumably formed relatively soon after the onset of ice recession, estimated between 1850 and 1900 (Fig. 3c). The ponds are perennial, being sustained by snowfall and glacial melt throughout the year, and appear to exist today in a near-pristine natural state. Ponds and their margins have been colonized by *Distichia muscoides* (cushion bogs) and aquatic *Myriophyllum* and *Potamogeton*.

Higher still than site D are much more recently developed ponds at sites E and F (Fig. 4a). The ice recession analysis shown in Fig. 1d indicate that deglaciation of sites E1–5 (5369–5376 m) occurred between 1931 and 1962, and sites E6 and E7 (5381–5400 m) within

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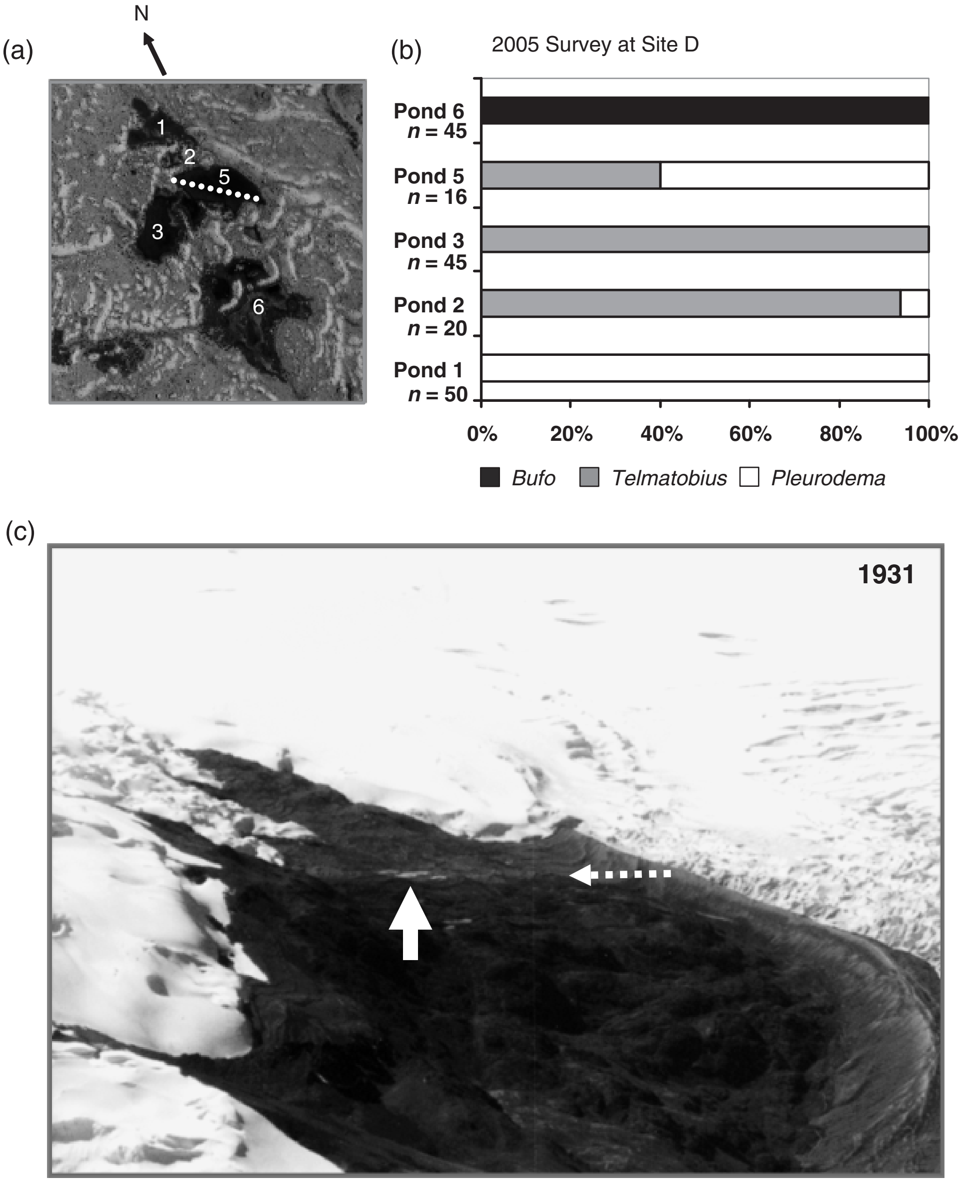


Fig. 3 Three species of anurans inhabiting permanent ponds in deglaciated terrain (5244 m). (a) Aerial view on 15 March 2005 of high ponds at site D identified by number. Dotted line represents 42.2 m. (b) Relative abundances of tadpole species within individual ponds at site D during the wet season of 2005. Percentages are derived from the number of tadpoles observed/survey hour (n = individuals counted). (c) Cropped section of 1931 air photo showing ponds at site D (5244 m, solid arrow) below receding ice margin. Margin of the LIA maximum is indicated (dotted arrow); view is looking N. [Negative No. O-86 Photo: Shippee–Johnson Collection. Department of Library Services, AMNH].

the last 10 years. Site F (5348 m) developed within the past 35–40 years (Fig. 4b and c). In March 2005, *Pleurodema* tadpoles and adults were found in both seasonal and permanent ponds surveyed within the pass corridor at sites E and F (Fig. 4d–f). Diurnal warming had melted nocturnal ice cover by the time of observation (1200–1300 local time). Tadpoles were observed in dense clusters of up to 950 ± 50 individuals, mostly in water of 1–2 cm depth, where temperatures ranged from 18.2 °C in E4, to 22.2 °C in E2 (Fig. 4e and f). These findings of recently established anuran populations are consistent with our surveys on the vigorous colonization by plants such as *Senecio graveolens*, *Calamagrostis* spp., *Ephedra rupestris*, and *Valeriana* cf. *nivalis*, recorded at elevations of 5400–5513 m within areas of recent glacial recession (data not shown).

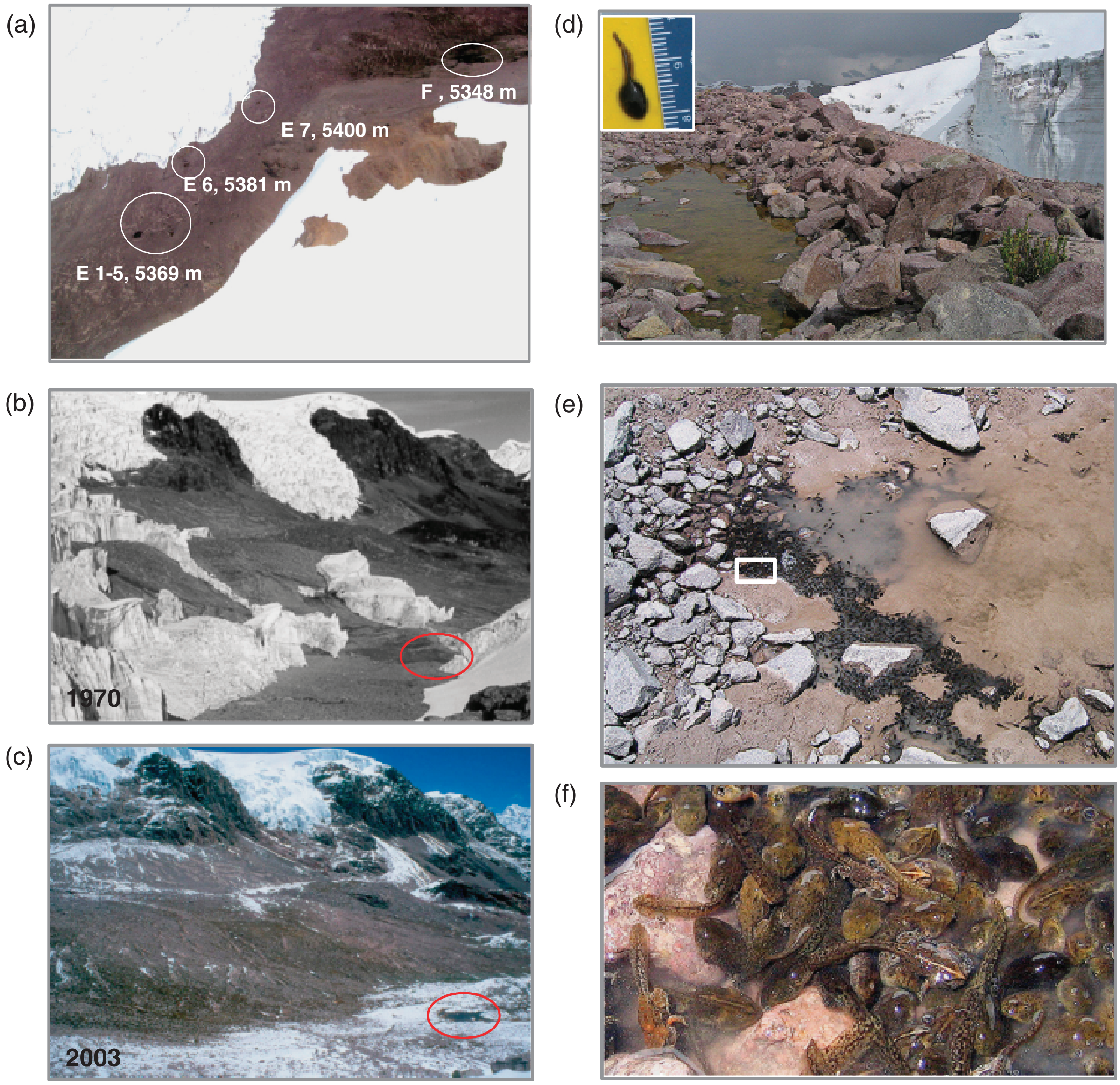
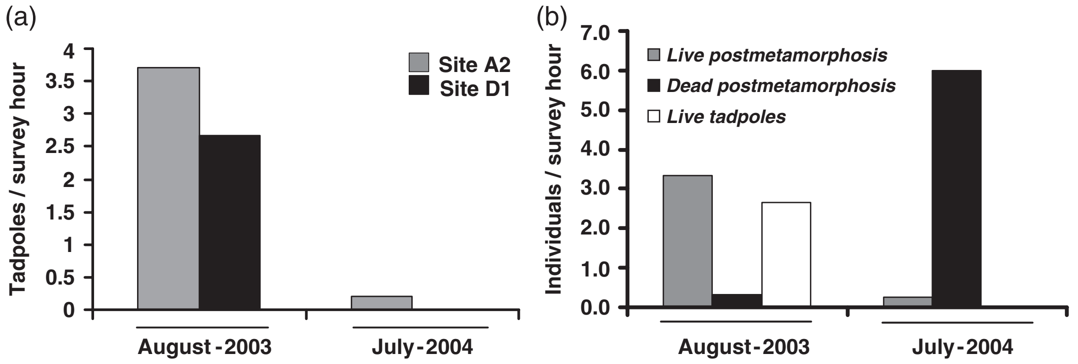


Fig. 4 Sites within recently deglaciated terrain above 5300 m with the highest anuran communities. (a) Near-vertical aerial perspective of high pass in March 2005. The locations of the seasonal ponds (sites E1–7) and perennial pond (site F) are circled. Minimum width of the ice-free corridor is 238 m. (b) Pond at site F (circled) in 1970 emerging from disintegrating ice field [photo by J. Ricker]. (c) Repeat of 1970 image from August 14, 2003 showing site F (circled), marked glacial recession, and upward advance of vegetation. (d) Seasonal pond (site E7) on the pass summit at 5400 m, the highest site where *Pleurodema* tadpoles were found (inset) Photos: March 20, 2005. Receding glacier in the background is 45 m from the pond; *Senecio graveolens* is also pictured (lower right). (e) *Pleurodema* tadpole cluster (black area, 950 ± 50 individuals) observed in a $\sim 1\,m^2$ area of site E1 (5369 m). (f) Enlargement of area indicated by small rectangle in (e) showing tadpoles clustering in 1–2 cm of water.

Repeated population surveys at Sites A2 and D1 revealed a significant reduction in *T. marmoratus* tadpoles from August 2003 to July 2004 at both sites (Fig. 5a). Adult *Telmatobius* were present at A2 in July 2002, but were not found on subsequent visits (not shown). At site D1, a 90% decrease in the number of live post-metamorphic *Telmatobius* was observed from August 2003 to July 2004 (Fig. 5b). No tadpoles were recorded in 2004 when 24 dead adult frogs were found within 4 survey hours ($6\,h^{-1}$), indicating a recent die-off, and consistent with the observed absence of breeding.

Reports from local highland inhabitants suggested that anuran populations had been disappearing rapidly. As *Bd* has been linked to the rapid decline of amphibian populations worldwide and catastrophic loss of amphibian biodiversity (Daszak *et al*., 1999, 2003; La Marca



(c)

	A1/A2	B	C	D	E	F
Telmatobius	2002: 3/4	–	2003: 1/2	2003: 1/11	–	–
Pleurodema	2003: 2/7	2003: 1/4	–	–	–	2005: 1/2

Year:# individuals positive / # individuals tested

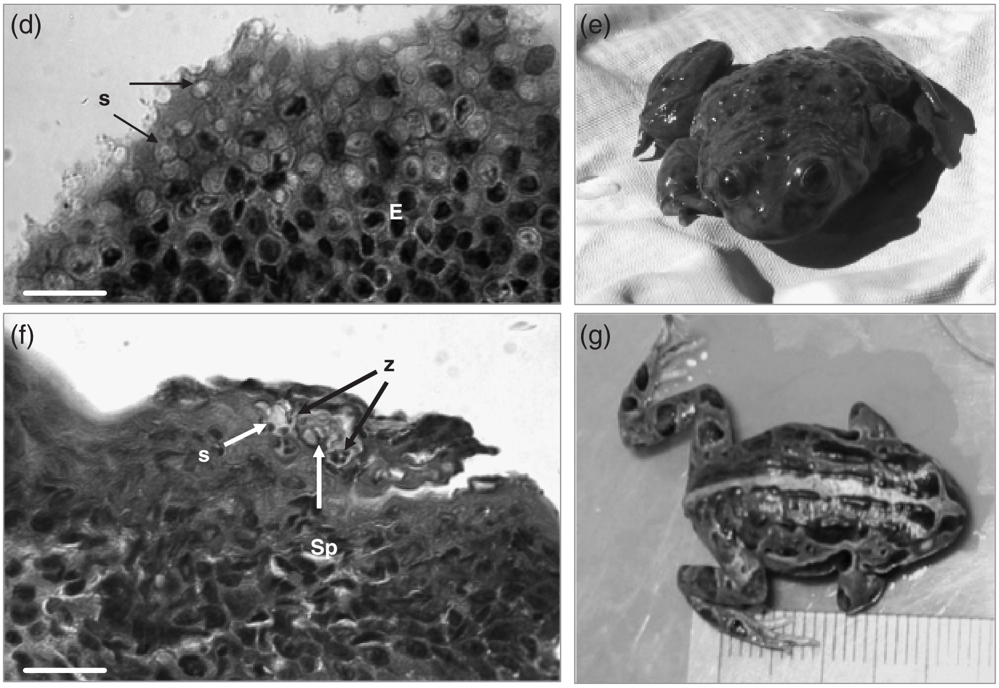


Fig. 5 Pathogenic *Batrachochytrium dendrobatidis* (*Bd*) afflicting high-Andean *Telmatobius* and *Pleurodema* communities. (a) Comparison of *Telmatobius* tadpole populations recorded during the dry seasons of 2003 and 2004 at sites A2 and D1. (b) Numbers of postmetamorphic live *Telmatobius*, post-metamorphic dead *Telmatobius*, and *Telmatobius* tadpoles are compared between dry seasons 2003 vs. 2004 at site D1. (c) Summary of the *Bd* results for sites A–F of *Pleurodema* and *Telmatobius*. Specimens that tested positive from sites A–F are scored for infectivity in Table 1. (d) Histological hematoxylin and eosin stained section from the ventral hindlimb skin of *Telmatobius marmoratus* (2003 site D1). The majority of the sporangia (s) within the stratum corneum are empty. Significant hyperkeratosis (three to five cell layers) is evident in the epithelial layer, E. Scale bar represents 25 μm. (e) Photograph of healthy adult frog of the species *T. marmoratus* collected at site D1 in 2003. (f) Histological hematoxylin and eosin stained section from the ventral hindlimb skin of *Pleurodema marmorata* (2003 site B). Lesion displays light hyperkeratosis (1–2 cell layers) and is focal. Zoospores (z) are evident in the majority of sporangia identifiable as small dark basophilic masses, some divided by a septum (sp). Scale bar represents 25 μm. (g) Adult *P. marmorata* that tested positive for *Bd* shown in (f).

Table 1 Results for *Batrachochytrium dendrobatidis* positive specimens collected at sites A–F

Year and location	Species	Infectivity	Signs of illness
2003 Site A1	*Pleurodema marmorata*	2.5	Healthy and active
2003 Site A1	*P. marmorata*	0.6	Healthy and active
2002 Site A2	*Telmatobius marmoratus*	2.4	Lethargic
2002 Site A2	*T. marmoratus*	Not determined	Dead
2002 Site A2	*T. marmoratus*	Not determined	Active
2003 Site B	*P. marmorata*	0.2	Healthy and active
2003 Site C	*T. marmoratus*	0.8	Irregular spotting
2003 Site D	*T. marmoratus*	1.3	Signs of Red-leg/blood in hindlimbs under skin
2005 Site F	*P. marmorata*	Not determined	Healthy and active

Increased severity of infection between individuals is indicated by the higher infectivity score (see 'Materials and methods'). Samples labelled 'not determined' could not be scored due to poor tissue preservation, insufficient material for microscopic examination, or samples were analyzed by polymerase chain reaction analysis.

et al., 2005; Lips *et al.*, 2005, 2006), we tested for the presence of this fungus at our six study sites. We analyzed skin samples from specimens collected in 2002 (Seimon *et al.*, 2005) and 2003 at sites A–D, and skin swabs from uncollected specimens at sites E and F in July 2005; results are summarized in Fig. 5c. Dead specimens found in July of 2004 were not tested for *Bd* as these specimens had been decomposing for at least several days, and perhaps weeks, before collection. Two species tested positive for *Bd*: *P. marmorata* and *T. marmoratus* (Fig. 5d–g). We did not find any evidence of *Bd* in *B. spinulosus* ($N = 2$). The fungus was found to be present at five sites, including sites D and F within recently deglaciated terrain. No anurans tested positive at the highest site, site E, in 2005. All *Pleurodema* specimens collected were energetic and did not exhibit signs of sloughing skin, a clinical sign of chytridiomycosis. In contrast, *Telmatobius* specimens collected exhibited characteristics consistent with clinical and pathological signs of fatal chytridiomycosis in captive and wild caught amphibians (Pessier *et al.*, 1999; Nichols *et al.*, 2001), including: lethargy upon handling; had more marked hyperplasia of the keratinaceous layer (Fig. 5d and f) and more intense infection (i.e. greater numbers of fungal sporangia scored for infectivity). A single specimen was found dead. These results are summarized in Table 1.

Discussion

Rapid deglaciation over recent decades associated with strong regional warming has exposed new terrain that is now suitable for anurans, whereas before the 20th century the ice advances of the LIA had depressed the elevational limit of potential habitat. Our findings on anuran distributions are consistent with a broader pattern of ecological succession in the Andean region and worldwide, where species' ranges have expanded vertically in response to warmer temperatures in high-alpine zones (Parmesan & Yohe, 2003; Halloy *et al.*, 2005). At lower elevations in Ecuador, recent observations of anuran communities have also identified new records in elevational range of *Eleutherodactylus* and *Hyla* species (Bustamante *et al.*, 2005). Our observations provide the first direct evidence that anurans are now colonizing terrain exposed by recent deglaciation at the upper limits of the biome. Furthermore, glacial recession in the Cordillera Vilcanota has opened a direct ecological corridor within the past 25 years between watershed habitats previously segregated by ice. The opening of this corridor may have long-term ecological consequences, such as distributional overlap of species, which may have genetically diverged, or facilitation of the spread of chytridiomycosis through host-species colonization, and is the subject of our future studies.

Our observations represent a new elevation record for amphibian species, exceeding the unsubstantiated account of 5200 m purported for *Scutiger alticola* in Tibet (Swan, 1990). In Latin America, records of up to 4800 m have been found recently in *Atelopus* species (La Marca *et al.*, 2005); up to 5000 m for *Pleurodema*, up to 4500 m for *Bufo,* and up to 5000 m, for *Telmatobius* (Pefaur & Duellman, 1980; Diaz-Páez & Ortiz, 2003).

Our observations indicate that *Pleurodema, Bufo,* and *Telmatobius* have colonized ponds at 5244 m within the deglaciated area, while *Pleurodema* has colonized newly formed ponds at even higher elevations, to 5400 m within the last 10 years. To survive at such elevations these species must possess adaptations to extreme environmental conditions, which include the hypoxic low-pressure of the mid-troposphere (~52% of mean sea level pressure at 5400 m), intense solar radiation (1000 W m^{-2}; Hastenrath, 1978), low absolute humidity, diurnal freeze–thaw cycles, and occasional deep snow

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cover that can persist for several weeks. Although direct measurements for atmospheric temperatures are not available from our field sites, during 2002 soil surface temperatures recorded at 5200 m at a site 160 m south of site D showed minima as low as −13.5 °C, and diurnal variability of up to 30 °C (data not shown). These results suggest that these anurans have capabilities for surviving frost. In line with these observations, *B. spinulosus* has been shown to survive body temperatures as low as −5 °C with no noticeable impact on vitality (Halloy & Gonzalez, 1993). This adaptation has also been documented in lizards such as *Liolaemus huacahuasicus* in Argentina (Halloy & Gonzalez, 1993), of a genus we have observed to 5450 m in the Sibinacocha area. The low-pressure environment at high-altitudes appears to enhance the freezing tolerance of these animals (Halloy & Gonzalez, 1993).

The upward range extension of amphibians parallels the upward shifts of other organisms. We have documented upward expansion of plants, which serve as anuran habitats and food sources, and insects, which act as pollinators for plants and food for amphibians. Glacial recession and snow melt also provide water sources for the development of year-round and seasonal ponds, terrestrial and aquatic vegetative growth, aquatic microorganisms, amphipods, diptera, butterflies, bivalves, and insect larvae, and a drinking source for mammals such as vicuña (*Vicugna vicugna*), viscacha (*Lagidium* spp.), fox (*Dusicyon* spp.), puma (*Felis concolor*), and other cats (*Oncifelis* spp. or *Oreailurus* spp.).

We show chytridiomycosis in anuran communities at much higher elevations in the Neotropics than previously recognized. The existence of severe *Bd* infections in amphibians at site D1 in August 2003 correlates with the subsequent die-off observed there in July 2004, and the apparent disappearance of both post-metamorphic and pre-metamorphic populations of *T. marmoratus* by March 2005. As our quantitative surveys span less than 2 years we cannot rule out the possibility that the disappearance of *Telmatobius* at sites D1 and A2 are part of an interannual fluctuation within these populations. However, given the high degree of infectivity observed in histological sections, the abundance of dead and sick specimens collected within the last 2 years, of which several were *Bd* positive, and that *Telmatobius* species are disappearing in Ecuador (Merino-Viteri *et al*., 2005) and Bolivia (Ergueta & Morales, 1996), we believe it likely that *T. marmoratus* is in decline due to chytridiomycosis. In contrast to *Telmatobius*, *Pleurodema* populations appear to be relatively robust at the highest elevations, and the *Bd*-positive specimens examined did not appear sick. Furthermore, *Pleurodema* also tested positive on the more isolated north side of the pass at site F, which deglaciated 35–40 years ago, raising important questions on how *Bd* arrived at this remote location that should be addressed in future research. The Sibinacocha watershed is home to several species of waterfowl, mammals (described above), and domesticated animals such as alpaca and llama that travel through the area, and thus could potentially facilitate the spread of *Bd*. Further studies will also be needed to ascertain whether *Pleurodema* exhibits an innate capacity to survive chytridiomycosis, and is therefore a potential vector for this disease.

The discovery of chytridiomycosis in a high alpine setting in the Andes is at odds with elevational (much higher) and climatic (much colder) characteristics of environments where chytridiomycosis have been reported elsewhere in the Neotropics. Niche modeling analysis based on observations from 44 localities predicts the most likely occurrence of *Bd* in the Andes is above 1000 m, with a median elevation for pathogenic *Bd* found at 1714 m and maximum at 4112 m (Ron, 2005). Our observations demonstrate that *Bd* is occupying an area of the biome that was previously unstudied. By confirming the presence of chytridiomycosis 1236 m above the previously reported maximum, our findings also significantly increase the spatial domain of potential pathogenicity because much of the central Andean region, which includes the vast Altiplano plateau, lies within this elevational range. Using linear regression analysis, Pounds *et al*. (2006) predicted that above 3500 m in the Neotropics, daily minimum and maximum temperatures would fall well below the optimal temperature range of 17–25 °C for *Bd* growth, and become increasingly inhospitable at higher elevations. However, our results clearly indicate that neither temperatures far below freezing nor large diurnal variations are limiting factors for *Bd* pathogenicity. Although atmospheric temperature means in tropical alpine zones fall well below the window of optimal *Bd* growth, our observations suggest that intense solar heating during midday hours temporarily raises water temperatures in shallow ponds to within this range, even at extreme elevations around glacial margins above 5300 m. Thus, solar heating may provide a thermal opportunity to allow *Bd* to survive and grow in these environments: this conjecture could be investigated in a controlled laboratory setting. The infectivity of the *Bd* within these extreme environments suggests that frogs responding to climatic warming are unlikely to escape this pathogen by moving to higher, colder, and more pristine areas. Thus, it is likely that *Bd* may drive changes in species assemblages in the high Andes by promoting susceptible species loss – as already seen with the disappearance of three species of *Telmatobius* in Ecuador (Merino-Viteri *et al*., 2005) – or by promoting the expansion of resistant species. There are 55 known species of *Telmatobius* (De la Riva *et al*., 2005), yet given the efficiency of pathogenic *Bd* to propagate, our results suggest an ominous future for the sustainability of this genus. Furthermore, this study offers an example of how the expansion of biota into previously unsuitable habitat can bring with it the introduction of new diseases. In this case it is chytridiomycosis, however, with other fauna it may encompass other pathogens of unknown host ranges and impacts.

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