Amphibians' expansion to record elevations influences *Batrachochytrium dendrobatidis* (Batrachochytriaceae) infection dynamics

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ABSTRACT

Climate change impacts emerging infectious disease events through multiple mechanisms, but the influence it exerts through driving host range shifts has been little explored. Not only might we expect range shifts to affect pathogen transmission by altering the connectivity of host populations, but range expanding hosts and pathogens will have different physiological responses to the suites of novel conditions they are exposed to, influencing infection outcomes. We studied the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) on three amphibians in the Cordillera Vilcanota, Peru: *Pleurodema marmoratum*, *Telmatobius marmoratus*, and *Rhinella spinulosa*. There, these species have undergone a climate-driven range expansion into recently deglaciated habitat to become both the highest elevation amphibians and the highest elevation cases of *Bd* infection globally. We analyzed *Bd* genetics, infection metrics, and apparent sublethal impacts along the colonization front (3,900—5,400 m asl) to explore how elevational range expansion affected host-pathogen dynamics. Amphibian range shifts have enabled their new connectivity across the once continuously glaciated Cordillera Vilcanota, but genetic evidence suggests that *Bd* disperses so frequently and extensively that this novel connectivity has not contributed significantly to overall *Bd* dispersal. Although amphibians have not escaped *Bd* infection outright through upslope expansion in the Cordillera Vilcanota, *Bd* growth does appear to be constrained at the highest reaches of the Vilcanota. Finally, we present evidence that *Bd* infection has different sublethal costs for amphibians at the new elevations they have colonized, though whether the costs are mitigated or exacerbated by extreme elevation may be moderated by amphibian microhabitat use.

KEYWORDS:

climate change— Cordillera Vilcanota, Peru — disease triangle — range expansion—sublethal effects — synergisms — transmission

INTRODUCTION

The rising incidence of emerging infectious diseases (EIDs) is a critical issue in both conservation and public health (Fisher *et al.* 2012; Jones *et al.* 2008). Climate change may be contributing to increased outbreaks by providing pathogens with opportunities to switch hosts, expand geographically, or become more virulent (Harvell *et al.* 2002; Hoberg & Brooks 2015; Liang & Gong 2017), while climate-driven range shifts are also expected to influence the infection dynamics of several important pathogens. In Hawaii, the upslope range shifts of mosquitos are predicted to drive the continuing decline of endemic birds currently escaping avian malaria at high elevations (Zamora-Vilchis *et al.* 2012), and in Europe the northwards expansion of ticks is predicted to increase the spatial extent of Lyme infections (Jaenson & Lindgren 2011). However, the data we currently have on the interaction of climate-driven range shifts and EIDs tends to come from vector-borne systems, as many vectors are highly sensitive to climate (Harvell *et al.* 2002). Though little documented, the infection dynamics of many pathogens that rely on direct transmission will presumably be affected by the widespread climate-driven range shifts of host species (e.g. Freeman *et al.* 2018; Moritz *et al.* 2008; Parmesan *et al.* 1999). As hosts undergo range shifts, their exposure to novel environments may alter host-pathogen dynamics—potentially exacerbating or mitigating infections, changing transmission patterns, or exposing hosts to new pathogens.

The emergence of *Batrachochytrium dendrobatidis* (*Bd*), the pathogen causing chytridiomycosis that has contributed to devastating global amphibian declines (Scheele *et al.* 2019; Skerratt *et al.* 2007), has been linked to climate change through a few proposed mechanisms (Li *et al.* 2013). Climate change could expand the spatial extent of optimal *Bd* growth conditions (Bosch *et al.* 2007). Meanwhile, more frequent droughts compromise amphibian immunity and enhance *Bd* transmission when amphibians aggregate in wet or humid microhabitats (Burrowes *et al.* 2004; Lampo *et al.* 2006). Higher climatic variability may also favor rapidly-adapting pathogens over hosts, resulting in worse infections (Raffel *et al.* 2013;

Rohr & Raffel, 2010). Finally, as parasites generally have broader thermal tolerances than hosts, frogs are likely to be exposed to suboptimal temperatures before *Bd*, placing them at a disadvantage (Cohen *et al.* 2019; Cohen *et al.* 2017). The mounting evidence of climate-driven amphibian range shifts (e.g. Bustamante *et al.* 2005; Enriquez-Urzelai *et al.* 2019; Raxworthy *et al.* 2008) suggests this as an additional mechanism that could connecting climate change and amphibian-*Bd* dynamics.

The Cordillera Vilcanota, in southern Peru, presents an ideal system for exploring how climate-driven range shifts impact EIDs. Field surveys of this heavily glaciated tropical mountain chain in the early 2000s documented the first known *Bd* infections in southern Peru (Seimon *et al.* 2005), but also revealed that three frog species had expanded their elevational ranges by hundreds of vertical meters into passes that had deglaciated since the end of the Little Ice Age (~150 years ago). The Marbled four-eyed frog (*Pleurodema marmoratum*) had expanded upslope to 5,400 m asl, making it the highest elevation amphibian in the world; the Andean toad (*Rhinella spinulosa*) and Marbled water frog (*Telmatobius marmoratus*) had expanded to 5,244 m asl (Seimon *et al.* 2007). Chytridiomycosis was recorded at the upper limits of these species distributions, and a die-off event was observed in *T. marmoratus* at 5,244 m asl (Seimon *et al.* 2007). Today, all three species persist at lower abundances in the Vilcanota. Host-pathogen dynamics have transitioned into a more stable, enzootic state (Seimon *et al.* 2017), as can also be said of neighboring amphibian communities downslope (Catenazzi *et al.* 2017).

Moving upslope in the Vilcanota, *Bd* and frogs are challenged by progressively more intense UV, deep frozen precipitation, and a partial oxygen pressure 50-60% of that at sea level

(Poremba *et al.* 2015; Seimon *et al.* 2017; Wang *et al.* 2014). At the apex of the mountain passes, soil temperature can fluctuate between –12° C at night and 25° C during the day, with a soil freezing rate exceeding that measured from any site on Earth (1.8° C/ hr; Schmidt *et al.* 2009). Resident frogs are exposed to an even broader operative temperature range (−3.5 to 44° C; Reider 2018). Here, we asked how range expansion into these new, challenging elevations may have influenced *Bd* infection dynamics. We used genetic data to inform our understanding of the local history of *Bd*, then examined site infection metrics and apparent sublethal infection impacts to understand how conditions at newly-colonized elevations might sway infection outcomes. We anticipate that *Bd* in the Vilcanota is from the global panzootic lineage (*Bd*GPL), the lineage largely responsible for *Bd* epizootics in South America (James *et al.* 2015), though these highest elevations inhabited by *Bd* presumably impose strong selective pressures that could conceivably result in a limited number of locally-adapted strains proliferating. If *Bd* remains dispersive despite extreme high-elevation conditions, and particularly if it was introduced to the Vilcanota just before local die-offs in the 2000s, *Bd* will be spatially unstructured, a common finding elsewhere *Bd* population genetics have been studied (e.g. Alvarado-Rybak *et al.* 2021; Basanta *et al.* 2021; Byrne *et al.* 2019). However, if the environmental persistence of *Bd* is depressed by harsh, high elevation conditions, the genetic structure of *Bd* is more likely to reflect its gradual spread between watersheds by way of host dispersal along the corridors provided by deglaciated passes (Haddad *et al.* 2014). The present study is the first to examine *Bd* infection dynamics above 4000 m asl (3,900—5,400 m asl); though *Bd* infection prevalence and intensity tend to initially increase with elevation, studies sampling to 4000 m asl suggest that these metrics may begin to decline again with increasing elevations (Catenazzi *et al.* 2011; Muths *et al.* 2008). For this reason, we expect *Bd* infection to have lower sublethal impacts for amphibians at newly-colonized, extreme high elevation sites. Alternatively, if the stress of high elevations and *Bd* infection impact amphibians synergistically, the sublethal impacts of *Bd* may increase with elevation. These analyses provide the first insights into how the climate-driven range shifts of hosts may influence EID events.

METHODS

1. Fieldwork

We sampled *P. marmoratum*, *R. spinulosa,* and *T. marmoratus* in the Cordillera Vilcanota (13° 44' 24"˚S, 71° 5' 24"˚W), an 80 km-long mountain chain in the southern Peruvian Andes, during the transition between the wet and dry seasons (March-May) in 2018 and 2019. *T. marmoratus* breed year-round, while *P. marmoratum* and *R. spinulosa* are in their final breeding months at this time. We sampled at 76 sites from 3,967—5,333 m asl, including transects across two deglaciated passes (Figure S 1; see Supporting Information for details). We expended equal search effort to capture 15 adults per species per site, supplementing as needed first with juveniles and then with tadpoles. We sampled 695 *P. marmoratum* (317 adults), 173 *Rhinella spinulosa* (82 adults)*,* and 232 *T. marmoratus* (23 adults). All individuals were dry swabbed to assess *Bd* infection (MW113, Medical Wire & Equipment Co., Ltd., Corsham, UK; Hyatt *et al.* 2007; see Supporting Information for details). We noted snout-vent length (SVL), mass, sex, and signs of disease in adults (lethargy, excessive sloughing, reddened skin). We also recorded SVL, mass, and signs of disease in juveniles; as well as SVL, mass, and Gosner developmental stage

(Gosner 1960) in tadpoles. Samples were transported to the University of California, Berkeley for -80˚C storage.

Paired DS1921G Thermocron Temperature Loggers (OnSolution Pty Ltd, Sydney, Australia; hereafter, 'iButtons') were placed in aquatic and terrestrial microhabitats at 20 sites during the 2018 sampling season to measure temperature every four hours (see Supporting Information for details). Data was downloaded from recovered iButtons during the 2019 sampling season.

2. *Bd* quantification and genotyping

We focused on gaining a comprehensive understanding of *Bd* dynamics in our most widely-sampled frog, *P. marmoratum.* We estimated *Bd* zoospore equivalents (ZE) per swab using triplicate quantitative PCR reactions (Hyatt *et al.* 2007; see Supporting Information for details). Amplicon sequencing libraries were prepared on a Fluidigm Access Array for 96 *Bd*+ extracts and sequenced with Illumina Miseq (Byrne *et al.* 2017).

3. Phylogenetic, structure, and redundancy analyses

We aligned filtered ambiguity sequences for Vilcanota samples and previously published sequence data representative of global *Bd* diversity (Byrne *et al.* 2019a,b) in MUSCLE (v3.32; Edgar 2004), estimated a ML gene tree for each amplicon using RAxML (v4.0), and estimated an unrooted species tree in ASTRAL (v5.7.4; see Supporting Information for details). We examined standing genetic variation of Vilcanota *Bd* with an ANGSD Site Frequency Spectrum (SFS; v0.933-106-gb0d8011). We processed BAMs separately for the subset of Vilcanota samples retained in phylogenetic analyses and the larger dataset including globally representative *Bd*GPL samples (Byrne *et al.* 2019a,b). We conducted a principal component analysis with a NIPALS PCA (Korneliussen *et al.* 2014; see Supporting Information for details). We tested for population structure at the scale of sampling sites and watersheds using AMOVA in Poppr (Kamvar *et al.* 2014). We also assessed the proportion of genetic variance that could be explained by elevation in R by conducting a redundancy analysis in Vegan (v2.5-7) and an ANOVA of the resulting model in Stats (v4.1.1; see Supporting Information for details).

4. Infection dynamics across elevations

We analyzed the site-level effects and apparent sublethal impacts of *Bd* in R (v4.0.2; see Supporting Information for details). Adult counts per site did not correlate with elevation using GLMs in MASS (v7.3-54) for *P. marmoratum* ($n_{sites} = 63$; Figure S3a) or *T. marmoratus* ($n_{sites} = 17$; Figure S3b), so though adult counts of *R. spinulosa* declined slightly with elevation ($n_{sites} = 25$, $p < 0.001$, Figure S3c, Table S3), we did not include this proxy of frog density in subsequent models of site-level infection metrics. To investigate whether elevation was a good predictor of site infection metrics (site prevalence, site mean infection intensity, and site maximal infection intensity), we tested the fit of linear and quadratic regression models at *Bd*+ sites. We did not record signs of disease like excessive sloughing with sufficient frequency to report relationships between these symptoms and elevation.

We examined whether frog body size (SVL) and condition (SMI; as per Peig & Green, 2009) responded to putative site infection status (*Bd*+/-), elevation, and the interaction between these terms using GLMMs in lme4. We built models for the species-life stage combinations for which we had sufficient data: *P. marmoratum* adults, *P. marmoratum* tadpoles, and *T. marmoratus* tadpoles. We included site as a random effect in all models, and Gosner developmental stage as a partial correlate predicting tadpole SVL. We dropped sex as a variable from models of adult data as it was not a significant contributor. Published literature does not yield expectations for how body size or condition relates to elevation in these species, so we examined samples at *Bd*- sites to establish baseline trends. To explore how thermal regimes along the elevational gradient might relate to observed trends in infection metrics and apparent sublethal impacts, we plotted March-April temperature data from recovered iButtons relative to the temperature-dependent logistic growth rate (*r*) of a tropical *Bd* strain (Voyles *et al.* 2017a,b), the CT_{max} of adult *P. marmoratum*, and the mean temperature tolerated by *P. marmoratum* adults that recovered following freezing (Reider *et al.* 2020).

RESULTS

1. Phylogenetic placement, spatial genetic structure, and local adaptation

Vilcanota *Bd* samples from *P. marmoratum* (*n* = 44) nest within the *Bd*GPL-2 clade of the *Bd*GPL lineage but do not cluster together relative to a panel of globally-derived *Bd*GPL genotypes in a consensus gene tree or PCA (Figure 1, Figure 2a). Vilcanota *Bd* is geographically unstructured, with samples failing to cluster in a PCA (Figure 2b) despite containing many low-frequency variants after stringent filtering (73.4% variants have an allele frequency of ~1%). Watersheds or sites were not differentiated according to an AMOVA ($p > 0.05$, Table S1). Elevation was a significant predictor of genetic variance according to redundancy analysis but only explained 1.2% of genetic variance ($p < 0.05$, Table S2).

2. Site infection metrics across elevations

We detected *R. spinulosa* up to 4,895 m asl. We recorded adult *Pleurodema marmoratum* and *Telmatobius marmoratus* at the highest part of the mountain passes, at a maximal elevation of 5,333 and 5,226 m asl respectively. We were able to sample *P. marmoratus* (but not the other species) at regular intervals across the entirety of both deglaciated mountain passes, suggesting that this species now has connectivity between populations north and south of the Vilcanota.

Bd prevalence in *P. marmoratum* was 30.0% among juveniles ($n = 120$) and 24.0% among adults ($n = 317$), but not detected in tadpoles ($n = 256$). The best model by AIC relating elevation to prevalence was a quadratic model, where prevalence peaked at approx. 4700 m asl (Figure S5a, Table S4). The predictors in this model were significant and explained 20% of variance in prevalence ($n_{sites} = 18$, $p < 0.05$, Table S4). Visual inspection of site infection intensity metrics against elevation suggested that these metrics declined with increasing elevation (Figure S5b-c), but the data supported neither a linear nor a quadratic relationship ($n_{sites} = 18$).

3. Sublethal impacts across elevations

In *Telmatobius marmoratus* tadpoles, a full interactive model including elevation and putative site infection status explained 11% of variance in body condition and minimized AIC (n = 77, n_{sites} = 6, Table S5), though this model may be overfit. All predictors were significant ($p < 0.05$) except the slope of SMI against elevation at *Bd*+ sites. This model suggested that tadpoles had higher body conditions at lower elevations for *Bd*- sites but not *Bd*+ sites (Figure 3a). Similarly, *T. marmoratus* tadpole body size (SVL) was best predicted by a full interactive model that included elevation, putative site status, and developmental stage. All predictors were significant, with fixed effects explaining 68% of the variance (n = 77, n_{sites} = 6, $p < 0.001$, Table S6). Consistent with our findings regarding SMI, this model suggested that tadpoles are longer relative to developmental stage at lower elevations but only at *Bd*- sites (Figure 3b). The relationships between *T. marmoratus* tadpole SMI or SVL and elevation cannot be attributed to differences in phenology across elevation (Figure S7d).

The model with lowest AIC predicting *Pleurodema marmoratum* tadpole SMI from elevation and putative site status included elevation only. According to this model, body condition declined with increasing elevation, but the model predictors were not significant, and the fixed effects explained only 4% of variance in SMI (n = 128, n_{sites} = 15, $p > 0.05$, Table S5). Visual inspection of these data revealed that SMI appeared reduced for lower elevation tadpoles at *Bd*+ sites, similar to trends detected for *T. marmoratus* tadpoles (compare Figure 3a and Figure S6a). Indeed, a model including site status and its interaction with elevation was within a ΔAIC of only 0.02 of the elevation-only model, explained 7% of variance in SMI, and included a significant slope and intercept at *Bd*- sites (Table S5). Meanwhile, *P. marmoratum* tadpole SVL was best predicted by elevation alone. Tadpoles were longer relative to developmental stage at higher elevations regardless of site infection status (Figure S7a). The model explained 18% of variance in SVL, but not all predictors were significant (n = 128, n_{sites} = 15, $p > 0.05$, Table S6). Differences in *P. marmoratum* phenology across elevations could potentially contribute to relationships between elevation and body condition (Figure S7c), but this phenomenon would presumably impact *Bd*+ and *Bd*- sites alike.

In *P. marmoratum* adults, the best model of SMI by AIC was an additive model that included elevation and putative site infection status, though these predictors were not significant and explained only 3% of variance in SMI (n = 339, $\mathrm{n_{sites}}$ = 53, $p > 0.05$, Table S5). Based on this model, adults had lower body conditions at high than at low elevations, and at *Bd*+ than at *Bd*- sites. Visual inspection of these data reflects the trends that emerged for *T. marmoratus* tadpoles (compare Figure 3a and Figure S6b): SMI was depressed for lower elevation tadpoles at *Bd*+ sites. However, the full interactive model was not well supported by AIC-based model comparison (Table S5).

In adult *P. marmoratum*, SVL was best predicted from a full interactive model of elevation and putative site infection status. Fixed effects in this model explained 9% of variance in SVL, but though all the predictors were significant the intercept was not (n = 339, n_{sites} = 53, $p > 0.05$, Table S6). According to this model, SVL in adult *P. marmoratum* increases with elevation, regardless of site infection status. However, while SVL increases rapidly at *Bd*- sites

(6.5 mm per 1,000 m of elevation), SVL increases only gradually at *Bd*+ sites (1.4 mm per 1,000 m; Figure S7b).

Temperature data along the elevational gradient demonstrated that higher elevations were characterized by lower average daily temperatures and larger fluctuations in daily temperature (Figure 4a). Ephemeral pond habitats experienced less thermal variability than adjacent terrestrial habitats (Figure 4a). It follows that, at higher elevations, *Bd* is characterized by a lower average growth rate, but frogs also experience greater exposure to their physiological tolerance limits (Figure 4b).

DISCUSSION

Although how infection dynamics are influenced by pathogen or vector range shifts has been the subject of frequent study over the past decade (e.g. Dudney *et al.* 2021; Jaenson & Lindgren, 2011; Romanello *et al.* 2021; Zamora-Vilchis *et al.* 2012), we still know little about how they are influenced by the climate-driven range shifts of hosts. Here, we demonstrate that climate-driven range shifts may impact the infection dynamics of direct-transmission pathogen systems, as well as vector-borne ones. We used genetic analyses and modeled the relationship among elevation, site infection metrics, and measures of individual energetic status to understand how the range expansion of three species may have impacted the course of their infection with a shared pathogen. We learned that one of the species studied is using mountain passes cleared by contemporary deglaciation as a new dispersal corridor, and that *Bd* is resilient to, and can disperse extensively at, the highest elevations used by amphibians. Finally, we found evidence that some site infection metrics and apparent sublethal impacts of infection are affected by elevation, suggesting that hosts' elevational range shifts have had implications for infection outcomes.

Genetic evidence suggests the recent introduction and extensive dispersal of ***Bd*GPL-2**

Whether global *Bd* epizootics were provoked by a novel or endemic pathogen remains an open question (Rosenblum *et al.* 2013). *Bd* was often present in areas long before recorded outbreaks (e.g. Basanta *et al.* 2021; De León *et al.* 2019). Indeed, the earliest known *Bd* was swabbed from a Titicaca water frog (*Telmatobius culeus*) collected 300 km southeast of the Cordillera Vilcanota in 1863 (Burrowes & De la Riva 2017). Detections of *Bd* that predate epizootic events are often hypothesized to represent local *Bd* strains, with regional *Bd*-associated declines instead being attributed to the introduction of a novel strain in the 1990s (Becker *et al.* 2016; Burrowes & De la Riva 2017)—usually the global panzootic lineage (*Bd*GPL), the lineage most frequently associated with disease outbreaks, whose lack of genetic structure even at global scales suggests its recent expansion (James *et al.* 2015; O'Hanlon *et al.* 2018; Rosenblum *et al.* 2013; Schloegel *et al.* 2012).

Bd samples sequenced in this study belong to *Bd*GPL, consistent with previous studies genotyping South American *Bd* west of the Brazilian Atlantic Forest (Alvarado-Rybak *et al.* 2021; Byrne *et al.* 2019; O'Hanlon *et al.* 2018; Russell *et al.* 2019). Further, Vilcanota *Bd* was all *Bd*GPL-2, the more derived and globalized of two *Bd*GPL sub-clades (James *et al.* 2015). However, it is worth noting that *Bd*GPL can outcompete other strains during coinfections, so may have displaced potentially pre-existing endemic strains in the Vilcanota (Farrer *et al.* 2011; Jenkinson *et al.* 2018). Additionally, selecting the highest intensity swabs to sequence at each site could introduce bias against less-virulent strains (Byrne *et al.* 2017; Farrer *et al.* 2011).

We found Vilcanota *Bd*GPL-2 to be spatially unstructured using our genetic markers, consistent with most previous genetic studies of *Bd*GPL, which have found it to be spatially unstructured from local to continental scales (Alvarado-Rybak *et al.* 2021; Basanta *et al.* 2021; Byrne *et al.* 2019; Rothstein *et al.* 2021; Velo-Antón *et al.* 2012). This lack of structure has often been interpreted as evidence for its recent introduction to the sampled area, though might simply indicate high rates of dispersal. One exception to the general lack of spatial structuring in *Bd* has been the Sierra Nevada of California, suggesting the western U.S.A. as a potential origin for *Bd*GPL (Rothstein *et al.* 2021). Sierran *Bd* demonstrates that *Bd*GPL can develop spatial structure, even despite cool, high-elevation conditions that may constrain rates of evolutionary change.

Though unstructured, Vilcanota *Bd* has substantial low-frequency variation, representative of the global variation in *Bd*GPL-2. Our data cannot exclude the possibility that *Bd*GPL circulated in the Vilcanota prior to local epizootics of the early 2000s, but in the context of prior regional studies (Burrowes *et al.* 2020; Catenazzi *et al.* 2011; Lips *et al.* 2008) it is more likely that *Bd*GPL-2 housed substantial standing genetic variation upon its introduction or was introduced multiple times. Our analyses place Vilcanota *Bd* in a large, global polytomy in *Bd*GPL-2, so we cannot determine whether *Bd*GPL spread from Ecuador via the Andes (Lips *et al.* 2008) or from Brazil via Bolivia (Burrowes *et al.* 2020; Catenazzi *et al.* 2011). However, the timing of *Bd*GPL-2's arrival likely coincides with the amphibian die-offs in the Vilcanota in the early 2000s (Seimon *et al.* 2007; Seimon *et al.* 2005), as this corresponds to declines in adjacent lowland and cloud forest regions (Catenazzi *et al.* 2011).

Regardless of the provenance and timing of introduction, our data demonstrates that *Bd*GPL-2 has undergone extensive local dispersal. Its movement across the Cordillera Vilcanota would not have been limited to transmission by frogs along the terrestrial corridors provided by deglaciated passes. Processes facilitating its dispersal might include the trade of *T. marmoratus* for urban consumption (Catenazzi *et al.* 2010), the aquaculture of nonnative fish (Martín-Torrijos *et al.* 2016; Ortega & Hidalgo, 2008), the movements of Andean waterbirds (Burrowes & De la Riva, 2017), or even precipitation (Kolby *et al.* 2015).

Amphibians cannot escape *Bd* by range shifting upslope

Early studies generally found that *Bd* infection prevalence or intensity increased with elevation (Brem & Lips, 2008) and epizootics impacted highland sites more severely (Berger *et al.* 2004; Lips *et al.* 2006; Lips 1999). These relationships are consistent with the preference of *Bd* for cool temperatures (Piotrowski *et al.* 2004; Woodhams *et al.* 2008) and the impaired function of amphibian immune systems and skin microbiomes at colder, more thermally variable high elevations (Daskin *et al.* 2014; Jackson & Tinsley 2002). Early field studies did not sample above 2,500 m asl, but empirical work demonstrating that suboptimal temperatures retard *Bd* growth led to a hypothesis that *Bd* pathogenicity was restricted to below 4,000 m asl (Piotrowski *et al.* 2004; Pounds *et al.* 2006; Ron 2005; Woodhams *et al.* 2008). Subsequent studies of *Bd* dynamics up to 4,000 m asl reported declining infection prevalence or intensity with increasing elevation (Catenazzi *et al.* 2011; Muths *et al.* 2008), perhaps due as much to increasing aridity as to decreasing temperatures (De la Riva & Burrowes 2011).

The idea that *Bd* pathogenicity had an upslope bound was later undermined by severe infections documented above 4,000 m asl in the Vilcanota and other sites (Knapp *et al.* 2011; Seimon *et al.* 2007; Seimon *et al.* 2005). Here, we find limited evidence that elevational extremes constrain *Bd* growth. *Bd* infection metrics appear to decline at the upper reaches of the elevational gradient colonized by Vilcanota frogs (3,967—5,333 m asl)—both for the significant and well-fit quadratic model of prevalence and for the inherently noisy infection intensity data. Such declines would be consistent with our expectations following from our iButton temperature data, considered in light of a study profiling the thermal dependence of *Bd* growth (Voyles *et al.* 2017): *Bd* likely grows more slowly at higher elevations in the Vilcanota (Figure 4).

Upslope range shifts may mediate infection outcomes through exposure to thermal variability

We investigated whether extreme elevations compounded or ameliorated the apparent sublethal impacts of *Bd* by comparing frog body size and condition at putatively *Bd*+ and *Bd*- sites. Body size and condition signal important information about nutritional history in amphibian larvae, juveniles, and adults; and can predict important fitness components such as fecundity, the ability to respond effectively to environmental stress, and lifespan (Brodeur *et al.* 2020; Hector *et al.* 2012; Maccracken & Stebbings, 2023; Martins *et al.* 2013; Metcalfe & Monaghan 2001). To call sites as *Bd*- we required infection intensities for at least 10 postmetamorphic frogs at that site, with individual frog infection statuses being called from triplicate qPCR reactions; therefore, though we lacked longitudinal sampling that would more definitely classify site infection status, we believe our protocol minimizes the risk of both false negatives and false positives.

At *Bd*- sites, *Telmatobius marmoratus* tadpole condition and size declined with increasing elevations, which our examination of adult counts across elevations suggests we cannot attribute to increased competition. *T. marmoratus* tadpoles have a protracted larval development relative to *P. marmoratum* and *R. spinulosa*. During the estimated 5—19 months until metamorphosis (Lobos *et al.* 2018), they rely upon the resources of their natal stream (Catenazzi *et al.* 2013; Rubio 2019). Therefore, declines in stream primary productivity with increasing elevation (Jacobsen 2008) might explain this trend in tadpoles. An alternative explanation could be that *T. marmoratus* tadpoles metamorphose more quickly at higher elevations, attaining a smaller size in the process (Licht 1975).

For larval *T. marmoratus*, circulating *Bd* was associated with lower body condition and size relative to developmental stage, but only at low elevations. This interaction is consistent with expectations from our temperature data. Aquatic microhabitats at extreme elevations are frequently at no- or low-growth temperatures for *Bd*, resulting in a depressed average growth rate, but at low elevation remain steadily at temperatures conducive to *Bd* growth (Figure 4, Figure S8). Studies of other amphibian species have shown that *Bd* infection can result in smaller, lighter tadpoles (Catenazzi *et al.* 2013; Parris & Cornelius 2004). Infected tadpoles may sacrifice body size and condition by diverting energy towards immune response or by accelerating metamorphosis (Warne *et al.* 2011). We expect that, for slow-developing *T. marmoratus*, the impact of *Bd* on tadpole mouthparts is particularly important: *Bd* infects keratinized tissues, which in tadpoles limits *Bd* growth to their mouthparts and causes oral deformities over time (Berger *et al.* 1998; Vredenburg & Summers 2001), as has been demonstrated for congenerics (Rubio 2019). Damaged mouthparts can reduce tadpole feeding efficiency and retard their growth (Rachowicz & Vredenburg 2004; Rowe *et al.* 1996; Rubio 2019).

Shared mechanisms may contribute to a similar trend in larval *P. marmoratum* body condition. Their body condition declines with increasing elevations at *Bd*- sites, but *Bd* infections appear to take a larger energetic toll on *P. marmoratum* tadpoles at lower elevations. Unlike larval *T. marmoratus*, larval *P. marmoratum* increase in body size relative to developmental stage with elevation regardless of site infection status. This trend may reflect a reproductive strategy that is advantageous in harsh environments and has been documented in several frog species (e.g., Liao *et al.* 2014; Lüddecke 2002; Räsänen *et al.* 2005), that females produce fewer offspring of higher quality in harsher environments, or given the decline in abundance of *R. spinulosa* with increasing elevations could possibly result from decreasing competition since these species can share larval habitat.

Adult *P. marmoratum* body size also increases with elevation, perhaps owing to reduced competition with *R. spinulosa* or selection for larger size at metamorphosis and/or larger females capable of greater maternal investment (Chen *et al.* 2013; Liao *et al.* 2014; Womack & Bell 2020). However, adult *P. marmoratum* body size increases dramatically with elevation at *Bd*-

sites, while only gradually at *Bd*+ sites (6.5 mm vs. 1.4 mm per 1,000 m of elevation), suggesting that *Bd* may have a larger sublethal toll at higher elevations. While terrestrial frogs experience lower average *Bd* growth rates at extreme elevations, they are also not as thermally buffered from harsh temperature extremes as frogs inhabiting aquatic microhabitats. Indeed, we found that adult *P. marmoratum* are more exposed to their thermal tolerance limits at extreme high elevations (Figure 4, Figure S8), placing them at a fitness disadvantage relative to the *Bd* pathogen (Cohen *et al.* 2019, 2017). Their failure to respond phenotypically to high-elevation conditions could compromise their fecundity and survivorship, suggesting that extreme elevations may compound rather than ameliorate the stress of *Bd* infection for *P. marmoratum* adults.

It is important to note that all association between *Bd* site status and apparent sublethal impacts to individuals are correlative in this study. We did not conduct trials and cannot know the *Bd* exposure history of any individual frog. One important implication is that we do not know the direction of causality: we interpret that *Bd* could be incurring sublethal impacts, but alternately *Bd* might be more likely to affect amphibian populations with lower energy reserves. If this were the case, observed interactions between body condition or size, elevation, and site *Bd* status require different mechanistic explanations.

Conclusion

Climate change will continue to drive range shifts, with many species expanding into more thermally-variable regions like the Vilcanota. It is important that we understand how the stress of novel, frequently less optimal, habitats impact host-pathogen systems, particularly in the face of increasingly common emerging infectious disease challenges. In the case of amphibian-*Bd* systems, hosts cannot escape infection by range shifting upslope: *Bd* infects amphibians and disperses readily even at the edges of their physiological tolerances, though factors like exposure to thermal variability may mediate infection outcomes. This work can help inform and stimulate further questions around how host range shifts might in some cases exacerbate and in others mitigate emerging infectious disease events.

FIGURES

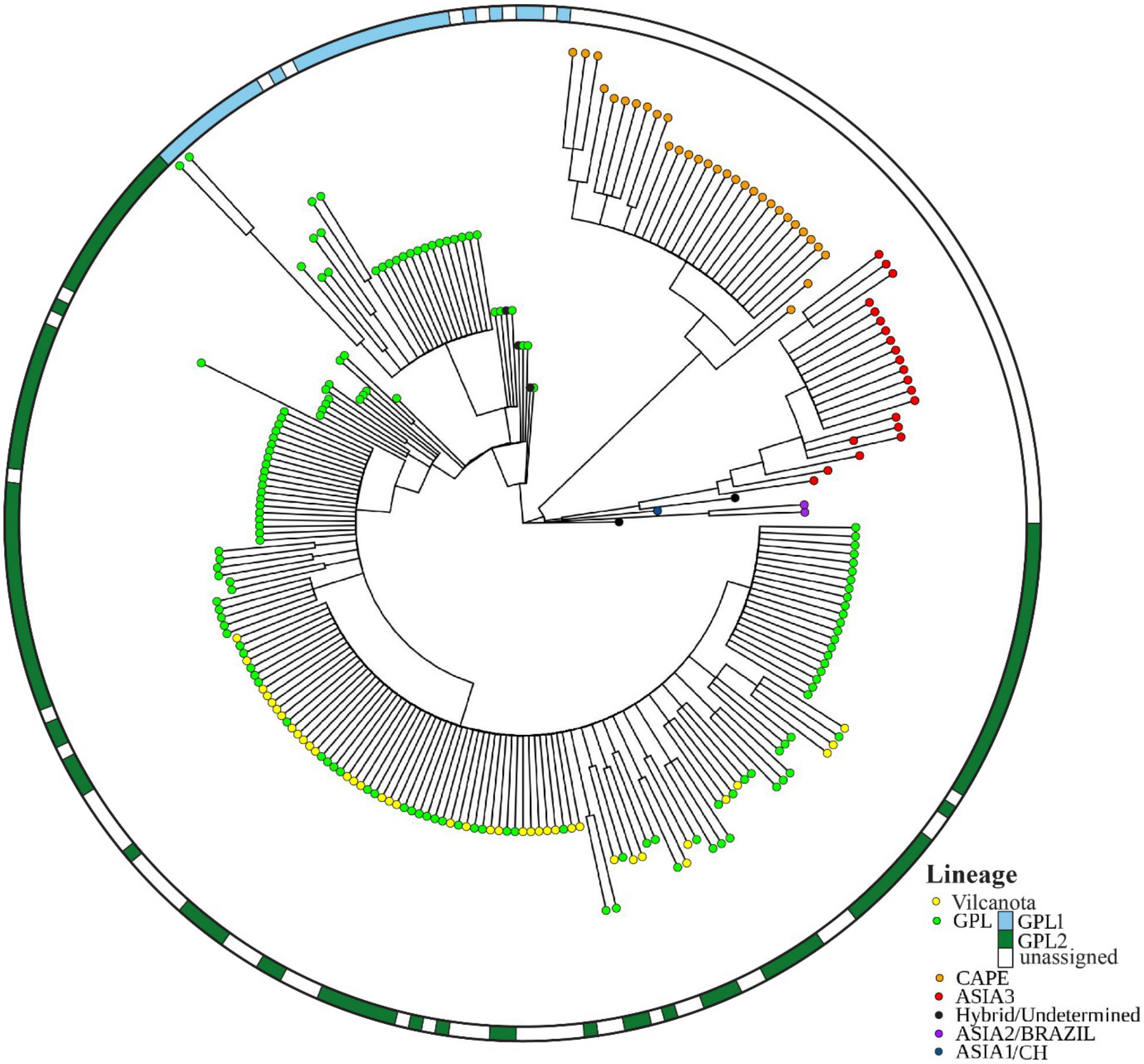

Figure 1. A midpoint-rooted consensus gene tree of Vilcanota *Bd* sampled from *Pleurodema marmoratum* (yellow tips) alongside previously-published samples representative of the five major *Bd* lineages (CAPE, ASIA3, ASIA2/BRAZIL, ASIA1/CH, GPL) and their hybrids (Byrne *et al.* 2019a,b). This tree has a normalized quartet score of 0.807 and includes only nodes with a posterior probability ≥ 0.7. Assignment of *Bd*GPL samples to *Bd*GPL-1 or *Bd*GPL-2 is included whenever assigned by previous studies (James *et al.* 2015; Rothstein *et al.* 2021; Schloegel *et al.* 2012) and is shown in the concentric mosaic. This tree is included with sample names and the continent of swab origin in the supplements (Figure S2).

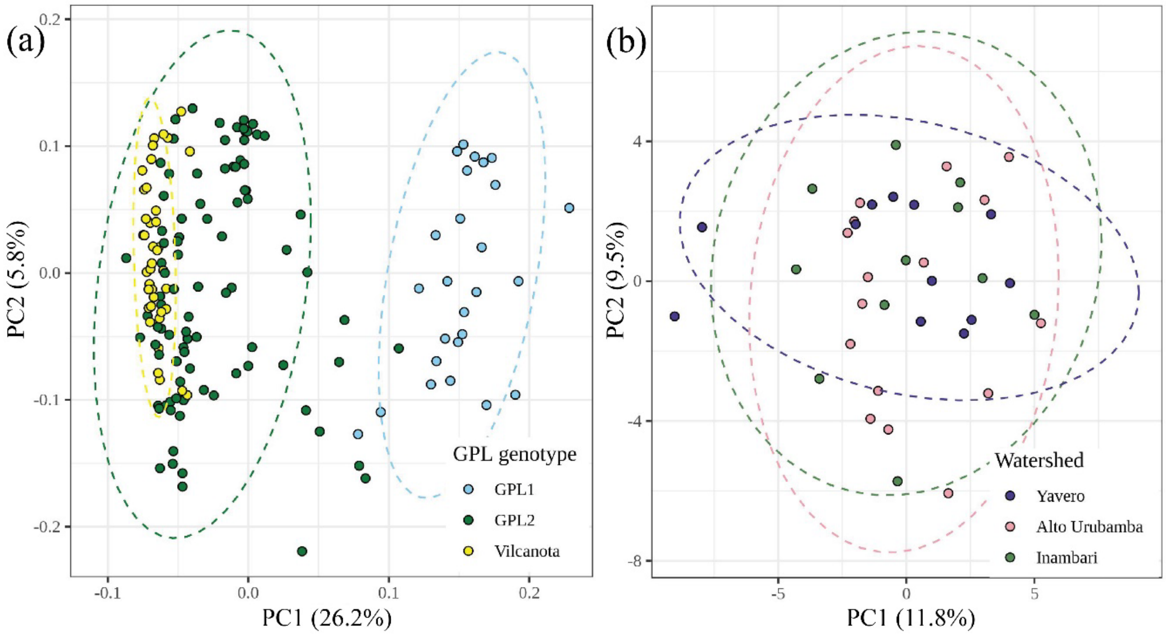

Figure 2. Principal component analyses (PCAs) of **(a)** global *Bd*GPL samples, demonstrating how *Bd* sampled from *Pleurodema marmoratum* in the Vilcanota is nested within the *Bd*GPL-2, and **(b)** Vilcanota samples, colored by watershed, showing their lack of spatial genetic structure.

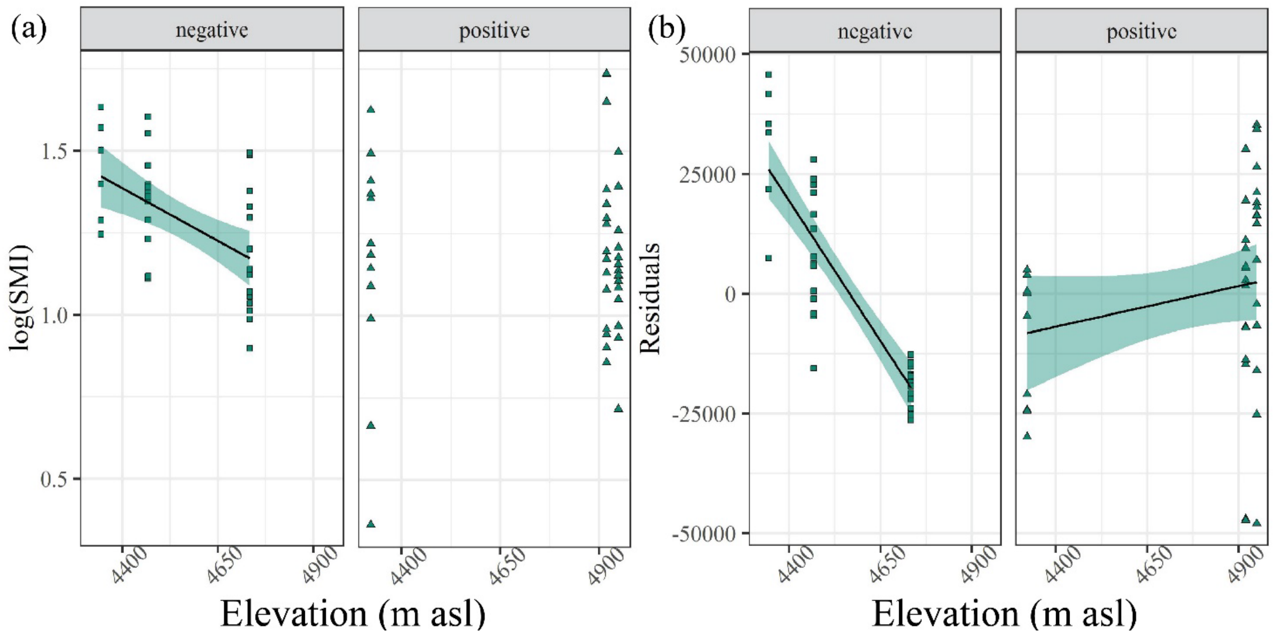

Figure 3. The relationship between elevation, site *Bd* infection status, and energetic status for *T. marmoratus* tadpoles. **(a)** Results of linear mixed model of *T. marmoratus* tadpole body condition (SMI) along the elevational gradient, displayed in separate panels for *Bd*-positive and *Bd*-negative sites as site infection status was a significant contributor to these models. **(b)** Results of linear mixed model of *T. marmoratus* tadpole residuals of body size (SVL) against Gosner stage, displayed in separate panels for *Bd*-positive and *Bd*-negative sites as site infection status was a significant contributor to these models. These trends do not appear to be attributable to differences in phenology across elevations (Fig S 7D).

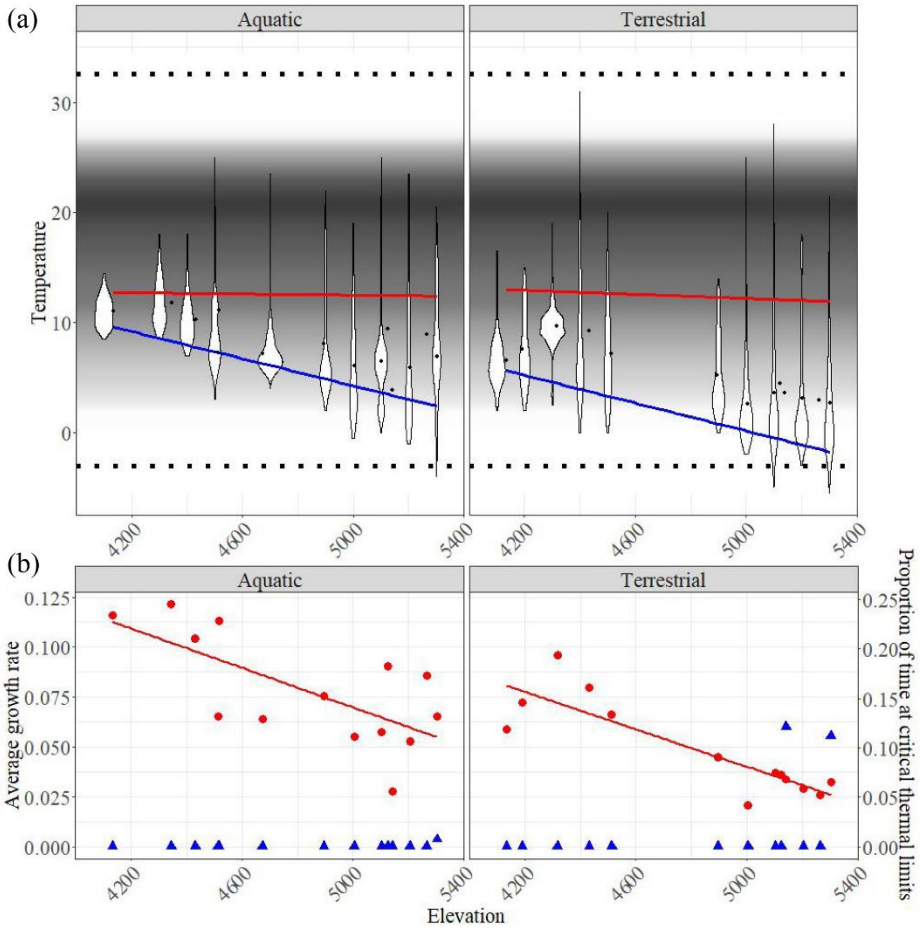

Figure 4. Thermal regimes along the elevational gradient and across microhabitats. **(a)** Violin plots of raw temperature data. Mean daily temperatures measured by a given iButton are displayed as black points. The red and blue lines represent linear regression of maximal and minimal daily temperatures, respectively. The greyscale gradient represents the logistic growth rate (r) of a tropical *Bd* strain quantified across temperatures (from Voyles *et al.* 2017a,b). The upper black dotted line represents the CT_{max} of adult *P. marmoratum* (32.56˚ C), and the lower dotted line represents the mean temperature tolerated by *P. marmoratum* adults that recovered following freezing (Reider *et al.* 2020). **(b)** Red points represent the average growth rate of *Bd* according to the temperatures recorded by a given iButton, and the red line represents a linear regression of this data. Blue triangles represent the proportion of time a given iButton was exposed to temperatures outside the physiological tolerance of *P. marmoratum* adults.

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was conducted according to the protocols AUP-2017-12-10585 and AUP-2018-12-11648, approved by the Animal Care and Use Committee at the University of California, Berkeley.

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