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Thermal tolerance and the importance of microhabitats for Andean frogs in the context of land use and climate change

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Abstract

1. Global warming is having impacts across the Tree of Life. Understanding species' physiological sensitivity to temperature change and how they relate to local temperature variation in their habitats is crucial to determining vulnerability to global warming.

2. We ask how species' vulnerability varies across habitats and elevations, and how climatically buffered microhabitats can contribute to reduce their vulnerability.

3. We measured thermal sensitivity (critical thermal maximum—CT_{max}) of 14 species of *Pristimantis* frogs inhabiting young and old secondary, and primary forests in the Colombian Andes. Exposure to temperature stress was measured by recording temperature in the understorey and across five microhabitats. We determined frogs' current vulnerability across habitats, elevations and microhabitats accounting for phylogeny and then ask how vulnerability varies under four warming scenarios: +1.5, +2, +3 and +5°C.

4. We found that CT_{max} was constant across species regardless of habitat and elevation. However, species in young secondary forests are expected to become more vulnerable because of increased exposure to higher temperatures. Microhabitat variation could enable species to persist within their thermal temperature range as long as regional temperatures do not surpass +2°C. The effectiveness of microhabitat buffering decreases with a 2–3°C increase, and is almost null under a 5°C temperature increase.

5. Microhabitats will provide thermal protection to Andean frog communities from climate change by enabling tracking of suitable climates through short distance movement. Conservation strategies, such as managing landscapes by preserving primary forests and allowing regrowth and reconnection of secondary forest would offer thermally buffered microhabitats and aid in the survival of this group.

1 | INTRODUCTION

The accelerated rate of global warming is driving a global extinction crisis (IPBES, 2019). As warming accelerates, species will have to shift their distributions to more favourable environments, adapt in situ or acclimate via pre-existing phenotypic plasticity (IPCC, 2014; McDonnell & Chapman, 2015). For organisms unable to move to more favourable environments (e.g. those living in high mountain environments incapable of moving upwards), physical shelters, such as epiphytes, tree holes and leaf litter, can provide refuge during unusually hot or dry conditions (González del Pliego et al., 2016; Scheffers, Edwards, Diesmos, Williams, & Evans, 2014). This is especially important in the tropics as organisms living in near-constant temperatures may be severely impacted due to their small thermal ranges (Deutsch et al., 2008).

Negative effects of global warming on biodiversity are likely amplified by other threats, including land use change (Hof, Araujo, Jetz, & Rahbek, 2011; Mantyka-pringle, Martin, & Rhodes, 2012). Under specific circumstances, human pressures and land use can result in switches towards hotter–drier conditions (González del Pliego et al., 2016). The habitat type thus interacts with individuals shaping their ability to cope with changes in climate. Tropical species may be particularly vulnerable, as the tropics suffer some of the highest rates of deforestation globally (Hansen et al., 2013). In some cases, these areas also experience high rates of secondary forest regrowth on abandoned farmland (Aide et al., 2013), which benefits threatened and endemic tropical biodiversity (Basham et al., 2016; Gilroy et al., 2014). As they age, secondary forests increase the density of thermally buffered microhabitats (González del Pliego et al., 2016). However, secondary forests might not have sufficient thermal refugia for biodiversity under predicted climate warming conditions.

The vulnerability of species assemblages to climate change largely depends on the environmental regimes they are exposed to and their sensitivity, determined by physiological responses (Williams, Shoo, Isaac, Hoffman, & Langham, 2008). For example, species community composition across habitats with different levels of deforestation is explained by the differences in species' thermal niches (Frishkoff, Hadly, & Daily, 2015). Ectotherms are especially dependant on environmental temperature and precipitation (especially water-dependent ectotherms) to perform their basic bodily activities (e.g. food intake, reproduction). Measuring species critical thermal maximum—CT_{max} (the thermal point at which an animal loses its locomotory ability to escape from conditions that will lead to its death; Cowles & Bogert, 1944) would reveal the tolerance to high temperatures of a given species. Moreover, determining which species currently live closest to their CT_{max} will help predict performance in warmer conditions.

We assessed physiological responses of frogs to projected temperature increases by testing whether CT_{max} varied across environmental conditions associated with elevation and habitat degradation in the western Colombian Andes. Specifically, we ask: (a) Are species in secondary forests more threatened by global warming than primary forest species considering the higher CT_{max} of species in secondary forest and the higher temperatures of secondary forests? (b) How important are microhabitats to amphibian conservation in reducing the impact of extreme maximum temperatures across forest types? (c) To what extent might microhabitats continue to provide thermal protection to amphibians in secondary forests under future climate change scenarios? By answering these questions, we assess species vulnerability to climate change and suggest landscape management strategies that can aid amphibian conservation.

2 | MATERIALS AND METHODS

2.1 | Study site

Our research was conducted on the western cordillera of the Colombian Andes, in the Reserva Mesenia-Paramillo cloud forest, Antioquia (−75.8895 lon, 5.4950 lat; Figure S1). The study area covered an altitudinal range of 2,150–2,690 m a.s.l., and encompasses primary forest, naturally regenerating secondary forests and an agricultural matrix dominated by cattle pasture, embedded within contiguous primary forest (<1,000,000 ha). This region supports one of the highest global diversities of threatened and endemic amphibians (Jenkins, Pimm, & Joppa, 2013). Our site has an annual rainfall between 5,000 mm and 12,000 mm/year and the average relative humidity is 97% (Poveda et al., 2005).

2.2 | Study organisms

Between July and August 2014, we collected individuals of 14 species of *Pristimantis* frogs. These small frogs (range 10–37 mm) are abundant and share similar microhabitats (Acosta-Galvis & Cuentas, 2016; Basham et al., 2016). Frogs were sampled in 25 × 10 m transects each separated by 200–300 m in primary forest (20 transects), old secondary forest (OSF) ≥19 years old (four transects) and young secondary forest (YSF) <19 years old (11 transects). Frog species were identified by a regional expert (ARA-G; see Supporting information).

2.3 | Sensitivity—Critical thermal maximum (CT_{max})

We use the loss of righting response as our CT_{max} proxy (Catenazzi, Lehr, & Vredenburg, 2014; Navas, Antoniazzi, Carvalho, Suzuki, & Jared, 2007). Frogs were acclimated at similar temperature in

the field laboratory for 3–5 days before experiments (Catenazzi et al., 2014). We placed frogs in transparent plastic cups with a thin layer of water (1.5 ml; Navas et al., 2007), and cups were positioned in a water bath. The temperature of the water bath was increased from approximately 17–34°C (Catenazzi et al., 2014) at a rate of 0.45°C ($\pm$0.005 SE) per minute. Although experimentally induced temperature increase might not reflect natural conditions, an increase rate of <1°C has provided satisfactory CT_{max} results in previous studies (Catenazzi et al., 2014; Frishkoff et al., 2015; Navas et al., 2007).

We used the probe of a two-channel digital thermometer (K-type; resolution: 0.1°C, accuracy: $\pm$0.1°C) to turn frogs on their backs at regular intervals, and incited individuals to righten by touching their flanks and inner thighs. After 5 s, if the frog was incapable of rightening, we registered its temperature by touching one flank with the probe (Catenazzi et al., 2014; Navas et al., 2007). We considered this temperature as the core body temperature, because the small size of these frogs facilitates heat transfer between the water and the frog's body (Catenazzi et al., 2014; Herrando-Pérez et al., 2019; Navas et al., 2007). Frogs were immediately placed in a cool container and observed for 24 hr to verify they were ready for release (all individuals recovered fully).

2.4 | Exposure—Environmental temperatures

To quantify the degree to which temperature and precipitation are projected to change in our study area, we collected the bioclimatic variables of monthly average maximum temperature and annual precipitation from WordlClim projections for 2070 (Fick & Hijmans, 2017). We obtained the highest available resolution (~1 km^2) projections for the most recent data (average 1970–2000) and the four future climate scenarios (RCP 2.6, RCP 4.5, RCP 6.0 and RCP 8.5) for 30 transects where frogs were collected (overlapping eleven ~1 km^2 projection grids; see Figure S2a).

Most projected warming relies on environmental temperatures taken at a much higher resolution than the organism's immediate habitat (Lenoir, Hattab, & Pierre, 2017; Potter, Arthur Woods, & Pincebourde, 2013), thus missing fine-scale variation that likely increase availability of suitable climates (Figure S2). To account for such variability, we recorded daily temperatures where individuals were sampled. We measured understorey ambient and microhabitat temperatures using 84 iButton loggers (model: DS1921G-F5; accuracy: 0.5°C) from March 2013 to February 2014. Loggers were placed within 16 amphibian sample transects (see Supporting information).

To capture understorey ambient temperature buffered by aboveground vegetation, we placed iButtons approximately 1.5 m aboveground (Scheffers et al., 2013) in 16 transects (PF: six, OSF: four, YSF: six). *Pristimantis* frogs are mainly found between the ground and 1.5 m above-ground (see Supporting information), and thus nocturnal understorey ambient temperature reflects the temperature to which these nocturnal frogs are usually exposed.

Temperature loggers were also deployed within microhabitats used by frogs during the daytime (see Supporting information for microhabitat use and selection), and in the same transects as ambient loggers. Data loggers were placed: (a) approximately 20-cm deep in soil ($n = 7$); (b) approximately 5-cm under leaf litter ($n = 16$); (c) within fern roots ($n = 16$); (d) inside holes at the base of trees ($n = 16$); and (e) between the leaves of bromeliads ($n = 13$). All loggers were placed within 8 m of the understorey ambient logger. Only one iButton was placed within a particular microhabitat per transect.

2.5 | Analyses

2.5.1 | Sensitivity

Previous research has found a strong phylogenetic signal of realized climate niches of amphibians around the world (Hof, Rahbek, & Araújo, 2010), a pattern supported with experimentally derived measurements of CT_{max} (Araujo et al., 2013), especially in *Pristimantis* (Pintanel, Tejedo, Ron, Llorente, & Merino-Viteri, 2019). Thus, to estimate the phylogenetic dependence of the data, we measured Pagel's lambda (λ) using the phylosig function in the R package PHYTOOLS (Revell, 2012). Lambda values tend to range from 1 (strong phylogenetic signal) to 0 (no phylogenetic signal). As we observed a strong lambda value from CT_{max} ($\lambda = 0.85$), we used a phylogenetic approach. We performed a linear mixed effect model using lmekin function from the COXME package (Therneau, 2020). This model assessed the impact of individuals' body size (SVL), elevation, forest type, and the interaction between elevation and forest type, on individuals' CT_{max}. To account for phylogeny, we used a randomly selected subsample of 20 phylogenetic trees from Jetz and Pyron (2018; for information about the phylogeny see Supporting information). The phylogenetic approach is described in detail under the *Vulnerability across elevation and habitat types* section.

We then used linear regressions to explore the relationship between (a) body size and elevation; and (b) CT_{max} and number of acclimation days, because this variable might influence CT_{max} variation (Hutchison & Rowlan, 1974). Finally, we performed an ANOVA to determine if CT_{max} values were lower for primary forest-restricted species compared to those that occur in secondary forests.

2.5.2 | Exposure

To quantify how much temperature and precipitation are projected to change in our study area, and to determine if both low and high elevation areas in our study area will warm evenly under climate change scenarios, we calculated the projected increase of temperature (°C) by subtracting the current annual average maximum temperature (1970–2000) from the annual average maximum temperature projected for 2070 for each transect under every climate change scenario (RCP 2.6, RCP 4.5, RCP 6.0 and RCP 8.5). We then performed linear models explaining: (a) the change in current and projected maximum temperatures across elevation and (b) the projected increase of temperature

depending on elevation across the four climate change scenarios. We also calculated the projected increase in precipitation for each transect under each climate change scenario.

2.5.3 | Vulnerability to extreme maximum temperatures (Thermal margin of safety)

To determine exposure to extreme maximum temperature we used the iButton data, as it represents the temperature that individuals experience more accurately than WorldClim data (Figure S2). For each individual we examined extreme annual average maximum temperatures from the understorey, and microhabitats from the iButtons located closest to that individual, considering both elevation and habitat type. We defined extreme average maximum temperature (or extreme outliers) as all temperature data values that are 1.5 times the interquartile range of our data (the difference in the response variable between its first and third quartiles) above the third quartile (for maximum temperatures; Scheffers et al., 2014).

We then calculated thermal margin of safety (TMS) as the degrees between the extreme average maximum temperature and CT_{max} (Deutsch et al., 2008).

$$TMS = CT_{max} - T_{ex}$$

TMS is calculated as each individual's critical thermal maximum (CT_{max}) minus the extreme average maximum temperatures that each individual experiences (T_{ex}), across understorey or the different microhabitats.

Thus, TMS indicates whether individuals have sufficient thermal tolerance to cope with extreme temperatures (i.e. whether exposure is close to lethal limits). We expected TMS from all individuals to be currently positive thus indicating 'safety'. However, negative values would indicate 'danger'. Note that we measured CT_{max} at loss of righting response rather than at onset of spasms and, as such, our estimates of TMS could be considered conservative.

2.5.4 | Vulnerability across elevation and habitat types

To determine the relationship between the TMS of amphibians across elevation, forest type, the interaction between elevation and forest type, and body size, we fitted a linear mixed effect model that accounts for phylogenetic dependence. We used the function lmekin in the R package COXME (Therneau, 2020) and a subsample of 20 phylogenetic trees from Jetz and Pyron (2018; see Supporting information and Figure S3 for comparison with cattle pasture). We performed these TMS models for understorey ambient and the different microhabitats. This model produces a z-value that represents the contribution of each predictor (Table 1). Our model included residual variance associated with both the individual measurement (e.g. species-specific variation in CT_{max}) and phylogeny (shown in Table S1). We specified the variance–covariance structure of the residual variance as a vector of three errors (e; following Freckleton & Rees, 2019; see Supporting information for a description of the residual variance vector of errors).

TABLE 1 Summary of linear mixed effect models accounting for phylogenetic effect of current thermal margin of safety of *Pristimantis* species in diurnal and nocturnal ambient temperature and different microhabitats across all pairwise comparisons of forests in Colombian Andes. YSF, young secondary forest; OSF, old secondary forest; PF, primary forest

| | z score; p value | Differences on thermal margin of safety across habitats | | | Interaction between elevation and habitat | | |
		YSF versus PF	OSF versus PF	YSF versus OSF	YSF versus PF	OSF versus PF	YSF versus OSF
Understorey diurnal	z	5.01	1.09	2.55	4.66	1.11	2.49
	p	<0.001*	0.28	0.01*	<0.001*	0.27	0.01*
Understorey nocturnal	z	0.71	1.04	1.22	0.53	1.07	1.19
	p	0.48	0.30	0.22	0.59	0.29	0.23
Bromeliad	z	0.85	1.62	1.32	0.99	1.64	1.28
	p	0.40	0.10	0.19	0.32	0.10	0.20
Tree fern	z	0.75	1.61	1.33	0.99	1.64	1.28
	p	0.45	0.11	0.18	0.32	0.10	0.20
Tree hole	z	0.80	1.62	1.33	0.99	1.64	1.28
	p	0.42	0.10	0.18	0.32	0.10	0.20
Leaf litter	z	0.80	1.61	1.32	0.99	1.64	1.28
	p	0.42	0.11	0.19	0.32	0.10	0.20
Soil	z	0.75	1.62	1.35	0.99	1.64	1.28
	p	0.45	0.10	0.18	0.32	0.10	0.20

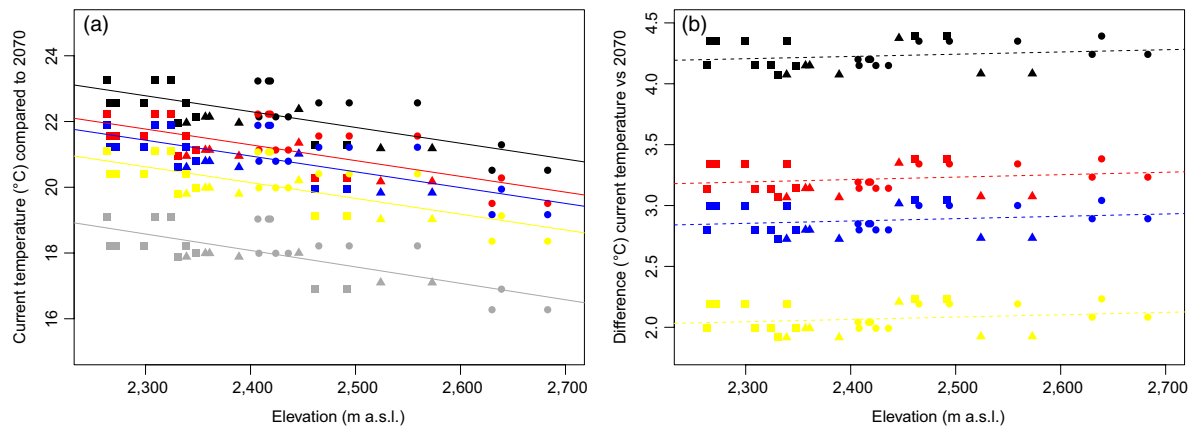*Significant relationships ($p < 0.05$). See Table S1 for the phylogenetic variances.

FIGURE 1 Annual maximum temperature currently and under different climate change scenarios across an elevational gradient in Colombia (a). Difference in annual maximum temperature between current temperature (represented by the 0 value in the y-axis) and projected temperature under different climate change scenarios (b). Each symbol represents one transect and habitat—squares: young secondary forest; triangles: old secondary forest; circles: primary forest. Colours represent different climate change projections—grey: current temperature (mean temperature 1970–2000); yellow: RCP 2.6; blue: RCP 4.5; red: RCP 6.0; black: RCP 8.5. Continuous line represents significant relationships ($p < 0.001$), segmented line represents non-significant relationship ($p > 0.05$)

We performed a piecewise regression (SEGMENTED package) to illustrate the two clear relationships in our data unveiled by the linear mixed effect model. This method revealed the elevational point at which the slope of the TMS was partitioned into two groups (2,321 m a.s.l. was the break point), and a regression model was fitted to each group for illustrative purposes.

2.5.5 | Vulnerability under future warming

Barring successful implementation of COP21 targets to limit warming well below 2°C, ambient temperatures in our study area are projected to increase across elevation by 2–5°C by 2070 (Figure 1). Therefore, we selected four different climate change scenarios to determine the impact on species sensitivity: (a) 1.5°C increase reflecting the COP21 aspirational limit; (b) 2°C increase pre-COP21 best-case scenario targets (RCP 2.6); (c) 3°C increase as a more feasible scenario (RCP 4.5, 6.0; Figure 1) and (d) a worst-case scenario (RCP 8.5) of a 5°C increase. All analyses were conducted using R (version 3.3.1 R Core Team, 2016).

3 | RESULTS

3.1 | Sensitivity

Primary and young secondary forest had the highest and lowest species richness respectively (for a closer look into community composition see Supporting information). Species' CT_{max} was primarily driven by the size of the individual ($z = 6.69$, $p < 0.001$). CT_{max} was constant across elevation ($z = -1.08$, $p = 0.28$), similar across forest types ($z < 1.30$, $p > 0.19$), was not affected by the interaction between elevation and forest type ($z < 0.99$, $p > 0.20$; Figure 2b) and was not driven by number of acclimation days ($F_{1,242} = 0.36$, $p = 0.54$; Figure S4a). Although CT_{max} was positively related to the size of the individual
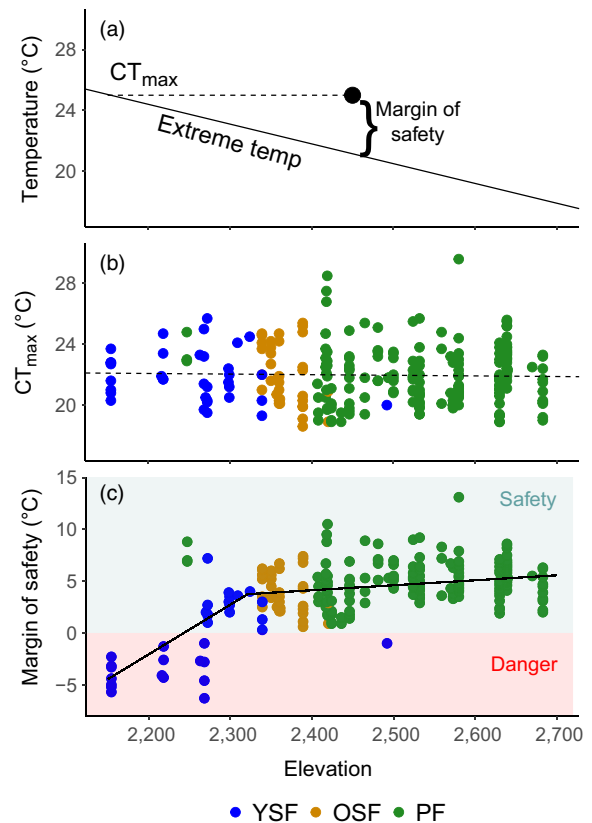

FIGURE 2 Graphical representation of thermal margin of safety as a function of the degrees between extreme maximum temperatures and critical thermal maximum—CT_{max} (a; see Figure S8 for temperatures across elevation). Linear model from the critical thermal maximum (b) and piecewise regression from the thermal margin of safety from understorey ambient (c) from *Pristimantis* frog species in different habitat types (blue: young secondary forest; orange: old secondary forest; green: primary forest) across an elevational gradient in the Colombian Andes. Each dot represents an individual. Dashed line represents non-significant relationship ($p > 0.05$); solid line represents significant relationship ($p < 0.05$)

($t = 7.77$, $R^2 = 0.19$, $p < 0.001$; Figure S4b), body size did not change with elevation ($t = 0.49$, $R^2 < -0.01$, $p = 0.61$; Figure S5).

3.2 | Exposure

The highest ambient temperatures were recorded in YSF (22.58°C), followed by OSF (18.12°C), and the lowest were recorded in PF (16.96°C). This was true for all microhabitats (Table S2). Although maximum temperatures decreased with elevation ($t = -5.34$, $p < 0.001$, Figure 1a), we found that both low and high elevation areas in our study area are predicted to warm evenly under every RCP climate scenario (Figure 1b; $p > 0.05$). Precipitation is also projected to increase in our study area by 11%–35% by 2070 under every future climate scenario and the percentage of change is similar across all habitat types ($F_{2,27} = 0.97$, $p = 0.38$; Figure S6). Precipitation projections, however, showed no linear temporal increase, meaning predictions as to the effect of future precipitation on amphibians were unattainable.

3.3 | Vulnerability

Our data showed that the TMS in understorey nocturnal ambient temperature (the temperature these frogs are exposed to when most active) was positive. There were no differences on the TMS across habitats, elevation or the interaction between both (Figure 3a; Table 1). Species TMS in understorey diurnal ambient was lower in YSF than that in both OSF and PF ($p < 0.05$; Table 1), yet TMS was similar between OSF and PF ($z = 1.09$, $p = 0.28$). Although TMS was not driven by elevation ($z = -0.73$, $p = 0.47$), the interaction between elevation and forest type proved to be significant when comparing YSF to OSF ($p = 0.01$) and when comparing YSF to PF ($p < 0.01$; Table 1). Maximum temperatures during the day currently exceed the thermal tolerance of most species in YSF, making frogs in YSF considerably more vulnerable than those in OSF and PF when exposed to diurnal ambient temperatures (Figure S7). Nevertheless, across all forest types, after incorporating the thermal buffering capacity of microhabitats, all species were safe under current diurnal levels of exposure (Figure 3b–f). There were also no longer differences in the TMS across habitats after incorporating the thermal buffering capacity of microhabitats (Table 1). Relative to understorey diurnal ambient temperatures, microhabitats in YSF were 5.58°C cooler on average, 2.03°C cooler on average in OSF and 1.56°C cooler on average in PF (Table S2).

3.4 | Vulnerability under future warming

Species TMS in the understorey diurnal ambient decreased across all habitat types even with the most conservative warming scenario (1.5°C increase; Figure S7). With a 3°C temperature increase
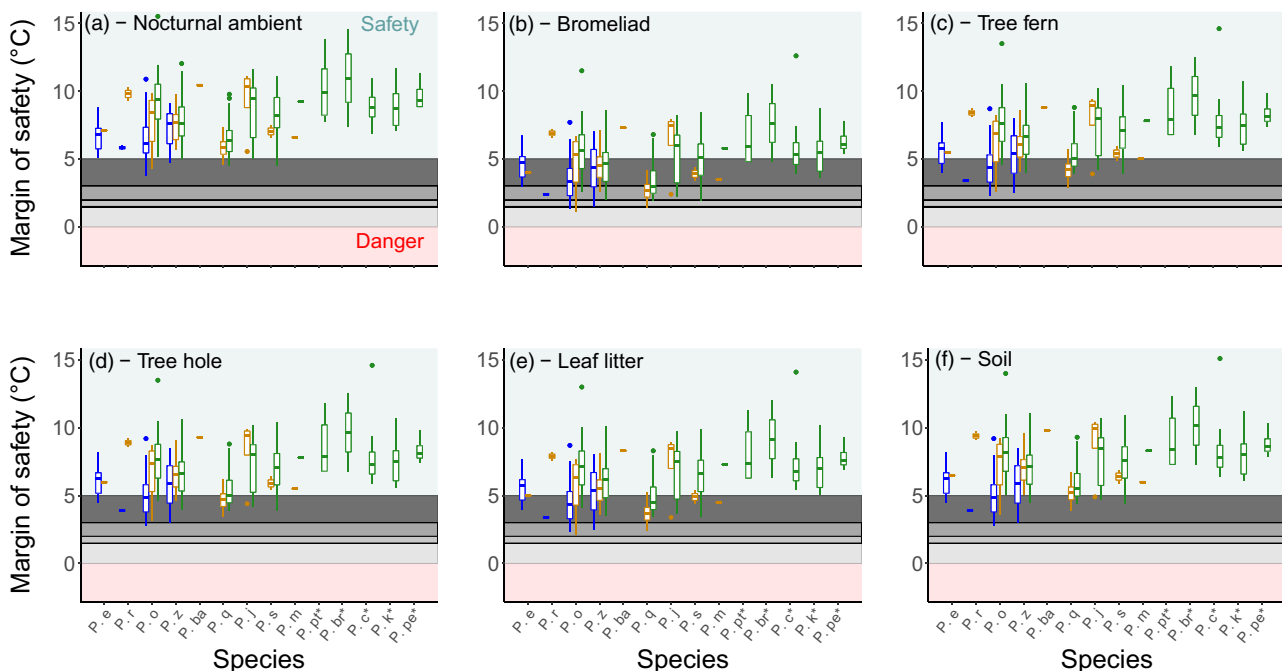

FIGURE 3 Amphibian thermal margin of safety (°C) in young secondary (blue), old secondary (orange) and primary forests (green) along an elevational gradient in the Colombian Andes. Boxplots of the predicted thermal margin of safety of amphibians experiencing the nocturnal mean temperatures of ambient air (a) and the diurnal extreme maximum temperatures within specific microhabitats (b–f). The grey bars represent the temperature increase for different global warming scenarios: light grey: 1.5°C; medium light grey: 2°C; medium dark grey: 3°C; dark grey: 5°C. Species: *P. e, Pristimantis erythropleura*; *P. r, P. ruedai*; *P. o, P. orpacobates*; *P. z, P. zophus*; *P. ba, P. baiotis*; *P. q, P. quantus*; *P. j, P. juanchoi*; *P. s, P. sp.*; *P. m, P. myops*; *P. pt, P. ptochus*; *P. br, P. brevifrons*; *P. c, P. calcaratus*; *P. k, P. kelephus*; *P. pe, P. permixtus*. Asterisk in the x-axis refers to primary forest-restricted species. The zero value in the y-axis is calibrated with respect to current conditions. Values below zero represent thermal danger. Values above zero represent thermal safety

in our study site, all (4/4) species from YSF, most species (5/9) from OSF and five species (5/11) from PF will become vulnerable to maximum temperatures if exposed to understorey diurnal ambient temperatures (Figure S7). Moreover, even if the aspirational COP21 limits of a 1.5°C temperature increase are met, all (4/4) species from YSF, three species (3/9) from OSF and four species (4/11) from PF will still become vulnerable to maximum temperatures if exposed to understorey diurnal ambient temperatures. Considering the worst-case scenario, a 5°C increase will put all but one species in peril (*P. permixtus*; Figure S7a). In contrast, species TMS in the understorey nocturnal ambient will continue to be positive unless temperatures increase 5°C (Figure 3a; Figure S7b). It is worth noting that the RCP scenarios are used as a baseline of potential temperature increase. Future TMS will depend on several factors, such as precipitation, forest regrowth and microhabitat availability.

Assuming that the buffering capacity of microhabitats will remain similar under global warming, under a 1.5°C temperature increase, species actively searching for thermally buffered microhabitats will be able to find thermal refuge (represented by the lower quartile being within the safety zone in Figure 3) across all habitat types (Figure 3b–f). A 2°C and 3°C temperature increase would result in most microhabitats, except bromeliads (Figure 3b), offering climatic refuge for all species. A 5°C temperature increase would only provide thermally buffered microhabitats for primary forest-restricted species (Figure 3), and would be lost in secondary forests. Moreover, soil would continue to provide a climatic refuge, unless temperature increases 5°C (Figure 3f).

4 | DISCUSSION

We found that while the sensitivity of frog species to extreme maximum temperatures is similar across habitats, the use of microhabitats reduces species' exposure to stressful temperatures. We found that species in young secondary forests displayed the lowest TMS, a trend worsening under future warming scenarios. Importantly, microhabitats can allow frogs to reduce climate exposure if future temperature increases do not surpass +2°C. Our study underscores the importance of microhabitats for amphibian survival. In an increasingly human-dominated era, protecting primary forests remains a critical conservation goal (Edwards et al., 2019; Gibson et al., 2011). However, managing landscapes by allowing the regrowth and reconnection of secondary forest will increase availability of thermally buffered microhabitats (Senior, Hill, & Edwards, 2019), thus protecting amphibians from threatening temperatures. These feasible strategies should represent an urgent investment for amphibian conservation.

4.1 | Sensitivity—CT_{max}

We discovered no variation in species' CT_{max} across elevation, contrary to studies showing that high-altitude species have lower CT_{max} than low-altitude species, both in insects (Garcia-Robledo, Kuprewicz, Staines, Erwin, & Kress, 2016) and amphibians (Pintanel et al., 2019). Yet, the outcomes of studies analysing the effect of elevation on CT_{max}—or of any other thermal gradient—have been mixed (Clusella-Trullas & Chown, 2014; Herrando-Pérez et al., 2020). Our results, however, may be a product of scale, as we used a relatively narrow elevational range (~10% of *Pristimantis* range) compared to other studies (Catenazzi et al., 2014; Pintanel et al., 2019; von May et al., 2017), or due to phylogenetic constraints on CT_{max} across lineages (Araujo et al., 2013).

Species CT_{max} also exhibited no variation between habitats, even when CT_{max} in ectotherms is known to vary across related species and habitats (Catenazzi et al., 2014; Nowakowski et al., 2016; von May et al., 2017). For example, forest-restricted species have lower CT_{max} than species from altered habitats (Nowakowski et al., 2016). The similar CT_{max} we observed across habitats may be a result of land use changes being too recent and current levels of exposure not being sufficiently extreme to drive differences in thermal tolerance in our system. Species thermoregulating (i.e. using microhabitats as a thermal refuge) might be reducing the need for local physiological adjustments to the thermal environment (Huey, Hertz, & Sinervo, 2003) because these frogs would experience similar minimum (nocturnal) temperatures (Figure S8). Also, the high precipitation in our study area could be buffering the relationship between temperature and CT_{max}, because heat resistance decreases when precipitation increases (Kellermann et al., 2012). This thermal insulation by precipitation would reduce the phenotypic response of these populations to hot temperatures (Kellermann et al., 2012), potentially leading to populations in YSF that are maladapted to hot temperatures (Angilletta, 2009).

4.2 | Exposure and vulnerability

Despite using a narrow elevational range, the effect of elevation and habitat on the TMS of species in YSF was evident, showing that multiple factors determine the overall vulnerability of species. Indeed, the TMS increased steeply in YSF up to 2,321 m a.s.l., after which the TMS remained constant across elevation, and OSF and PF. Considering the near-constant CT_{max} across the elevational gradient (Figure 2b), the near-constant lapse rate of ambient temperatures (Figure 1a), and the fact that elevation was unrelated to the TMS of species, the dramatic shift in TMS from low to high elevations (Figure 2c) is driven by local habitat temperatures in YSF and the interaction between YSF and elevation. Although thermal physiology is relevant in determining where species live (von May et al., 2017), our results suggest that the vulnerability of these *Pristimantis* frogs would mainly be driven from exposure rather than sensitivity. For example, frog communities appeared to be filtered by temperature conditions across a landscape of deforestation (Frishkoff et al., 2015). Forest vegetation decreases understorey temperature across all habitat types (González del Pliego et al., 2016), meaning that species living in denser, more

structurally complex forests (i.e. PF) experience lower temperatures than species living in less complex forests (i.e. YSF). Also, microhabitats are less abundant in YSF, where they are potentially more necessary due to higher maximum temperatures (González del Pliego et al., 2016). Lower TMS in YSF means that frogs are considerably more vulnerable than those found in OSF and PF. Most mountain tops in our study area still retain PF, and species in PF have larger TMS and thus should be less impacted by peak temperatures (Duarte et al., 2012).

4.3 | Vulnerability under future warming

During extreme maximum temperatures, the ability of different species to find refuge in thermally buffered microhabitats will depend on the overall temperature increase (Figures 1 and 3). It will also depend on the microhabitat complexity of the forest, the thermal refugia available (González del Pliego et al., 2016; Pinsky, Eikeset, McCauley, Payne, & Sunday, 2019) and the effect that future projected increases in precipitation will have on the buffering ability of microhabitats. The warming rate in the Tropical Andes has already tripled since 1939 (Vuille & Bradley, 2000), and even if we limit global warming to well below 2°C, as agreed in the 2015 Paris Agreement (COP21), the understorey diurnal ambient temperature will surpass the CT_{max} of most species living in secondary forests. However, with this 1.5°C increase in temperature, microhabitats across all habitat types will continue to provide a thermal shelter. Moreover, if temperature increases by 2°C or 3°C, most microhabitats would still offer climatic refuge during extreme maximum temperatures during the day. Our worst-case scenario, a 5°C increase, would result in the understorey nocturnal ambient temperature being higher than the CT_{max} of almost all species across all YSF and OSF, causing potential future local extinction from acute thermal stress.

5 | CONCLUSIONS

The impacts of land use and climate change will continue to create major challenges for species survival in the tropics (Edwards et al., 2019), especially among frogs (Hof, Araujo, et al., 2011). Biotic and abiotic variables affect the biological responses of ectotherms—such as deforestation, spread of pathogens, increasing temperatures and the projected increase of precipitation in our study area—increasing the uncertainty and complexity of predicting the impacts of climate change (Clusella-Trullas, Blackburn, & Chown, 2011; Hof, Araujo, et al., 2011). Indeed, indirect effects could increase species' vulnerability to climate change and cause population declines before temperatures in the microhabitats exceed their critical thermal maximum. Our results indicate that thermal limits are similar across habitats and elevations. How this pattern holds across this highly diverse genus comprised of over 500 species requires further consideration. Species in young secondary forests in particular are more vulnerable

to global warming. However, by use of thermal refuges, most species could avoid changing their distributions if the temperature increase does not surpass +2°C.

Our results contribute to understanding the important role microhabitat variability play in fostering species adaptation (see also Hof, Levinsky, Araújo, & Rahbek, 2011). As such, a useful tool in applied forest management would be the translocation of microhabitats (e.g. bromeliads) into medium-aged secondary forests, thus providing thermally protected shelters. Our analyses also suggest that conservation efforts should be put towards managing landscapes to preserve and reconnect primary forests and allow natural regrowth of secondary forests over long time frames—an on-the-ground conclusion that matches those of regional to global modelling projects of thermal connectivity (Alagador et al., 2012; Senior et al., 2019). We suggest that, wherever possible, species' vulnerability and landscape connectivity are considered to inform conservation decision-making and to establish management objectives.

COMPETING INTERESTS
The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS
P.G., B.R.S., R.P.F. and D.P.E. conceived the ideas and designed the methodology; P.G. and E.W.B. collected the amphibians; P.G., E.W.B. and A.R.A.-G. identified the species; P.G. performed CT_{max} experiments, analysed the data and led the writing of the manuscript; B.R.S., R.P.F., D.P.E., M.B.A., C.A.M.U. and T.H. gave important intellectual input. All the authors revised the manuscript critically.

DATA AVAILABILITY STATEMENT
Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.931zcrjhj (González-del-Pliego et al., 2020).

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