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# Ecophysiological strategies to daily air temperature variations in two key species in the northwestern slope of the superpáramo of the Antisana volcano

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Certifico que la tesis de grado de Magister de Biología de la Conservación del candidato Ricardo Mauricio Jaramillo Terán ha sido concluida de conformidad con las normas establecidas, por lo tanto puede ser presentada para la calificación correspondiente.

Erika Priscilla Muriel Mera, Ph.D.
Directora de tesis

Dedico este trabajo a todos quienes
nos esforzamos por la conservación
de la naturaleza

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# Ecophysiological strategies to daily air temperature variations in two key species in the northwestern slope of the superpáramo of the Antisana volcano

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## Abstract

***Background:*** Tropical alpine plants experience daily freezing risk, yet little is known about the interaction between their surroundings and thermal traits. Existent studies are based on information taken over short periods of time, which does not allow to understand the effects of inter-annual climate variations on plants.

***Aims:*** To evaluate if *Werneria nubigena* and *Xenophyllum rigidum* present similar response mechanisms to freezing, determine if their inner leaf temperature (ILT) is controlled by microhabitat conditions, and if the exotherm temperature variation depends on the correspondent response to freezing.

***Methods:*** We measured the ILT and the micro environmental temperature, and evaluated the exotherm and freezing injury temperature ($LT_{50}$), on the Antisana Volcano.

***Results:*** The thermal niche of both species was warmer than the registered ILT. The exotherm and $LT_{50}$ relation revealed that both species presented a *tolerance mechanism* to freezing with an inversed relation with elevation. Microhabitat substrates (rock and vascular plants) surrounding the plants determined the exposure to freezing for both species, having a significant inverse relation with the ILT.

***Conclusions:*** Our results indicate that independently of their tolerance mechanism, microhabitat conditions could be the primary filter when determining the exposure of these tropical alpine species to air freezing temperatures.

Keywords

Inner leaf temperature, equatorial Andes, freezing tolerance, microhabitat.

## Resumen

***Antecedentes:*** Las plantas alto andinas experimentan riesgos diarios por congelamiento, sin embargo se conoce todavía poco sobre la interacción de su ambiente y sus caracteres térmicos. Los estudios existentes se basan en información obtenida en períodos costos de tiempo, lo cual no permite entender los efectos de las variaciones climáticas inter-anuales sobre las plantas.

***Objetivos:*** Evaluar si *Werneria nubigena* y *Xenophyllum rigidum* presentan mecanismos de respuesta similares al congelamiento, determiner si la temperatura interna foliar (TIL) es controlada por el microhábitat y si la variación de la exoterma depende de la respuesta al congelamiento.

***Métodos:*** Medimos la TIL y la temprERatura micro ambiental y se evaluó la exoterma y temperatura de daño ($LT_{50}$)*,* en el Volcán Antisana.

***Resultados:*** El nicho térmico de ambas especies fue más caliente que el TIL de las dos especies. La relación entre la exoterma y $LT_{50}$ reveló que ambas especies presentan un mecanismo de tolerancia al congelamiento con una relacion inversa a la elevación. Los sustratos del microhábitat (roca y plantas vasculares) que roderon a las plantas determinaron la exposición de las mismas al congelamiento, teniendo una relación inversa significante con la TIL.

***Conclusiones:*** Nuestros resultados indicant que independientemente del mecanismo de tolerancia, el microhábitat podrían ser un filtro primario para determiner la exposición de estas especies tropicales alpinas al congelamiento.

Palabras clave

Temperatura interna foliar, Andes ecuatoriales, tolerancia al congelamiento, microhábitat.

## Introduction

Tropical high mountain ecosystems (Páramos hereafter) are stress-dominated habitats characterized by low air and soil temperatures and high frequency of frost occurrence which can take place in any night of the year (Márquez et al. 2006, Rundel et al. 2008); also, low atmospheric pressure, intense ultra-violet radiation and the drying effects of wind (Buytaert et al. 2006). Usually, the months between June–August correspond to a marked seasonality in the monthly rainfall patterns (~200 mm), triggering the frequency of night frosts events, ranging from -5 ºC to -10 ºC (Sklenář et al. 2008). Freezing temperatures events during day and night are not rare in Páramo habitats (Cuesta et al. 2016). The ability of plants to withstand freezing temperatures and the formation of ice crystals within its tissues under these extreme climatic conditions should be considered one of the most important abiotic determinants (i.e. filters) in structuring high Andean plant communities (Lortie et al. 2004), and a defining role determining its geographic distribution (Wisniewski et al. 2014). The relief in interaction with the wind influences the microclimate directly by affecting aerodynamic boundary layer, convective heat loss, evaporative cooling and the distribution of precipitation, snow in particular. Because slopes tend to be steeper in mountains these effects are more pronounced and climatic vectors are stronger here. This is of great importance because plant life becomes more dependent on decoupling from a “hostile” atmosphere the higher the elevation (Körner 2003). In Páramos environments the daily temperature variation greatly surpasses the seasonal variation and so, the climate on a daily basis can reach up to 28 °C at midday and decrease as low as below 0°C during the night (Hedberg 1995). Several studies have demonstrated that plants respond by either tolerating or avoiding daily freezing temperatures (García-Varela and Rada 2003, Rada et al. 2001, Rada et al.

1985, Rada et al. 1987, Sierra and Cavieres 2010, Sierra et al. 2009, Sierra-Almeida and Cavieres 2010, Sierra-Almeida and Cavieres 2012, Sklenář et al. 2012, Sklenář et al. 2010, Sklenár et al. 2016). While the former is associated with biochemical and physiological mechanisms that allow plants to tolerate the presence of ice in its tissues and dehydrative stress (Gusta and Wisniewski 2013), the latter is associated with structural aspects that determine when and where ice forms in a plant (Wisniewski et al. 2014) and can be obtained by two strategies: a) freezing prevention by protecting organs under 0ºC temperatures, by insulation, or b) prevention by the formation of ice under 0 ºC, avoidance of ice nucleation (Wisniewski et al. 2014). Tolerance mechanisms provide long-term protection against frost ceasing cells and tissues metabolic activity, while avoidance allows the survival of plants for short moderate freezing periods (Azócar et al. 2006).

The capacity of High Andean plant species to withstand extreme climatic events is of upmost importance given the observed climate warming and its likely impacts on the Páramos biota (Buytaert et al. 2011, Vuille et al. 2015), favouring selection and adaptive radiation among regional floras due to steep temperature gradients (Sakai and Larcher 1987). Thus, the necessity to generate information on the ecology and physiology of tropical alpine plants and their response to temperature changes is a primary step to design science-based conservation actions under a global change scenario. Nevertheless, to the best of our knowledge few studies have been carried out in the Andean Páramos related to plants ecophysiology (Azócar et al. 1988, García-Varela and Rada 2003, Márquez et al. 2006, Parmesan and Hanley 2015, Rada et al. 1985, Rada et al. 1987, Sklenář et al. 2012, Sklenář et al. 2010, Squeo et al. 1991, Squeo et al. 1996, von Fircks and Verwijst 1993). Most of these studies are based on information taken over short periods of time; therefore, they are not associated to a temporality that allows studying the effect of inter-annual climate changes on plants. Also, these studies are not associated to a thermal gradient and its relation to elevation and micro scale topography. This article deeps in previous works of Sklenář et al. (2010, 2012) by monitoring the inner foliar temperature of two key species (*Werneria nubigena* and *Xenophyllum rigidum*) of the superpáramo plant communities and environmental temperatures during six months with high daily resolution data (every 5 minutes). In this study, we intend to answer the following interrelated questions:

1) Do both study species have the same response mechanism to freezing events?

2) Is the inner leaf temperature of each species controlled by microhabitat conditions?

3) The exotherm temperature variation depends on the response mechanism to freezing events?

## Materials and methods

This study comprised the characterization of the thermal niche by 1) monitoring of the inner leaf and environmental temperature on the field during six months, and 2) the determination of the exotherm and damage by freezing under laboratory conditions.

### *Target species and study area*

We selected two common species of the superpáramo plant community based on their growth form, abundance and distribution patterns along the elevation gradient: *Werneria nubigena* Kunth (Asteraceae) and *Xenophyllum rigidum* (Kunth) V.A. Funk (Asteraceae). *Werneria nubigena* is a perennial rosette, which grows alone or in small groups on herbaceous páramos, it can be found from 4300 to 4900 m (personal observation). *Xenophyllum rigidum* is a conspicuous and common high Andean cushion plant. In Ecuador, it is common in the upper superpáramo (above 4500 m) plant communities (Sklenář 2000, Sklenář and Balslev 2007).

In the field, we recorded the inner leaf temperature of 16 and 15 individuals of *W. nubigena* and *X. rigidum* respectively (see below), along an elevation gradient (4300–5100 m). Additionally, for laboratory analyses we collected 14 and 15 individuals of *W. nubigena* and *X. rigidum*, respectively for the exotherm temperature determination, and 18 individuals of both species for the freezing damage analysis.

The study site is located at the superpáramo of the Antisana Volcano northwestern slope were the terrain of consists of glacial moraines, rocky and sandy slopes in the Eastern Ecuadorian Andes. The mean daily temperature reported for this area is 2.3 ºC, with a mean annual rainfall of 1098 mm (Sklenář et al. 2015). The site covers an elevation gradient from 4300 to 5100 m along lower superpáramo (4100 to 4500 m) and upper superpáramo (above 4500 m) (Sklenář and Balslev 2005). From 4200 to 4500 m, the site is dominated by grass species of the following genera: *Calamagrostis*, *Agrostis,* and *Festuca*. Additionally, sclerophyllous dwarf shrubs patches of *Loricaria* spp., *Chuquiraga jussieui*, *Valeriana microphylla* are common. Finally, there is an abundant diversity of rosettes, and creeping bushes and other herbaceous growth forms are common (Salgado et al. 2013, Sklenář and Balslev 2005). From 4500 to 5000 m the site is characterized for being an ultra humid subnival grassland, with disperse shrubs and cushions (*Xenophyllum spp.* and *Azorella spp.*) (Sklenář et al. 2008, Sklenář et al. 2010).

Ecuadorian Páramos, lying above the upper forest-line along the country's two mountain chains, consist of open herbaceous vegetation, grasslands, peat bogs, shrublands and sparse forest patches (Sklenář and Ramsay 2001). They are a tropical high altitude Andean ecosystem with cool temperatures (daily mean 2.6 ºC) (Sklenár et al. 2016) where the landscape has been shaped by glacial activity, tectonic uplift, and an active volcanic history (Hribljan et al. 2016). Páramos are characterized for seasonal and diurnal patterns of temperature changes. Low tropical latitudes have relatively constant levels of solar irradiance over annual cycles. The daily maximum irradiance is only 13% higher than the minimum level (Rundel et al. 2008). This low irradiance at tropical latitudes causes small changes in seasonal temperature, but strong diurnal patterns of change at high elevations, causing even a 14 ºC temperature range during the day. In addition to this, precipitation patterns are much more difficult to generalize because they vary depending on the region. Northern Andes (Colombia and Ecuador) are characterized by being a humid ecosystem in contrast with the central Andes, however, in general there are no specific precipitation patterns (Rundel et al. 2008, Sklenář et al. 2015).

### *Microclimate and internal leaf temperature (ILT)*

To reduce the intraspecific variation of the selected thermal traits, the individuals chosen for the field study had to comply with the following microhabitat conditions: similar orientation, slope, and exposure. For each individual, we recorded inner leaf temperature (ILT) every 5 minutes, using Cu-Co thermocouples associated to a data logger (Omega), between November 2014–February 2015 and between August–September 2015. We inserted the thermocouple at the base of the innermost leaves, trying to cause the least possible damage to the attached leaf. When the leaves presented symptoms of damage (e.g. change in colour), we removed the thermocouple and placed it into another leaf of the same individual. We also recorded soil and air temperature (-10 cm below ground and 10 cm above ground, respectively), at three altitudes (4460, 4500 and 4600 m.), every hour between October 2013–December 2015, to characterize microhabitat conditions of the study site. We defined freezing events as the lowest temperature (below 0 ºC) that was recorded in a time series (month, day) and freezing temperatures as sub-zero temperatures. Finally, we defined the thermal niche of both species by using the thermal optimum defined by the mean minimum and mean maximum temperatures of environmental data for each species.

### *Microhabitat conditions*

On the field, for each monitored individual we recorded micro topographic conditions for substrate cover (vascular plants, bryophytes and lichens, solid rock, scree, lichens, bare ground and litter) of its surroundings, using a 1 $m^2$ frame placed over the individual, following the GLORIA field protocol for vegetation monitoring (Pauli et al. 2015).

### *Exotherm measurement*

We collected a total of 14 and 15 individuals for *W. nubigena* and *X. rigidum,* respectively, along an elevation gradient (4300 to 5100 m), divided in three ranges every 200 meters (Table 1). We extracted whole individuals from the ground, and kept in plastic bags inside a cooler, at a mean temperature of 6 ºC before transporting them to the Ecophysiology Laboratory of the Pontificia Universidad Católica del Ecuador in Quito. At the laboratory, we inserted thermocouples into one of the leaves of each individual, which were then placed inside a freezer to get acclimatized at ~0ºfor 20 minutes. After this period of time, we lowered the temperature at a rate of approximately -5 ºC per hour, until reaching -26 ºC. After this threshold, we monitored constantly ILT in order to identify the exotherm (an abrupt rise of the inner leaf temperature), which is an indicator for water freezing in the leaf extracellular spaces (Poirier et al. 2010, Sklenář et al. 2012).

***Freezing damage***

We exposed six individuals of each species at each elevation range (a total of 18 plants per species) to the same freezing treatment as described above. After this, we detached leaves of each plant at regular temperature intervals (at about -0.18 ºC, -6.2 ºC, -10.8 ºC, -15.7 ºC, -21.8 ºC and -26.6 ºC). For both species, we used an area of 2 $cm^2$ of leaf tissue to assess freezing damage: for *Werneria nubigena* we obtained nine discs of 6 mm diameter, and for *Xenophyllum rigidum* we obtained longitudinal leaf cuttings until the standardized area of 2 $cm^2$ was reached. We placed cut leaves in 50 ml vials with 20 ml of deionized water. For each species, we used six vials per temperature treatment. We used a Revolver 360º Sample Mixer (Labnet International, Inc.) to shake the samples for 1 hour, and after that, the samples had to remain still for another hour. We defined a control sample for each species as an individual that would not be subject to freezing. We measured sample conductivity, using a CDH-SD1 conductivity meter (Omega, USA), for the samples subjected to freezing and for one unfrozen control sample per species. To control for size differences among the leaves, we standardized the degree of damage by freezing to the maximum conductivity value for each sample, which we obtained after boiling the sample for 15 minutes. The Percentage of Electrolyte Leakage (PEL) was defined as:

Eq. (1) $\mathrm{PEL} = \frac{e_s}{e_t} \times 100.$

Where $e_s$ is the conductivity of the sample after the freezing treatment and $e_t$ is the conductivity of the sample after boiling (Pérez-Harguindeguy et al. 2013). We estimated the freezing injury temperature at which 50% damage to the tissue occurred ($LT_{50}$), using the following linear equation:

Eq. (2) $f(x)= mx + b.$

Where f(x) is the freezing injury temperature ($LT_{50}$); x is the Percentage of Electrolyte Leakage (%PEL) and b is the intercept in the temperature axis; parameters of equation (m and b) vary between each individual from the data obtained from each PEL treatment. Then we calculated the corrected PEL as the difference between the observed PEL for a given freezing treatment and the PEL for the control treatment, to control for intrinsic differences in membrane permeability, experimental manipulations and differences in injury when leaf disks or fragments are cut:

Eq. (3) Corrected PEL = PEL in the freezing treatment - PEL in the control treatment.

### *Freezing response mechanism*

For each species the freezing resistance mechanism was determined by comparing $LT_{50}$ with exotherm obtained in the thermal analyses. When $LT_{50}$ was observed at a lower temperature than exotherm, the plant was classified as tolerant to extracellular ice formation. On the contrary, when $LT_{50}$ was not significantly different from exotherm, the resistance mechanism was classified as freezing avoidance (Sklenář et al. 2012).

### *Statistical analysis*

First, we submitted data of inner foliar temperature and exotherm determination to normality tests. We subtracted outliers from the tests, by eliminating the 95% and 5% percentiles, which correspond to extreme values of ILT and exotherm. We performed a non–parametric Wilcoxon test to compare pair means of ILT and environmental temperature between both species. Also, in order to determine the response mechanism of each species to freezing we performed a non-parametric Wilcoxon test between the means of the exotherm and $LT_{50}$ and considered a significant difference at $p<0.05$ to indicate freezing tolerance (i.e. extracellular ice formation), while we considered the lack of significance a freezing avoidance mechanism (Pescador et al. 2016, Sierra-Almeida and Cavieres 2012). Finally, a Principal Components Analysis was carried out to see how the local microhabitat conditions (biotic and abiotic) explained the differences between ILT differences between both species and $Lt_{50}$ and exotherm.

## Results

### *Microclimate and inner leaf temperature (ILT)*

The thermal niche of both study species was almost identical in spite of their significant difference (Z= -5.94, P<0.0001). For *Werneria nubigena* the thermal niche varied between -2.45 ± 0.01 ºC and 14.51 ± 0.02 ºC in contrast to *Xenophyllum rigidum* which varied between -2.38 ± 0.01 ºC and 14.46 ± 0.03 ºC. Individuals of *W. nubigena* and *X. rigidum* were exposed to freezing temperatures during six months of sampling at each elevation range (Figure 1). For both species the mean microenvironmental temperature was near 4 ºC and had a similar temperature range (near -5 ºC to ~23 ºC) (Figure 2). The temperatures to which individuals of both species were subjected for a longer period of time varied between -2 ºC and 3 ºC. Environmental freezing events were more intense during the months of December and September, with temperatures of -5.42 ºC and -4.98 ºC occurring in 19.4 % and 60% of days, respectively (Table 2). Daily minimum air temperature was different ($X^2$= 364.6968, P<0.05) throughout the sampling months, except for December and August, which had similar temperatures (Z= -0.7685, P= 0.4422). Significant differences ($X^2$= 674.7682, P<0.0001) were found in daily maximum air temperature for every month, except for December and November (Z= 1,3959, P= 0.1627) and September and February (Z= -1,2505, P= 0.2111). The warmest temperatures above 17.48 ºC (absolute maximum 23.8 ºC) and 19.24 ºC (absolute maximum 23.75 ºC) occurred in 70.9 % and 80% of days in December and January, respectively. Lastly, we calculated the daily temperature range for each month (Figure 3) where September was the only month to be significantly different from all the months (January: Z=1.96, P=0.04; August: 2.26, P=0.02; December: Z=-2.27, P=0.02; November: -2.49, P=0.01) except from February (Z=0.5, P=0.1).

We found significant differences between the inner leaf temperature (ILT) for both species sampled at three elevation ranges (*Werneria nubigena* $X^2$ = 116.5240, DF= 2, P<0.0001; *Xenophyllum rigidum* $X^2$ = 517.8969, DF= 2, P<0.0001) (Table 3). For both species, the lowest mean ILT was recorded from individuals at the highest elevation range (*W. nubigena:* 4500–4700 m and *X. rigidum*: 4900–5100 m). In *W. nubigena*, the lowest ILT was recorded at 4500–4700 m (-5.93 ºC), whereas in *X. rigidum* the lowest ILT was recorted at 4700–4900 m (-10.48 ºC). When comparing both species *W. nubigena* presented warmer ILT than *X. rigidum* (*X2*=4394.134, DF=1, P<0.0001). The daily mean ILT for *W. nubigena* (4.35 ± 6.34 ºC) was warmer than *X. rigidum* (2.28 ± 6.26 ºC) by 2.07 ºC ($X^2$= 4394.134, DF= 1, P<0.0001). Daily minimum ILT was ca. 4 ºC lower in *X. rigidum* (-6.67 ± 1.36 ºC) than in *W. nubigena* (-2.65 ± 0.70 ºC). *X. rigidum* had -4.55 ºC ($X^2$= 600, DF= 1, P<0.0001) lower extreme freezing ILT than *W. nubigena* (-5.93 ºC) and we did not find significant differences in hours of duration of extreme freezing events between both species (*W. nubigena:* 2.18 ± 1.66 hours; *X. rigidum*: 1.34 ± 0.85 hours, $X^2$= 4.425, DF= 1, P= 0.1194). On the other hand, *X. rigidum* (3.91 ± 0.48 h) freezing events had a longer duration than *W. nubigena* (2.32 ± 0.21 h) and environment (2.38 ± 0.08) (P= 0.008, Z= 2.62 and 0,002, Z=3.08, respectively) (Figure 4). Also, daily minimum ILT of each species was different every month (*Werneria nubigena* $X^2$= 472.3525, DF= 5, P<0.0001; *X. rigidum* $X^2$= 60.9079, DF= 5, P<0.0001). Finally, freezing temperatures (below 0ºC) started at 19h00m for both species (*W. nubigena*: -2.15 ± 018 ºC and *X. rigidum*: -5.87 ± 1.06 ºC, $X^2$= 17.47, DF=1, P= 0.0001) and ended at 08h00m for *X. rigidum* (-5.14 ± 0.76 ºC) and 10h00m for *W. nubigena* (-2.38 ± 0.49 ºC). We found significant differences between freezing temperatures between both species at all hours of the day ($X^2$= 4130.42, DF= 1, P= 0.0001).

### *Microhabitat conditions*

Microhabitat conditions of each monitored individual had a stronger relation with vascular plant and rock cover with the intra specific variation of ILT observed in both species. Also, minimum absolute and mean temperature explained an important portion of the variation (Figure 5). The PCA axis 1 (61.57%) separated individuals according to biotic parameters such as minimum absolute and mean temperature (0.71) values of ILT. Individuals located in A and C quadrants presented a lower ILT than individuals in quadrants B and D of both species. On the other hand, PCA axis 2 (44.64%) divided the individuals according to substrate parameters, of which rock and vascular plants cover (-0.61) were the ones with the higher values. Individuals located in quadrants A and B were found near a lower cover of vascular plants and a higher cover of rock, meanwhile the opposite pattern (higher cover of vascular and lower cover of rock) was seen for individuals in quadrants C and D.

### *Exotherm measurement*

Exotherm in *Xenophyllum rigidum* occurred at -3.72 ± 1.69 ºC and warmed up to -1.47 ± 1.04 ºC, raising its temperature 2.25 ± 1.37 ºC, whereas, *Werneria nubigena* exotherm occurred at -5.33 ± 1.69 ºC warming up to -1.86 ± 0.58 ºC, with a warming range of

3.47 ± 1.39 ºC (Table 4). Significant differences were found in minimum exotherm temperature ($X^2$= 7.4588, DF= 1, P=0.0063) and warming range ($X^2$= 6,2054, DF= 1, P=0.0127) between both species. Nevertheless, we did not find differences once both species reached the warmest temperature after the exotherm event ($X^2$= 1,7206, DF= 1, P=0.1896). The duration of the exotherm event was longer in *X. rigidum* (95 ± 160.64 seconds), but was not significantly different from that recorded for *W. nubigena* (84.43 ± 117.88 seconds) ($X^2$= 0.3593, DF= 1, P=0.3593) (Table 5). When analysed by elevation gradient, the lowest exotherm for *W. nubigena* was -6.08 ± 1.84 ºC at an elevation of 4500 – 4700 m, while the warmest was -3.7 ± 1.61 ºC at its highest elevation (4700 – 4900 m). The same pattern occurred with *X. rigidum* (Table 4). We did not find differences between the three elevation ranges in both species (*W. nubigena*: $X^2$= 4.6345, DF= 2, P=0.0985; *X. rigidum*: $X^2$= 3,9855, DF= 2, P= 0.1363). When compared the exotherm of shared elevation ranges for both species, we found significant differences in 4500 – 4700 m (*W. nubigena*: -6.08 ± 1.84 ºC; *X. rigidum*: -3.64 ± 1.45 ºC, $X^2$= 4.3902, DF= 1, P= 0.0361) in contrast with 4700 – 4900 m (*W. nubigena*: -3.7 ± 1.61 ºC; *X. rigidum*: -3.97 ± 1.24 ºC, $X^2$= 0.2667, DF= 1, P= 0.6056).

### ***Freezing damage ($LT_{50}$)***

Damage by freezing temperatures ($LT_{50}$) was lower in *W. nubigena* at -12.43 ± 0.8 ºC compared with -10.15 ± 0.5 ºC in *X. rigidum* ($X^2$= 4.4619, DF= 1, P=0.0347) (Figure 6). When we analysed $LT_{50}$ in relation to elevation ranges, we found significant differences for both species between ranges (*W. nubigena:* $X^2$= 12.538, DF= 2, P= 0.0019 and *X. rigidum:* $X^2$= 4.9399, DF= 2, P=0.846).

### *$LT_{50}$ and exotherm relation*

The relation between exotherm and $LT_{50}$ was significantly different (*W. nubigena* $X^2$= 19.7569, DF= 1, P=0.0001; *X. rigidum* $X^2$= 22.1118, DF= 1, P<0.0001) for both species (Figure 6).

## Discussion

We found that tolerance was the response mechanism for both study species due to differences between $LT_{50}$ and exotherm temperature. Also, the inner leaf temperature is influenced by the microhabitat conditions which are strongly associated with the presence of vascular plants and rocks. Finally, the exotherm temperature does not depend on the response mechanism to freezing temperatures being both exotherm and $LT_{50}$ temperatures significantly different ($P<0.05$) between both species which are freezing tolerant species.

In spite of the thermal aseasonality of the Páramo ecosystems of the humid tropics, its daily air temperature regime greatly exceeds the seasonal annual variation (Cuesta et al. 2016). The daily temperature range can be as high as 28 ºC. Our results showed that for 32 nights during the six months of sampling, the temperature dropped below -6.6 ºC. These extreme cold temperatures are colder than the temperature values reported by Sierra-Almeida et al. 2012 for the Chilean Puna at 32 °S, where the lowest freezing event recorded was -5.3 ºC. Temperature variability in this ecosystem are driven by two factors: the elevation gradient and the air humidity, determined by the local climate (Buytaert et al. 2006). These conditions affect directly the physiology of the plants, stressing them in many ways, since they provoke cell dehydration, photobleaching at sunrise, rupture of tissues, loss of biomembrane semipermiability, loss of ion active transport, phospholipid degradation, among other factors that plants have to cope with (Azócar et al. 2006, Sakai and Larcher 1987). In fact, during six months of monitoring, the individuals of *Werneria nubigena* and *Xenophyllum rigidum* experienced lower freezing ILT values than the temperatures we identified as environmental freezing (Figure 1 and Table 2). The individual that presented the lowest temperature was a *X. rigidum* cushion, which cooled down to -10.48 ºC in one night during February, with an average daily minimum temperature of -5.24 ± 2.8 ºC and a duration of 4.73 ± 3.09 h. Several studies report that cushions are an efficient growth form at decoupling from ambient temperature and heat accumulation, under all mountain climatic conditions (Körner 2003, Larcher 2012, Mark et al. 2008, Sklenár et al. 2016). The same is known for rosette growth forms, such as *W. nubigena* (Diemer 1996, Körner 2003). Both cushions and rosettes, which are found closer to the ground than other growth forms, heat up more easily than erect plants (Körner 2003, Larcher 2012). For *W. nubigena* the lowest ILT recorded was -5.93 ºC, during one night of February, with a mean duration of 2.61 ± 2.43 h, for a corresponding average daily minimum temperature of -2.55 ± 1.21 ºC. Despite the fact that *W. nubigena* had warmer ILT conditions, we registered lower ILT temperatures than the environment for both species, meaning that they were not as effective in decoupling from the ambient temperature as reported by Sklenář *et al.*, 2016. Sklenář *et al.* (2016) found that cushions (*Azorella pedunculata* and *A. aretioides*) had an absolute minimum temperature of -4.1 ºC and -2.9 ºC, respectively, being 6.3 and 7.5 ºC warmer than what we recorded for *X. rigidum*. Nevertheless, we found that, during mornings, *W. nubigena* spent two hours more than *X. rigidum* experiencing freezing temperatures, which might suggest that rosettes are not as effective as cushions at decoupling from environmental temperatures. On the other hand, Sklenář *et al.* (2016) also reported an absolute minimum temperature of -5.8 ºC for *W. nubigena,* similar to the extreme freezing event of -5.93 ºC which we registered. The low inner leaf temperatures we recorded for the *X. rigidum* cushions might be due to their larger height, compared to the *W. nubigena* rosettes (Sklenář et al. 2010, Sklenář et al. 2016).

Environmental temperature is an important aspect when determining the ILT variation in plants. Sklenář *et al.* (2015) found that the western side of Antisana volcano experienced greater temperature oscillations than the eastern slopes and that those temperatures were the lowest. Also, they reported that the longest freezing periods of 16 hours were recorded at the western side. Our environmental records showed that during the six months monitored, extreme freezing events with temperatures lower than -4 ºC occured with a duration of more than 1 h, for the majority of months. Also, freezing temperatures under 0 ºC (e.g. daily mean minimum temperature under-2 ºC), indicated that for both species the ambient temperature and the high variability of temperature during the day forced them to drop their ILT to such extreme values, as a response mechanism to reduce heat loss due to these daily air extreme temperature conditions (Körner 2003).

From another perspective, we found that the response mechanism to freezing temperatures for both species was tolerance, due to the significant differences between $LT_{50}$ and exotherm. For *W. nubigena* the exotherm temperature was lower than for *X. rigidum*, nevertheless the ILT presented the opposite pattern meaning that under natural conditions the exotherm temperature of *Werneria nubigena* (-5.33 ºC) was similar to the extreme freezing event recorded on field measurements (-5.93 ºC). This could mean that, despite having a lower ILT in the field, both species can cope with even lower temperatures, before reaching $LT_{50}$ limit, independently of elevation. This is supported by our PCA results, that showed elevation was not as important as substrates — such as rock and vascular plant cover— for determining the ILT (Figure 5). Our results suggest that the surrounding microhabitat characteristics are the ones that define the stress magnitude to which plants are exposed (Scherrer and Körner 2011). We found that a higher rock and vascular plant cover create favourable microclimatic conditions for individuals of both species, decreasing the extreme temperature conditions individuals experience on a daily basis. Individuals that were surrounded by a greater cover of vascular plants (quadrant D, Figure 5) had the higher mean ILT (4.79 ºC), in comparison with individuals with less microclimatic buffering, such as the ones in quadrant C, which had lower cover of vascular plant and rock cover (mean temperature: 0.76 ºC and minimum temperature: -7.36 ºC).

As reported by Sklenář et al. (2015 and 2016), plants inhabiting the northwestern slope of Antisana volcano, are exposed to less extreme temperature conditions than plants from the eastern side, but the minimum air temperatures (-4.1 ºC absolute minimum) are lower and occurred at a higher frequency in the western side of Antisana volcano due to more frequent clear sky conditions. Our results showed that slope and orientation in study site of the studied plants were not as influent in ILT as expected (Daly et al. 2010, Dobrowski 2011, Dobrowski et al. 2009), in comparison to the effect of rock and vascular plants cover. Plants that live between rocks, or are protected by vascular plants, are likely to be less exposed to windward of Antisana volcano. This indicates that the micro-topography surrounding the individuals acts as a buffer, reducing the extreme environmental conditions to which they could be subjected (Scherrer and Körner 2010, Scherrer and Körner 2011 Spasojevic et al. 2014). These type of mountain terrains (rocky ones) often promote temperature inversions making them candidates for microrefugia (Dobrowski 2011, Scherrer and Körner 2011). Individuals surrounded by a larger cover of vascular plants or rock were the ones with warmer mean temperatures, while individuals with a smaller cover of both substrates had lower mean temperatures (Figure 5).

## Conclusions

Our study provides new evidence that rugged topography in mountainous landscapes, resulting in a great variety of micro-habitats, create large differences in climatic conditions over short distances, raising the possibility for species to survive rapid climate change through localized movement (Scherrer and Körner 2010, Spasojevic et al. 2014). Knowledge of limiting climatic factors is important for identifying the physiographic mechanisms by which micro-refugia can ameliorate climatic constraints on a species (Dobrowski 2011). Also, a deeper knowledge of how species are responding to climate change is necessary to understand how microclimatic conditions affect the distribution patterns (e.g. population density) of species along different elevation ranges.

## Acknowledgements

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# Figures

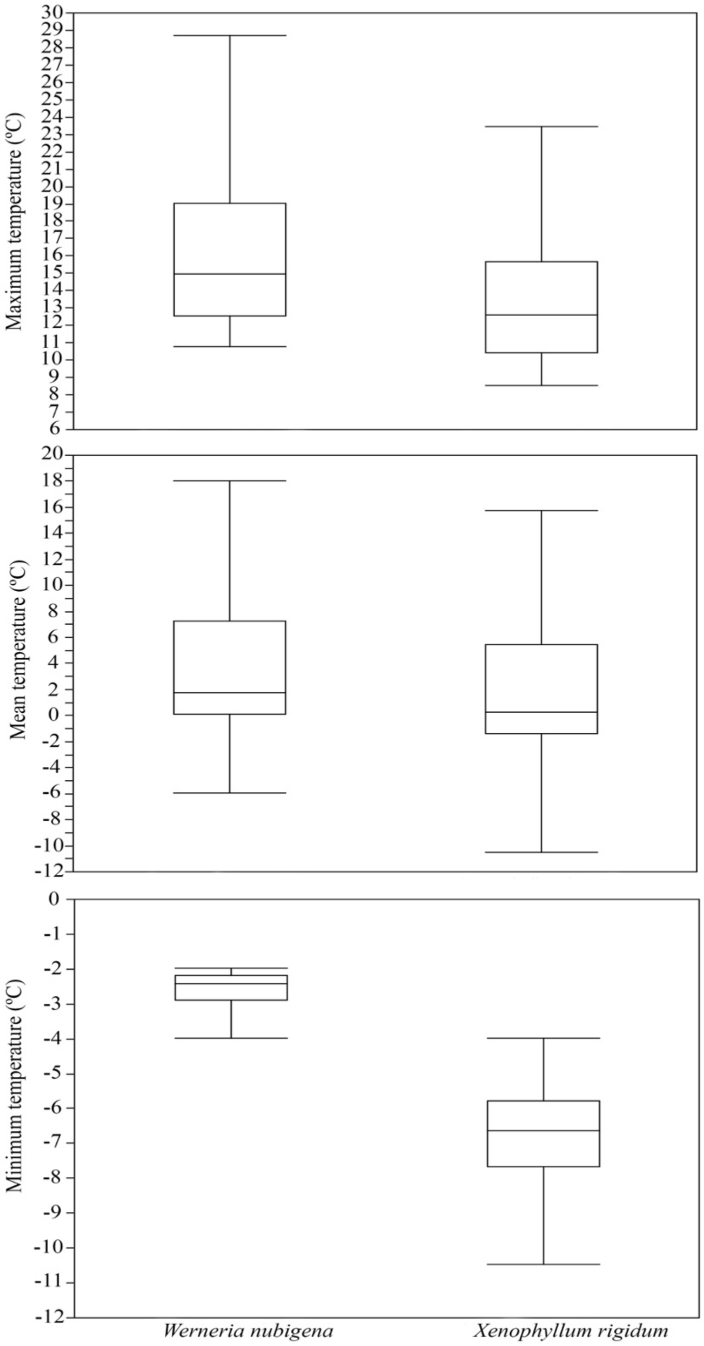


Figure 1. Inner leaf temperature of *Werneria nubigena* and *Xenophyllum rigidum* during sampling months, at the northwestern slope of Antisana Volcano. Temperatures are shown as maximum, mean and minimum. Bars are temperature range.

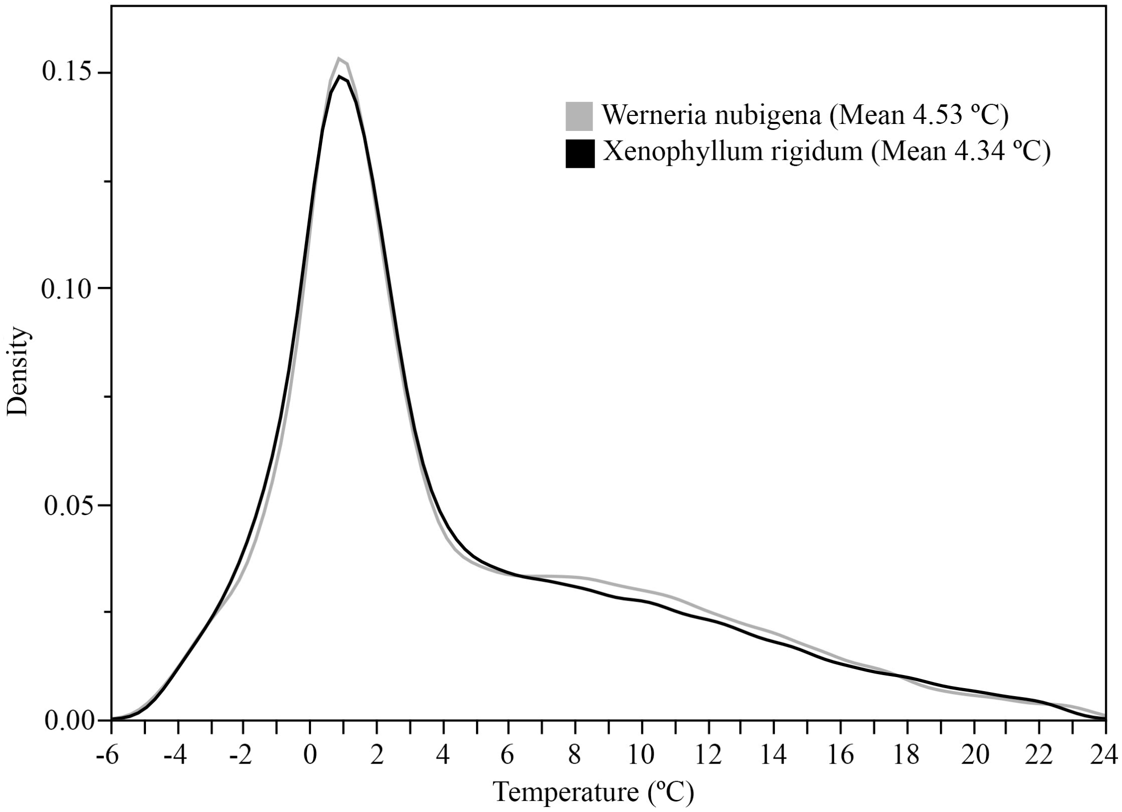


Figure 2. Thermal niches of *Werneria nubigena* and *Xenophyllum rigidum* recorded from November 2014–February 2015 and between August–September 2015, in a 240 m elevation gradient at the northwestern slopes of Antisana Volcano.

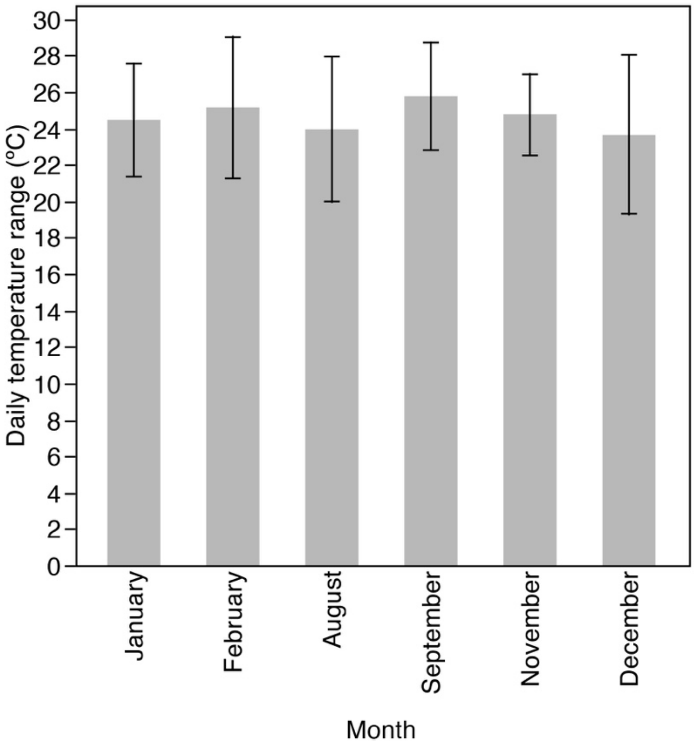


Figure 3. Daily temperature range (from the daily absolute minimum and maximum) of air temperature at 10 cm above ground during sampling months (November 2014–February 2015 and between August–September 2015) on the northwestern slope of Antisana Volcano. Bars represent de error.

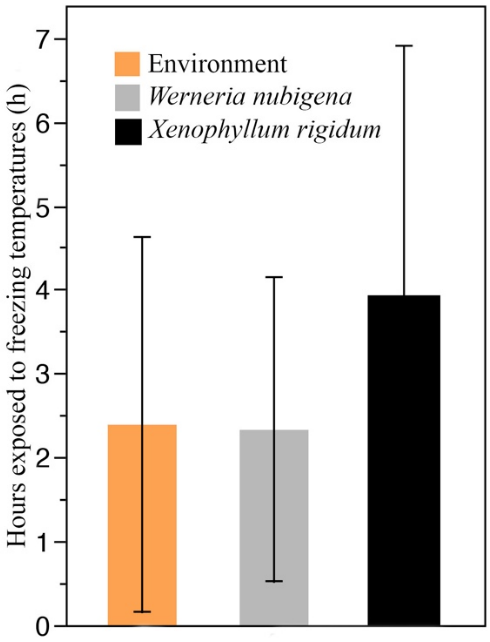
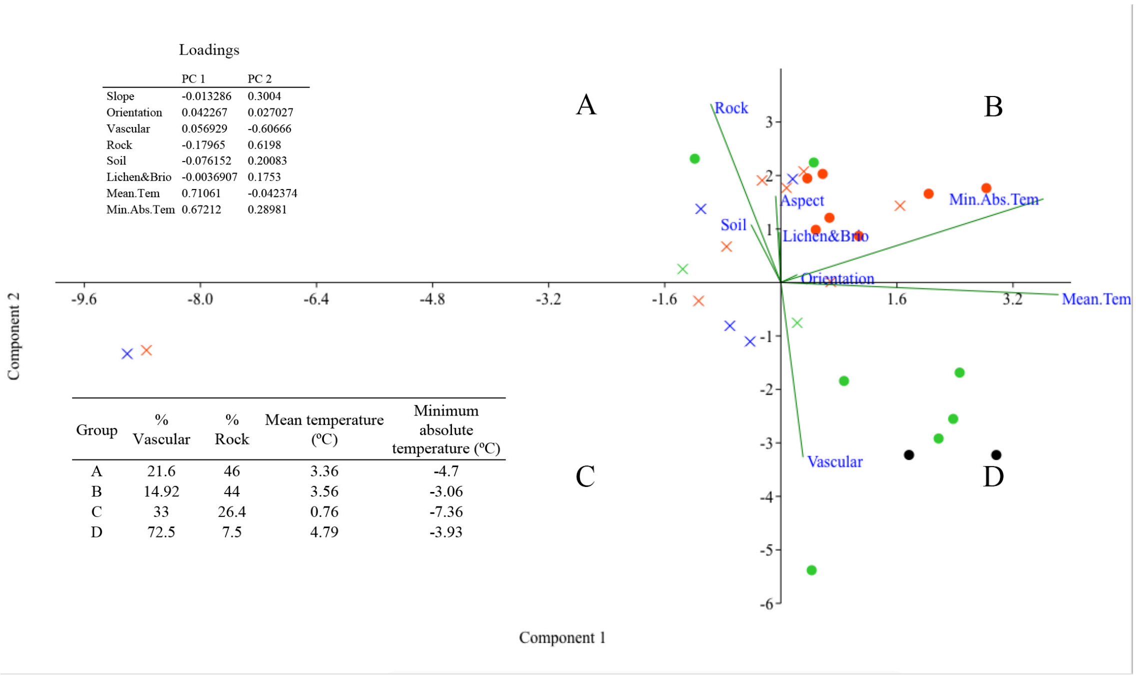


Figure 4. Hours of freezing temperature exposure for *Werneria nubigena, Xenophyllum rigidum* and the environment.



Loadings

|  | PC 1 | PC 2 |
|---|---|---|
| Slope | -0.013286 | 0.3004 |
| Orientation | 0.042267 | 0.027027 |
| Vascular | 0.056929 | -0.60666 |
| Rock | -0.17965 | 0.6198 |
| Soil | -0.076152 | 0.20083 |
| Lichen&Brio | -0.0036907 | 0.1753 |
| Mean.Tem | 0.71061 | -0.042374 |
| Min.Abs.Tem | 0.67212 | 0.28981 |

| Group | % Vascular | % Rock | Mean temperature (ºC) | Minimum absolute temperature (ºC) |
|---|---|---|---|---|
| A | 21.6 | 46 | 3.36 | -4.7 |
| B | 14.92 | 44 | 3.56 | -3.06 |
| C | 33 | 26.4 | 0.76 | -7.36 |
| D | 72.5 | 7.5 | 4.79 | -3.93 |

Figure 5. Principal components analysis (PCA) for all individuals of *Werneria nubigena* (Dot symbol) and *Xenophyllum rigidum* (X, symbol). Each symbol represents one individual of *X. rigidum* and *W. nubigena*. Colors stand for altitude ranges (Black: 4300–4500 m, Green: 4500–4700 m, Orange: 4700–4900 m and Blue: 4900–5100 m).

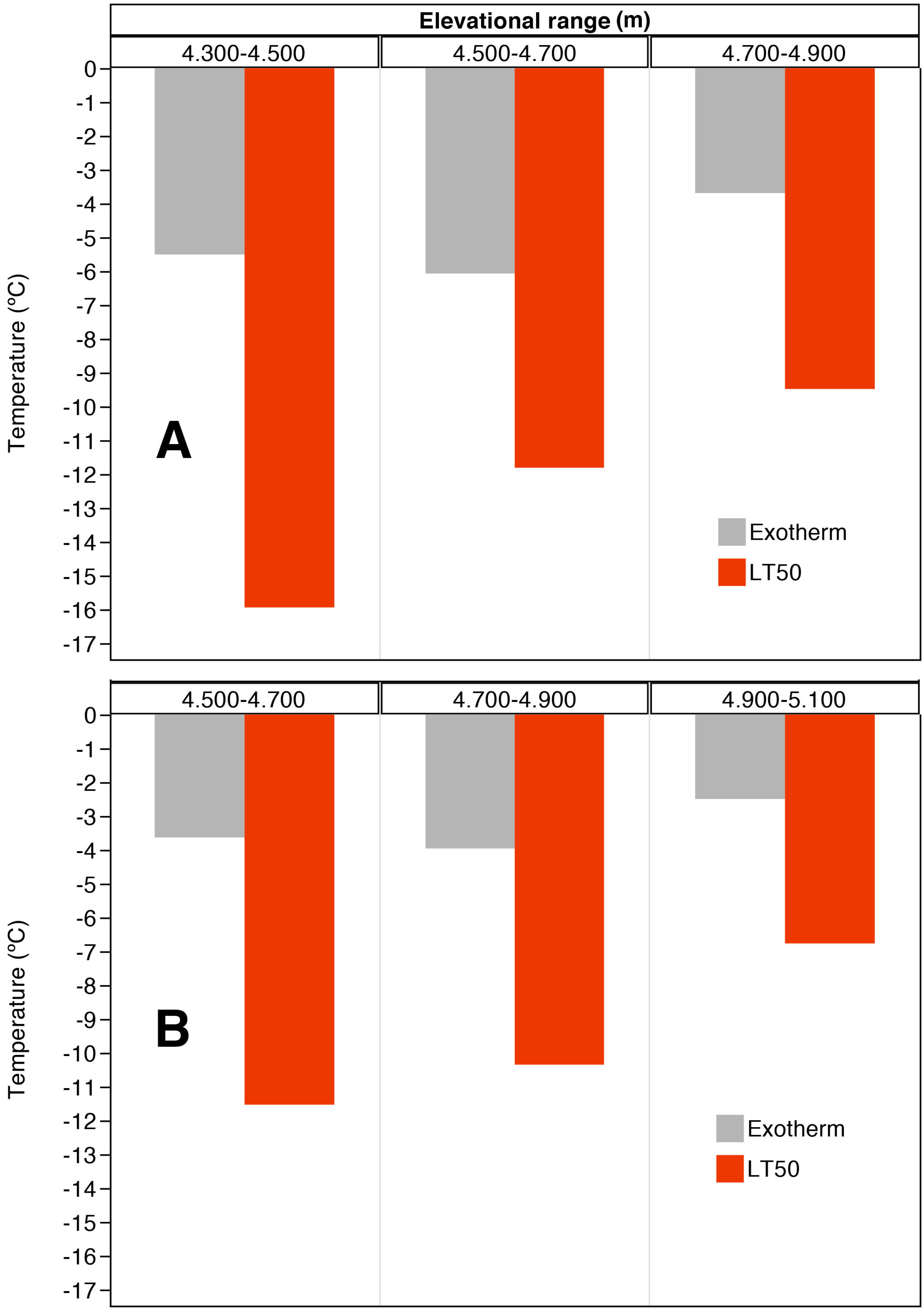


Figure 6. Comparison between Exotherm and $LT_{50}$ for both species on every elevation range. A= *Werneria nubigena* and B= *Xenophyllum rigidum*. All comparisons were significantly different ($P<0.05$).

# Tables

Table 1. Number of individuals of *Werneria nubigena* and *Xenophyllum rigidum* per elevational gradient monitored for exotherm temperature determination.

| | Studied individuals | |
|---|---|---|
| Elevational gradient | *Werneria nubigena* | *Xenophyllum rigidum* |
| 4300–4500 m | 6 | n/a* |
| 4500–4700 m | 5 | 5 |
| 4700–4900 m | 3 | 6 |
| 4900–5100 m | n/a* | 4 |

* The species was absent in this range in the study area.

Table 2. Environmental temperatures by month (between November 2014–February 2015 and between August–September 2015)

| Temperature | January | February | August | September | November | December |
|---|---|---|---|---|---|---|
| Mean | 4.28 ± 0.04 | 4.76 ± 0.04 | 4.76 ± 0.04 | 4.93 ± 0.05 | 4.16 ± 0.04 | 3.95 ± 0.04 |
| Std Dev | 5.59 | 6.09 | 5.83 | 6.27 | 5.11 | 5.15 |
| Absolute minimum | -4.86 | -4.97 | -4.95 | -4.98 | -4.91 | -5.42 |
| Absolute maximum | 23.75 | 23.8 | 23.75 | 23.79 | 23.75 | 23.8 |

Table 3. Inner leaf temperature values for individuals of *Werneria nubigena* and *Xenophyllum rigidum* at three elevation ranges (*W. nubigena:* 4300–4500 m, 4500–4700 m and 4700–4900 m; *W. rigidum*: 4500–4700 m, 4700–4900 m and 4900–5100 m).

| | *Werneria nubigena* | | | *Xenophyllum rigidum* | | |
|---|---|---|---|---|---|---|
| Elevation range | 4300–4500 | 4500–4700 | 4700–4900 | 4500–4700 | 4700–4900 | 4900–5100 |
| Mean | 5.59 ± 0.13 | 4.97 ± 0.06 | 3.98 ± 0.03 | 3.29 ± 0.06 | 2.12 ± 0.04 | 1.62 ± 0.07 |
| Std Dev | 7.95 | 7.29 | 5.7 | 6.19 | 6.14 | 6.52 |
| Min | -4.67 | -5.93 | -4.18 | -5.65 | -10.48 | -10.28 |
| Max | 32.88 | 37.75 | 28.37 | 24.92 | 23.17 | 22.88 |
| Range | 37.55 | 43.68 | 32.55 | 30.57 | 33.65 | 33.16 |
| p value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

Table 4. Exotherm temperature of *Werneria nubigena* and *Xenophyllum rigidum*. Temperature is shown as mean ± SD for each elevation range. Differences among species exotherm are indicated by NS (no significance) and * (significant differences).

|  | *Werneria nubigena* |  | *Xenophyllum rigidum* |  |
|---|---|---|---|---|
| Elevation range | n | Mean freeze temperature (ºC) | n | Mean freeze temperature (ºC) |
| 4300–4500 | 5 | -5.52 ± 1.25 |  |  |
| 4500–4700 | 5 | -6.08 ± 1.84 * | 5 | -3.64 ± 1.45 * |
| 4700–4900 | 3 | -3.7 ± 1.61 $^{NS}$ | 6 | -3.97 ± 1.24 $^{NS}$ |
| 4900–5100 |  |  | 5 | -3.52 ± 2.55 |

Table 5. Duration of exotherm events for *Werneria nubigena* and *Xenophyllum rigidum*.

| Species | Freeze event duration (s) | Mean freeze temperature (ºC) | Mean maximum warming temperature (ºC) | Mean raise temperature (ºC) |
|---|---|---|---|---|
| *Xenophyllum rigidum* | 95 ± 160.64 | -3.72 ± 1.69 | -1.47 ± 1.04 | 2.25 ± 1.37 |
| *Werneria nubigena* | 84.43 ± 117.88 | -5.33 ± 1.69 | -1.86 ± 0.58 | 3.47 ± 1.39 |
| P value between both species | 0.3709 | 0.0067* | 0.1971 | 0.0127* |