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Invisible patterns: Changes in floral UV pigmentation along an elevational gradient in the Colombian Andes

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Abstract

Floral pigmentation is shaped by both biotic and abiotic pressures. UV absorbing pigments serve a dual role in plants, they display visual cues to pollinators and protect reproductive structures from UV radiation and temperature stress. Since climate change could be altering the conditions that determine flower pigmentation, and páramos are one of the most vulnerable ecosystems, we characterized floral UV pigmentation in the páramo's flora along an elevation gradient of ca. 600 m in Cocuy National Park. We also tested how changes in UV radiation and temperature are related to floral UV pigmentation. We sampled inflorescences of the plant family Asteraceae along the gradient, and by UV photography and spectrophotometry, we determined the UV pigmentation patterns and UV-absorbing pigment concentrations. Additionally, we also measured UV irradiance and temperature along the gradient and determined that the highest elevations have lower temperatures and higher UV irradiance. Most of the species have contrasting reproductive structures with UV-absorbing disc florets and UV-reflective pollen, which acts as a visual cue to pollinators. The UV-absorbing pigments of species with inflorescences facing the sun (from the genera *Monticalia*, *Baccharis*, *Werneria*) were significantly affected by elevation, while species with inflorescences that do not receive direct radiation were not significantly affected by elevation (*Culcitium* and *Espeletia*). In addition, species that do not change UV pigment concentrations may prioritize pollination against abiotic stress, while species that change pigment concentrations prioritize protection from abiotic stress. This is the first study to characterize UV pigmentation in the páramo's flora.

Keywords: Asteraceae, páramo, PNN Cocuy, pollinators, temperature, UV irradiance.

Resumen

La pigmentación floral ha sido moldeada por presiones bióticas y abióticas. Los pigmentos que absorben radiación UV (pigmentos UV) cumplen un rol dual en las plantas, pues presentan señales visuales a sus polinizadores y protegen las estructuras reproductivas de la radiación UV y temperatura. Se caracterizaron los patrones de pigmentación UV en la flora de páramo, utilizando un gradiente altitudinal de aprox. 600 en el PNN Cocuy, y se evaluó cómo se relacionan la radiación UV y temperatura con la pigmentación UV. Se realizó un muestreo de inflorescencias de plantas de la familia Asteraceae en el gradiente y se estimaron las concentraciones de pigmentos mediante fotografía UV y espectrofotometría. Adicionalmente, mediciones de irradiancia UV y temperatura a lo largo del gradiente permitieron determinar que a mayor altitud se presenta mayor irradiancia UV y menores temperaturas. La mayoría de especies muestreadas tienen estructuras reproductivas contrastantes, ya que presentan flores de disco que absorben radiación UV, y polen que refleja la radiación UV; este patrón corresponde a una señal visual para los polinizadores. La altitud afectó significativamente a los pigmentos en plantas cuyas inflorescencias apuntan hacia el sol (*Monticalia*, *Baccharis*, *Werneria*), quienes priorizan la protección frente al estrés abiótico; mientras que no tuvo efectos sobre inflorescencias que no reciben radiación directa del sol (*Culcitium*, *Espeletia*), quienes priorizan mantener la señal visual a sus polinizadores. Este es el primer estudio en caracterizar la pigmentación UV en la flora de los páramos.

Palabras clave: Asteraceae, páramo, PNN Cocuy, polinizadores, temperatura, irradiancia UV.

1.Introduction:

The striking diversity of angiosperm floral traits allows plants to display several signals to their pollinators, including shape, colour patterns, odours and electrical signals . Depending on their conspicuousness, these signals attract certain pollinators, which have evolved sensorial systems that can detect them (Schiestl & Johnson, 2013). Although floral pigmentation has been widely studied (Altshuler, 2003; Brock et al., 2016; Gray et al., 2018; Narbona et al., 2021; Tai et al., 2020), one of its most relevant aspects, pigments visible in the ultraviolet spectrum (UV pigments), have not been well studied.

UV absorbing plant pigments are mainly flavonoids that serve a dual role: display attractive patterns to pollinators (i.e., bullseye patterns or nectar guides) and protect plant tissues against abiotic stress. For the first role, it has been shown that the proportion of UV pigments in the flowers can alter the amount of flower visitors. For example, sunflowers receive more visits from honeybees when UV pigments cover between 50% and 80% of the petal surface area (Todesco et al., 2022). Pollinators' sensorial systems evolved before the emergence of angiosperms, therefore, pigment coverage has been under strong pollinator selection (Chittka, 1996; Schiestl & Johnson, 2013). For the second role, UV pigments can ameliorate abiotic stress caused by temperature and UVB radiation. Given that pigments absorb UV radiation, they can prevent cellular tissue damage from radiation (Koski, 2020; Ferreyra et al., 2021). Other abiotic factors can limit the production of these pigments, such as carbon assimilation conditions, nitrogen soil availability and temperature (Ferreyra et al., 2021). Thus, abiotic conditions also have a strong pressure on floral pigmentation.

Climate change has caused a series of alterations in those abiotic conditions. The ozone layer depletion has increased the exposure to UVB radiation, which has detrimental effects on the organisms (Koski, 2020). In consequence, plants exposed to high UVB radiation have increased the production of UV absorbing pigments (Koski, 2020; Piri et al., 2011). In addition, changes in temperature can also alter flavonoid synthesis, including UV absorbing pigments (Ferreyra et al., 2021). Surprisingly, only few studies have assessed how floral UV pigmentation has changed in the context of global climate change. Koski et al. (2020) found that global UV pigmentation has increased approximately 2% per year during the last 76 years, with a positive correlation with respect to UVB radiation. Even though Koski et al. (2020) included specimens from Australia, Eurasia and North America, specimens from tropics were not well represented in their sampling. Therefore, we do not know how UV absorbing pigments vary in tropical areas as climate change progresses.

Páramos are a high altitude tropical Andean ecosystem known to display unique environmental conditions such as extreme daily variation in temperature and high UV exposure (Hofstede et al., 2014; Rada, 2016), which has led to high levels of endemicity and specialized adaptations in its flora (Madriñán et al., 2013). This makes páramos one of the most threatened alpine ecosystems by climate change and land cover transformation (Cresso et al., 2020; Eguiguren-Velepucha et al., 2016; Ruiz Carrascal et al., 2011). Páramos also encompass an elevational gradient of more than 1,500m, from altitudes between ca. 3,200 to 4,700 m. Wide altitudinal ranges are ideal to test the effects of climate in floral pigmentation since temperature and UV radiation varies with elevation (Gray et al., 2018; Piazena, 1996). Given that not much is known about how pigmentation patterns change with climatic conditions in the tropics, the páramos present an excellent opportunity to establish a baseline of floral pigmentation.

Consequently, our study aims to understand the relationship between changes in temperature and UV radiation and floral pigmentation, using an elevational gradient of ca. 600 m in the Colombian Andes. We hypothesize that there will be an increase in UV pigment concentration in plants at higher elevations, because flavonoid production is induced by physiological stressful conditions such as exposure to high UV radiation and low temperatures (Piri *et al.*, 2011; Koski & Ashman, 2016; van der Kooi *et al.*, 2019; Ferreyra *et al.*, 2021). However, floral disposition may change this pattern, as reported in Koski et al. (2020). To this end, we studied species from the plant family Asteraceae that were found along most, if not all, the elevational gradient.

2. Methods:

2.1 Study Area

The study was conducted in El Cocuy National Natural Park (PNN Cocuy). This area belongs to the National System of Protected Areas of Colombia, and is located in the Departments of Boyacá, Arauca, and Casanare (Figure 1). With the greatest elevational gradient of the Colombian System of Protected Areas, PNN Cocuy has ecosystems that range from lowland rainforests (600 m.a.s.l.) to superpáramo (above 4,500 m). Most of the protected area (170 km^2) is covered by highland ecosystems (above 3,000 m; Blanco, 2005). Temperatures range between 0 and 25°C and annual precipitations in the highlands are ca. 1,114 mm (Blanco, 2005). Flower sampling was carried out in the northern sector of the National Park, known as Parada de Romero, between the elevations of 3,995 and 4,531 m. Temperatures were measured in the southern sector of the Park, in a place known as Valle de Lagunillas, about 15 km away from

Parada de Romero (see below). Both sectors belong to the Western slope of the Eastern Cordillera and share similar (if not the same) environmental conditions (Blanco, 2005).

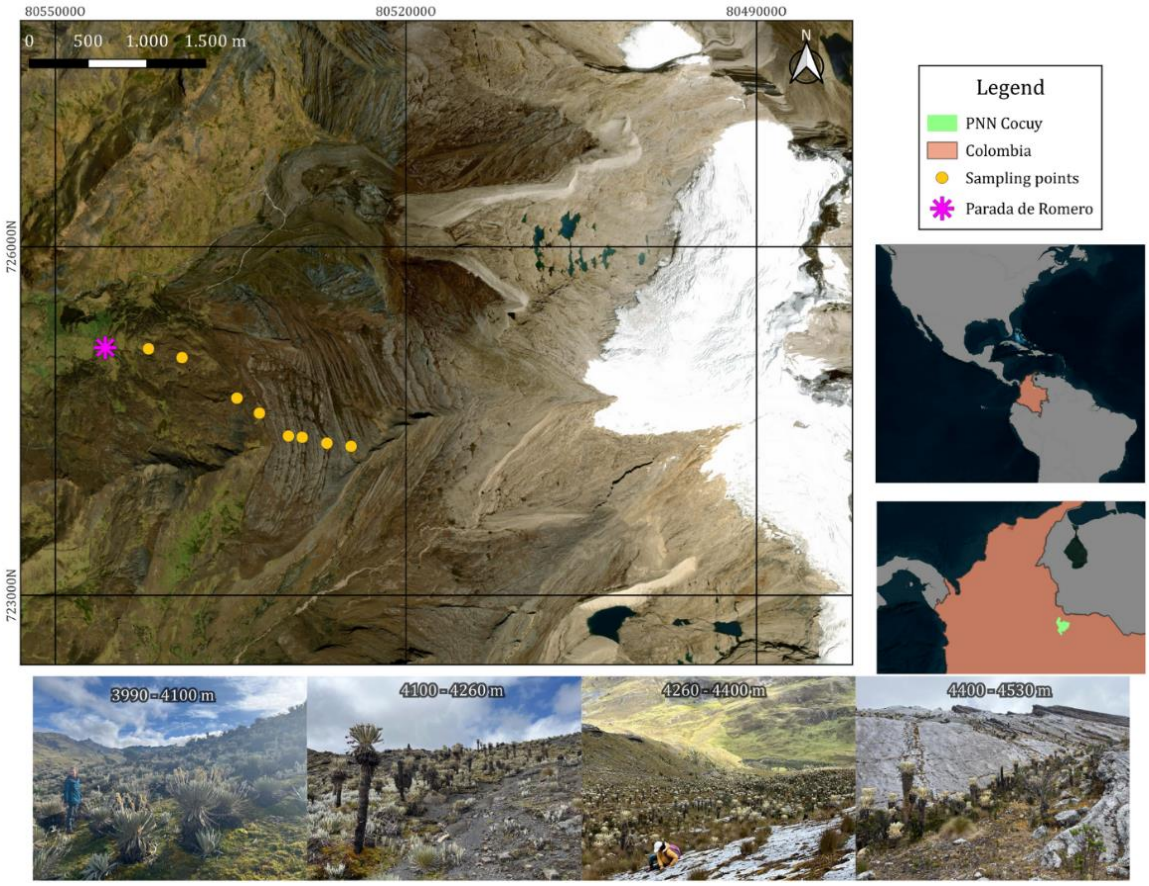

Figure 1. Map of the study area. PNN Cocuy is located in the Cordillera Oriental of Colombia. Eight sampling points were established in the northern sector of the PNN, known as Parada de Romero. These points encompassed an elevational gradient of 3,990 – 4,530 m. This gradient was divided into four ranges, as seen in the photographs below the map: 3,990 – 4,100 m; 4,100 m – 4,260 m; 4,260 – 4,400 m; 4,400 – 4,530 m.

2.2 UV irradiance and Temperature measurements

Along the elevational gradient in both sectors, Parada de Romero and Valle de Lagunillas, UV irradiance data was taken using a portable UV light meter (UV340B Amtast USA Inc., Florida, USA), along with data of the sky conditions by visually identifying cloud types when present. These recordings were taken in ten different days, always holding the UV light meter perpendicular to the sky. UV irradiance data was taken in clear days between 1100 h and 1400 h. Clear days were categorized as days with no clouds covering the solar orbit. These conditions guaranteed that the UV light meter received direct solar radiation. Hourly measurements of soil

temperature were recorded between 2011-2015 using Geo-precision dataloggers (model MLog5W, Geo-precision, Baden-Württemberg, Germany), belonging to the GLORIA network (J. Jácome, pers. comm; Grabherr *et al.*, 2000). Four dataloggers were placed in each of the cardinal directions (North, East, South and West) and in each of the four GLORIA summits in the PNN Cocuy (elevation of each summit: 4,033, 4,197, 4,324, and 4,403 m). The GLORIA summits are located in the southern part of the PNN, in the Valle de Lagunillas sector.

2.3 Flower sampling

Along the elevational gradient, we established eight sampling points (4,078, 4,150, 4,273; 4,280, 4,380, 4,405, 4,472, and 4,531 m; see Figure 1). At these elevations, Asteraceae is one of the most common and diverse plant families, and species flower throughout the year (Luteyn, 1999). In each site we searched for all the morphotypes of Asteraceae present, and collected three inflorescences per individual, of at least two individuals of each morphospecies (Table S1). The collected inflorescences per morphotype had the same exposition to solar radiation. Flowers were stored in paper bags, which were later stored in silica gel, to dry the samples and prevent fungal growth. It has been demonstrated that UV absorbing pigments are not lost or damaged when flowers are dried (Koski, 2020). Each individual was photographed *in situ* for further identification along with its flower samples. Individuals were identified at species level, except for the genus *Werneria*. Since species of the genus *Culcitium* (*C. canescens* and *C. cocuyanum*) showed the same morphological floral features (Figure 2; Figure S1), analyses were performed at genus level.

2.4 Ultraviolet Induced Fluorescence Photography

For all the individuals sampled, photos of inflorescences and individual disc florets were taken using natural light and a handheld UV lantern (wavelength peak at 365 nm) in a dark room. By using UV light, the UV absorbing pigments showed a darker coloration, whereas reflecting UV pigments and structures acquired bright coloration (Figure 2). To test if this photography technique was effective to visualize floral UV pigments, we tested it with a sample of *Helianthus* sp., which is known to have a remarkable UV bullseye pattern (Figure S1).

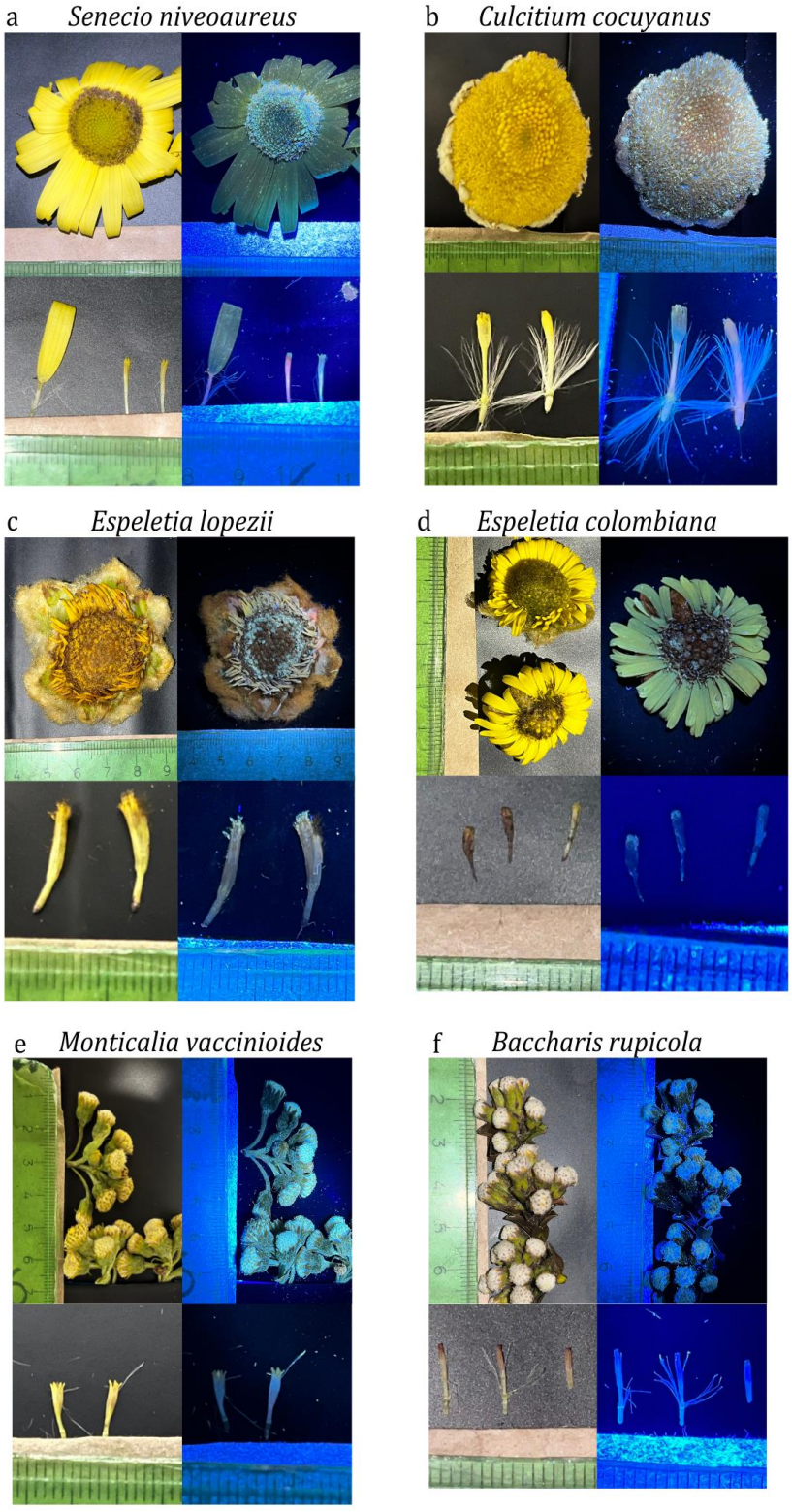

Figure 2. Pigmentation patterns of six species found along the elevational gradient in the PNN Cocuy. Photos were taken in a dark room with a UV light (peak at 365 nm) of the whole inflorescence and individual disc and ray florets. Disc florets are those found in the centre of

the inflorescence, while ray florets are placed in the periphery, and can be seen with petals (mostly visible in *Senecio* and *Espeletia*).

2.5 Spectrophotometry

Given that Asteraceae have two different flower types: disc and ray florets, we analysed them separately (Figure 2). For pigment extraction, 0.2 g of flower content was macerated with 6 mL of anhydrous ethanol as solvent (Tzanova et al., 2020). If less tissue was available, 0.1 g of flower content was macerated with 4 mL of solvent. Absorbance data was recorded from pigment extracts by UV-Visible Spectrophotometry (GENESYS 150 UV-Vis Spectrophotometer – Thermo Fisher Scientific Inc., Massachusetts, USA), between the wavelengths of 250 – 800 nm with intervals of 2 nm. To estimate the concentration of the UV-absorbing pigments, we divided the absorbance of the sample by its concentration (mass of flower content obtained / volume of solvent added). The volume was typically 0.2 g of flower content per 6 mL of anhydrous ethanol. We called this metric weighed absorbance.

2.6. Statistical analysis

We calculated the mean UV irradiance per elevation, and the mean temperature of each month, including recordings of all dataloggers placed in all summit areas. In order to test how UV irradiance and temperature changed along the elevational gradient, we performed linear regressions for both UV mean irradiance and mean monthly temperature as response variables to elevation.

Weighed absorbance data was segmented into two spectral bands comprising the UV spectrum: UVB (280 – 315 nm) and UVA (315 – 400 nm), as in Gray *et al*. (2018). For each individual sampled, mean, maximum and minimum weighed absorbance were calculated in both UVA and UVB bands. We tested if elevation was related to differences in UV weighed absorbance by performing ANOVAs and T-test for each species along the elevational gradient. We also performed linear regression models for each species, with UV weighed absorbance metrics as response variables to elevation. We did not test directly the effects of temperature in UV pigments as it was recorded at four elevations.

All statistical analyses were performed in R 4.2.2. (R Core Team, 2022) using the libraries tidyverse (Wickham et al., 2019), car (Fox & Weisberg, 2019), and performance (Lüdecke et al., 2021), whereas data visualization was performed in Python 3.7.6 (Van Rossum & Drake, 2009), using the libraries Seaborn (Waskom, 2021) and Matplotlib (Hunter, 2007).

3. Results:

3.1 Effects of elevation in UV irradiance and temperature

Mean UV irradiance increased significantly with elevation (Figure 3A; y = 1.32x - 3570.79; p = 0.01; R^2 = 0.04). However, UV irradiance is highly variable, with values varying from 500 mW/cm^2 to more than 4,000 mW/cm^2 (Figure 3A). The highest UV irradiance values in clear days were near 8,000 mW/cm^2, while cloudy and foggy days reduce them dramatically, to values well below 1,000 mW/cm^2 (Figure S2). In comparison, mean monthly temperatures decreased significantly with elevation (Figure S3; y = -0.01x + 29.50; p < 0.001; R^2 = 0.21). Mean temperatures were more variable at higher elevations (Figure 3B; Figure S3).

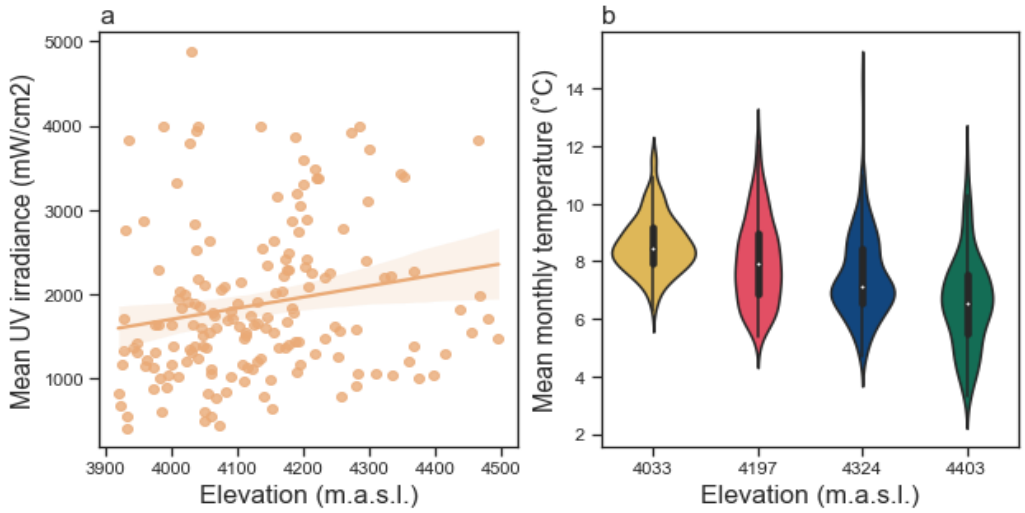

Figure 3. Changes in mean UV irradiance and temperature along the elevational gradient. (A) shows values of sunny days taken between 1100 h and 1400 h, adjusted to a linear regression model (y = 1.32x - 3570.79; p = 0.01; R^2 = 0.04). (B) shows the distribution of mean monthly temperatures in each elevation, which is adjusted to a linear regression model (y = -0.01x + 29.50; p < 0.001; R^2 = 0.21).

3.2 Flower sampling and characterization of floral UV patterns

We sampled a total of 62 individuals belonging to eight species along the elevational gradient (Table S1). The genera *Culcitium* and *Espeletia* were present all along the gradient, whereas *Baccharis rupicola*, *Werneria* sp. and *Senecio formosoides* were only found at higher elevations (between 4,260 and 4,530 m). *Senecio niveoaureus* was only found at lower elevations (3,900 – 4,260 m).

The UV induced fluorescence photography showed that all species sampled displayed three main patterns regarding their pigmentation in the UV spectrum: (a) contrasting reproductive structures, (b) uniformly UV-reflecting inflorescence, and (c) bullseye pattern. The genera *Culcitium*, *Espeletia*, *Werneria* and *Senecio* have a contrasting pattern between immature and mature disc florets (Figure 2; Figure S1). Since all disc florets were UV-absorbing they appeared dark when exposed to UV light, while the pollen present in mature florets appeared bright under the UV light, which means it is UV-reflective (Figure 2 a-d). Although *Monticalia vaccinioides* and *Baccharis rupicola* presented the same pattern in disc florets (UV-absorbing), the contrast between immature and mature florets was not as evident. This was due to the presence of abundant pubescence in the base of the florets, which is UV reflective and makes the inflorescence fully bright under UV light. Also, *M. vaccinioides* displayed a UV reflective pattern in the apex of the florets (Figure 2 e,f). Only in *Senecio niveoaureus* a bullseye pattern in the ray florets was evident, while all other species have uniformly UV-absorbing ray florets (Figure 2; Figure S1).

3.3 Changes in species' UV absorbance along the elevational gradient

Each species UV absorbance varied distinctively along the elevational gradient (Figure 4; Figure S4). *Espeletia colombiana* and *B. rupicola* decreased their overall UV absorbance at higher elevations, where *E. colombiana* showed the most dramatic decrease. In contrast, *Culcitium* spp. slightly increased its absorbance at higher elevations and *M. vaccinioides* had a peak absorbance between 4,260 m and 4,400 m, decreasing at higher elevations. Additionally, these changes varied depending on the region of the UV spectrum. For instance, *E. lopezii* and *M. vaccinioides* presented greater changes in their absorbance in the UVA spectrum, while changes in the UVB were not as pronounced (Figure S4). UV absorbance was significantly related to elevation in *B. rupicola* (UVA max absorbance: $p = 0.005$, $R^2 = 0.56$), and *M. vaccinioides* (UVB mean absorbance: $p = 0.01$, $R^2 = 0.70$; UVB max absorbance: $p = 0.04$, $R^2 = 0.70$; UVA max absorbance: $p < 0.01$, $R^2 = 0.90$; Figure 4; Table S2), while it was marginally significant in *Werneria* sp. (UVA mean absorbance: $p = 0.056$, $R^2 = 0.48$; UVA max absorbance: $p = 0.04$, $R^2 = 0.54$). However, *Werneria* sp. presented significant differences in all UV absorbance metrics along the elevation gradient (Table S3). We also found that at each elevational range, species had significantly different UV absorbance values, except for the range between 4,100-4,260 m. At the highest elevational range (4,400 – 4,530 m), all metrics were significantly different between all species (Table S4).

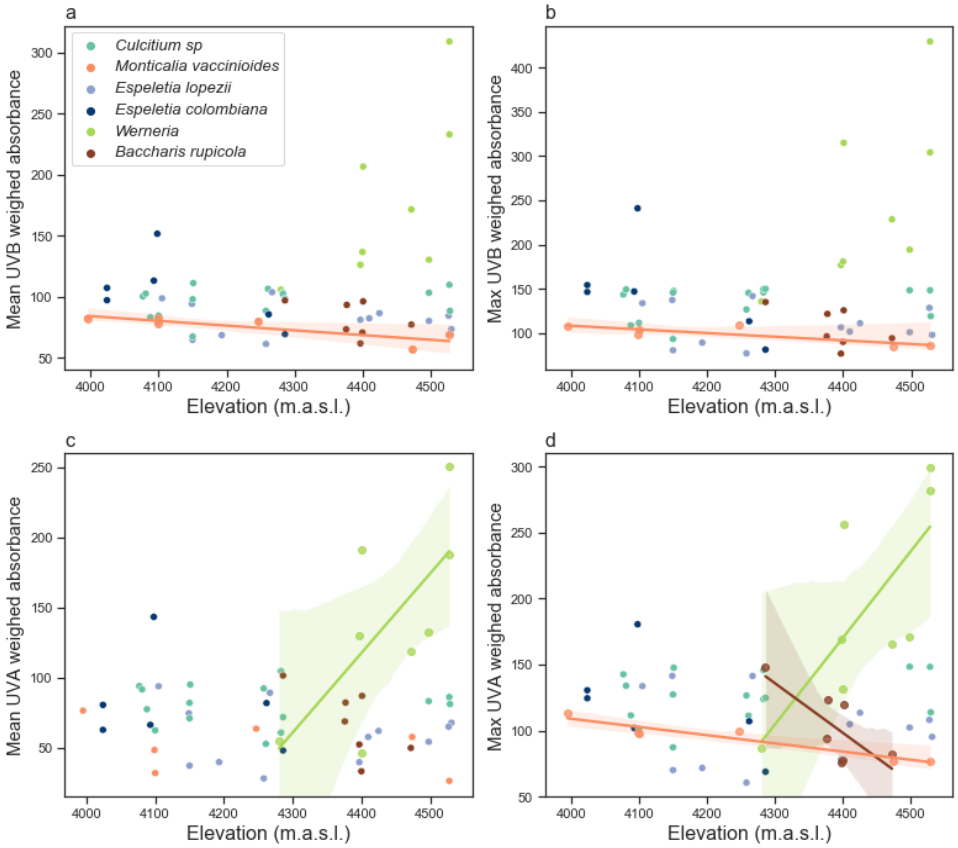

Figure 4. Scatter plot of UV absorbance metrics by species along the elevational gradient. UV absorbance metrics adjusted to a linear model in *Werneria* sp. (UVA mean absorbance: y = 0.57x -380.15, p = 0.056, R^2 = 0.48; UVA max absorbance: y = 0.66x – 2719.54, p = 0.04, R^2 = 0.54), *Baccharis rupicola* (UVA max absorbance: y = -0.38x + 1752.85, p = 0.005, R^2 = 0.56), and *Monticalia vaccinioides* (UVB mean absorbance: y = -0.04x + 238.84, p = 0.01, R^2 = 0.70; UVB max absorbance: y = -0.04x + 273.16, p = 0.04, R^2 = 0.70; UVA max absorbance: -0.06x + 357.77, p < 0.01, R^2 = 0.90).

4. Discussion:

To our knowledge, this is the first study to characterize UV floral pigments in the páramo ecosystem. We found three main strategies used by plants to respond to changes in temperature and UV irradiance along the elevational gradient: (a) increasing floral UV pigment concentrations, (b) decreasing pigment concentrations, and (c) not changing pigment concentrations. The only species that behaved according to our hypothesis and displayed the first strategy was *Werneria* sp., which significantly increased its UV pigment concentration along the elevational gradient. None of the other species had a significant pattern. For example, *Espeletia lopezii* tended to increase its UV pigment concentration while, *B. rupicola*, *M.*

vaccinioides and *E. colombiana* decreased in pigment concentrations. *Culcitium* spp. displayed the third strategy by not changing its UV absorbance values along the gradient.

4.1 UV irradiance, temperature and floral UV patterns

Our hypothesis was based on the assumption that UV irradiance increases while temperature decreases with elevation (Gray et al., 2018; Piazena, 1996). Our results are consistent with his pattern, where at higher elevations, plants tend to be exposed to higher UV radiation and lower temperatures. Not only UV radiation can produce damage in DNA and reproductive structures, but it can also decrease the photosynthetic capacity in leaves (Del Valle et al., 2020) and, in some cases, it can alter pollen production (Del Valle et al., 2020; Peach et al., 2020).

Three main patterns were found regarding flower's UV features: contrasting reproductive structures, uniformly UV-reflecting, and bullseye pattern, (Figure 2). Almost all species presented contrasting reproductive structures, meaning that their disc florets display a dark UV-absorbing background that contrasts with the conspicuous UV-reflective pollen. This pattern has been considered as a floral guide and, in other ecosystems, flowers with contrasting reproductive structures were found to be pollinated mainly by bees, flies and generalists, with pollen as their principal resource (Tunes et al., 2021).

4.2 Changes in species' UV absorbance along the elevational gradient

Our results suggest that certain species can respond distinctively in their floral UV features to changes in environmental conditions. Even though there is a generalised tendency to increase UV floral pigmentation in plants exposed to higher UV radiation and lower temperatures (Ferreyra et al., 2021; Koski & Ashman, 2016; Piri et al., 2011; van der Kooi et al., 2019), there are cases where this pattern does not occur. For instance, *Silene littorea* did not present significant changes in its concentration of UV-absorbing pigments but showed an increase in anthocyanins concentration when exposed to UV radiation (Del Valle et al., 2020). In comparison, *Clarkia unguiculata* decreased its bullseye pattern at higher elevations but also increased its anthocyanin concentration (Peach et al., 2020). Likewise, some páramo species may not increase their UV pigmentation at higher elevations.

The effect of floral angle or disposition in UV pigment patterns has not been studied, which could influence the concentration of UV pigmentation across elevation. For instance, some species have inflorescences that do not receive direct radiation from the sun (inflorescences protected by pubescence and facing towards the ground; Figure 5), like *Culcitium* spp. and *S. formosoides.* Species such as *Espeletia* spp. and *S. niveoaureus*, have

some degree of inclination in their inflorescences, which could ameliorate the effect of direct UV irradiance in floral pigmentation. *Werneria* sp., *B. rupicola* and *M. vaccinioides*, in contrast, have inflorescences that receive direct radiation, which can explain the significant effect of elevation on their floral pigmentation (Figure 4; Figure S4). Differences in UV pigmentation depending on the floral disposition have been found by Koski et al. (2020), where flowers with exposed anthers increase their UV pigmentation, while flowers with concealed anthers decrease their UV pigmentation, as a response to higher UV radiation. We propose that, in the same way that the disposition of anthers can change the effect of the environment on UV floral pigmentation, floral angle may also have an important role.

Figure 5. Species collected and their floral disposition. (A) *Senecio niveoaureus*, (B) *Senecio formosoides*, (C) *Culcitium cocuyanum*, (D) *Werneria* sp., (E) *Baccharis rupicola*, (F) *Monticalia vaccinioides*, (G) *Espeletia lopezii*, (H) *Espeletia colombiana*.

We found that the most significant differences in floral UV absorbance metrics were found at the highest elevation (Table S4). This result is similar to other studies conducted in subtropical regions, where a greater floral colour diversity has been found at higher elevations, despite having a lower species diversity, compared to lowland ecosystems (Shrestha et al., 2014; Tai et al., 2020). Although in this study we did not analyse floral colour diversity, changes in UV-absorbing pigment concentration can alter the optical properties of flowers, like hue and contrast (Gray et al., 2018). The highest elevation also showed the highest temperature

variability, which may cause more differences in plants responses, since climate is a predictor of floral UV pigmentation (Peach et al., 2020). In our study, *Culcitium* spp. maintained the same visual cues to its pollinators along the gradient, prioritizing pollination against protection from UV radiation. All other species may be more susceptible to changes in the environment and respond by modifying their UV-absorbing pigment concentrations. This could compromise the visual cues to their floral visitors.

The differences in floral colour diversity can also be attributed to shifts in the composition of pollinators assemblages along the elevational gradient. For instance, flies become more abundant as elevation increases, and bees are more relevant at lower elevations (Tai et al., 2020). Although the pollinators of our study site have not been identified at the species level, other research conducted in the Cordillera de Mérida (Pelayo et al., 2021) found that the diversity of plant and pollinator species decreases at higher elevations. However, at these elevations the relationship plant-pollinator is more specialised. Moreover, Pelayo et al. (2021) found that bumblebees and flies were the main pollinators in the páramos of the Cordillera de Mérida. These pollinators visit plant genera like *Espeletia*, *Monticalia*, and *Senecio*, which are also present in our study site.

Floral pigmentation is shaped by both biotic and abiotic pressures, and páramos' flowering plants have shown to respond with different strategies to the challenges of high altitude ecosystems. We also acknowledge that these strategies may depend on the disposition of the inflorescence. This is the first study that characterizes floral UV pigmentation in páramos along an elevational gradient, which establishes a baseline on how pollination-related features like UV pigmentation may be affected by changes in temperature and UV radiation. It is recommended in future studies to better understand the structure of the pollination network of the study site and include plant species abundance data, because it may show stronger colour-elevation relationships and it corrects the effect of rare species in a community level analysis (Gray et al., 2018). It is also crucial to have a better understanding of the role of pigmentation in plant-pollinator relations in a highly vulnerable ecosystem such as the páramo, and how these relations could be affected by climate change.

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Disclosure statements

Conflict of interest

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

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Supplementary Information

Table S1. Species and number of individuals collected along the elevational gradient.

	Elevational range (m.a.s.l)				
Species (*N*)	3990-4100	4100-4260	4260-4400	4400-4530	*Total*
Baccharis rupicola	0	0	4	3	7
Culcitium spp.	3	5	4	3	15
Espeletia colombiana	4	0	2	0	6
Espeletia lopezii	0	5	2	5	12
Monticalia vaccinioides	0	3	0	2	6
Senecio formosoides	0	0	0	4	4
Senecio niveoaureus	3	2	0	0	5
Werneria sp.	0	0	3	5	8
Total	11	15	14	22	62

Table S2. Results of linear models testing the effect of elevation in UV pigments absorbance metrics (both mean and maximum absorbance in UVA and UVB spectra) and p-values (p) on each species, by the equation y = m*x + b. Statistically significant values are marked with an asterisk (*).

Species	Model	Coefficients	Estimate	Sdt. Error	*t* value	*P*	R^2	Adjusted R^2	AIC
Baccharis rupicola	Mean UVB weighed absorbance ~ Elevation	Intercept	587.996	448.732	1.310	0.247	0.2032	0.044	60.220
		Elevation	-0.116	0.102	-1.129	0.310			
	Max UVB weighed absorbance ~ Elevation	Intercept	1126.663	626.244	1.799	0.132	0.347	0.217	64.886
		Elevation	-0.233	0.142	-1.631	0.164			
	Mean UVA weighed absorbance ~ Elevation	Intercept	1380.905	626.972	2.203	0.079	0.467	0.361	64.902
		Elevation	-0.299	0.143	-2.095	0.090			
	Max UVA weighed absorbance ~ Elevation	Intercept	1752.845	660.411	2.654	0.045*	0.555	0.4662	65.630
		Elevation	-0.376	0.150	-2.498	0.055*			
Culcitium spp.	Mean UVB weighed absorbance ~ Elevation	Intercept	10.993	84.255	0.130	0.898	0.073	0.002	120.584
		Elevation	0.020	0.020	1.015	0.329			
	Max UVB weighed absorbance ~ Elevation	Intercept	19.64097	133.890	0.147	0.886	0.005	-0.018	134.479
		Elevation	0.027	0.031	0.866	0.402			
	Mean UVA weighed absorbance ~ Elevation	Intercept	75.390	107.200	0.703	0.494	< 0.001	-0.077	127.805
		Elevation	0.001	0.025	0.046	0.964			
	Max UVA weighed absorbance ~ Elevation	Intercept	-18.426	131.827	-0.140	0.891	0.891	0.001	134.013
		Elevation	0.034	0.031	1.099	0.292			
Espeletia colombiana	Mean UVB weighed absorbance ~ Elevation	Intercept	682.234	411.816	1.657	0.173	0.330	0.163	59.562
		Elevation	-0.140	0.010	-1.405	0.233			
	Max UVB weighed absorbance ~ Elevation	Intercept	1290.179	770.115	1.675	0.169	0.355	0.194	67.074
		Elevation	-0.277	0.186	-1.485	0.212			
	Mean UVA weighed absorbance ~ Elevation	Intercept	359.311	578.303	0.621	0.568	0.055	-0.181	63.636
		Elevation	-0.068	0.140	-0.482	0.655			
	Max UVA weighed absorbance ~ Elevation	Intercept	897.237	539.274	1.664	0.171	0.343	0.178	62.798
		Elevation	-0.188	0.130	-1.444	0.222			
Espeletia lopezii	Mean UVB weighed absorbance ~ Elevation	Intercept	113.737	114.573	0.993	0.344	0.008	-0.091	100.816
		Elevation	-0.007	0.026	-0.281	0.784			
	Max UVB weighed absorbance ~ Elevation	Intercept	155.633	191.753	0.812	0.436	0.006	-0.093	113.176
		Elevation	-0.011	0.044	-0.244	0.812			

	Mean UVA weighed absorbance ~ Elevation	Intercept	102.496	179.729	0.570	0.581	0.006	-0.994	111.622
		Elevation	-0.010	0.042	-0.242	0.814			
	Max UVA weighed absorbance ~ Elevation	Intercept	170.274	242.422	0.702	0.498	0.008	-0.091	118.804
		Elevation	-0.016	0.056	-0.283	0.783			
Monticalia vaccinioides	Mean UVB weighed absorbance ~ Elevation	Intercept	238.840	54.365	4.393	0.012*	0.695	0.619	42.562
		Elevation	-0.039	0.013	-3.022	0.039*			
	Max UVB weighed absorbance ~ Elevation	Intercept	273.159	57.390	4.760	0.009*	0.699	0.624	43.212
		Elevation	-0.042	0.014	-3.051	0.038*			
	Mean UVA weighed absorbance ~ Elevation	Intercept	205.242	169.728	1.209	0.293	0.172	-0.035	56.224
		Elevation	-0.036	0.040	-0.912	0.413			
	Max UVA weighed absorbance ~ Elevation	Intercept	357.767	43.432	8.237	0.001*	0.9023	0.878	39.868
		Elevation	-0.062	0.010	-6.079	0.003*			
Werneria sp.	Mean UVB weighed absorbance ~ Elevation	Intercept	-2262.136	1074.586	-2.105	0.080	0.462	0.372	90.331
		Elevation	0.550	0.242	2.271	0.0636			
	Max UVB weighed absorbance ~ Elevation	Intercept	-3119.823	1552.337	-2.010	0.091	0.439	0.346	96.216
		Elevation	0.758	0.350	2.168	0.0732			
	Mean UVA weighed absorbance ~ Elevation	Intercept	-380.152	1067.196	-2.230	0.067	0.482	0.395	90.220
		Elevation	0.568	0.240	2.361	0.0562*			
	Max UVA weighed absorbance ~ Elevation	Intercept	-2719.538	1092.668	-2.489	0.047*	0.543	0.466	90.598
		Elevation	0.657	0.246	2.668	0.037*			

Table S3. Results of ANOVA/Kruskal/T-Student (T) test performed and p-values (p) of UV absorbance metrics by elevational range in each species. Statistically significant values are marked with asterisk (*).

Species	*N*	Weighed absorbance metric	Shapiro-Wilk		Levene		Test		
			W	*p*	*F*	*p*	$t / F / \chi^2$	*p*	
Baccharis rupicola	7	UVB mean	0.890	0.273	0.874	0.393	-0.001	0.997	T
		UVB max	0.922	0.488	0.795	0.414	0.242	0.819	T
		UVA mean	0.970	0.890	0.044	0.843	1.027	0.368	T
		UVA max	0.890	0.273	0.808	0.410	0.839	0.440	T
Culcitium spp.	15	UVB mean	0.962	0.445	0.962	0.445	0.975	0.439	ANOVA
		UVB max	0.762	0.000*	1.131	0.379	3.863	0.277	Kruskal
		UVA mean	0.972	0.883	1.386	0.299	0.641	0.605	ANOVA
		UVA max	0.930	0.268	0.269	0.846	0.617	0.618	ANOVA
Espeletia lopezii	10	UVB mean	0.971	0.919	1.484	0.277	-0.006	0.996	T
		UVB max	0.935	0.438	0.990	0.409	-0.309	0.769	T
		UVA mean	0.959	0.763	1.910	0.204	-0.520	0.629	T
		UVA max	0.934	0.425	1.732	0.231	-0.528	0.624	T
Werneria sp.	8	UVB mean	0.841	0.076	3.25	0.122	-2.690	0.054*	T
		UVB max	0.909	0.350	1.960	0.211	-3.001	0.031*	T
		UVA mean	0.948	0.693	0.155	0.707	-2.795	0.039*	T
		UVA max	0.922	0.449	0.735	0.424	-28.814	0.029*	T

Table S4. Results of ANOVA/Kruskal/T-Student (T) test performed and p-values (p) of UV absorbance metrics by species in each elevational range. Statistically significant values are marked with asterisk (*).

Elevation range	Species compared	*N*	Weighed absorbance metric	Shapiro-Wilk		Levene		Test		
				W	*p*	*F*	*p*	$t / F / \chi^2$	*p*	
3990-4100	*Culcitium* spp. *E. colombiana* *S. niveoaureus*	10	UVB mean	0.898	0.206	0.281	0.763	3.086	0.109	ANOVA
			UVB max	0.797	0.013*	0.134	0.876	10.549	0.005*	Kruskal
			UVA mean	0.951	0.679	0.897	0.450	4.693	0.051*	ANOVA
			UVA max	0.976	0.942	0.296	0.753	5.620	0.035*	ANOVA
4100-4260	*Culcitium* spp. *E. lopezii*	10	UVB mean	0.923	0.374	0.066	0.804	1.163	0.279	T
			UVB max	0.895	0.194	0.194	0.671	1.274	0.241	T
			UVA mean	0.895	0.196	0.820	0.392	1.843	0.116	T
			UVA max	0.901	0.222	0.466	0.514	1.246	0.307	T
4260-4400	*B. rupicola*. *Culcitium* spp. *Werneria* sp.	11	UVB mean	0.931	0.305	9.282	0.011*	7.282	0.026*	Kruskal
			UVB max	0.936	0.453	1.369	0.303	10.250	0.005*	ANOVA
			UVA mean	0.881	0.161	2.494	0.152	0.341	0.722	ANOVA
			UVA max	0.919	0.351	10.197	0.008*	6.545	0.038*	Kruskal
4400-4530	*Culcitium* spp. *E. lopezii* *Werneria* sp.	20	UVB mean	0.782	0.000*	3.519	0.030*	13.255	0.010*	Kruskal
			UVB max	0.783	0.000*	4.128	0.017*	13.778	0.008*	Kruskal
			UVA mean	0.833	0.002*	4.924	0.009*	10.071	0.039*	Kruskal
			UVA max	0.877	0.013*	7.336	0.001*	12.196	0.016*	Kruskal

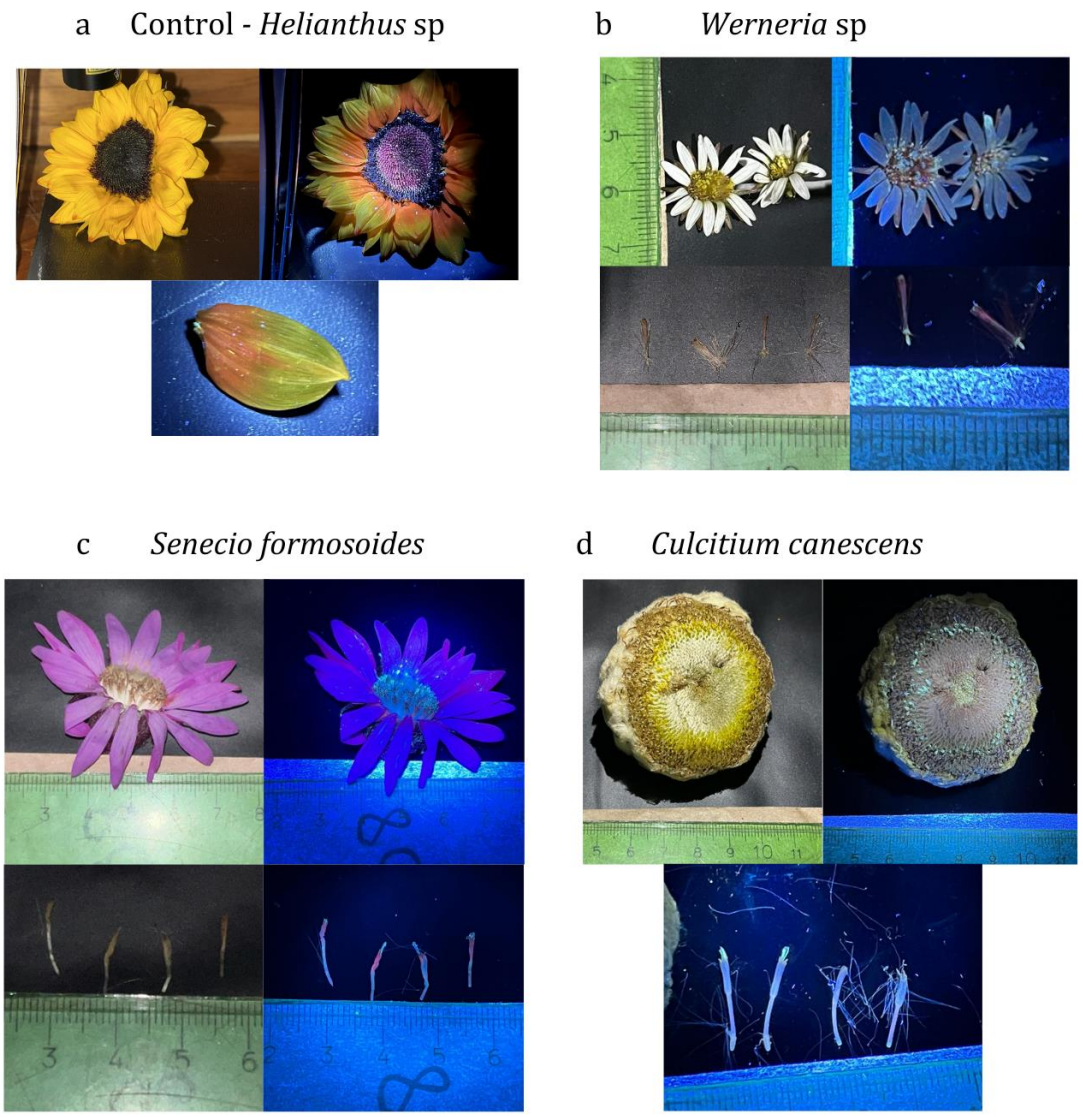

Figure S1. Pigmentation patterns of three species found along the elevational gradient. (A) Positive control using *Helianthus sp.* to show that our photography method can detect UV pigments. *Helianthus*'s bullseye pattern is visible as the base of the petals (ray florets) present darker colorations (UV-absorbing). Photos were taken in a dark room with a UV light (peak at 365 nm) of the whole inflorescence and individual disc florets.

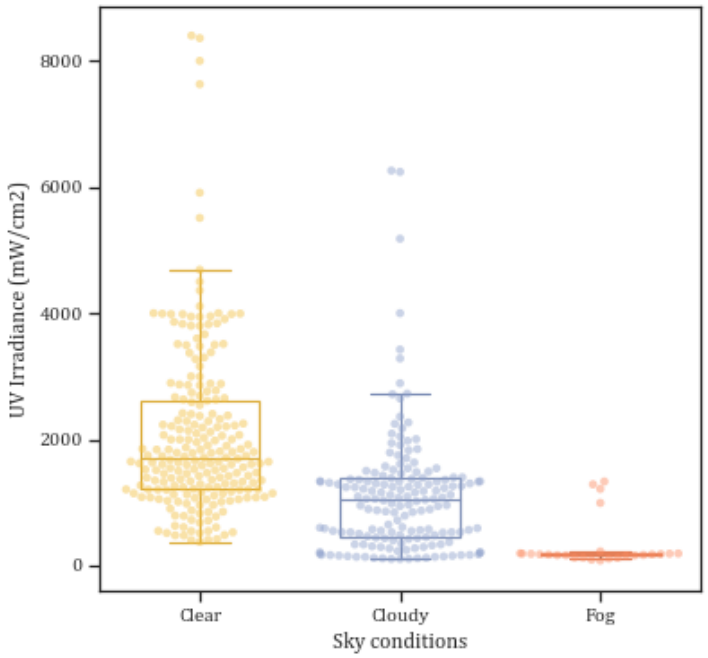

Figure S2. UV Irradiance measurements separated by sky conditions. Clear refers to days where no cloud covers the solar orbit, Cloudy refers to days where at least one cloud covers the solar orbit and Fog refers to foggy days.

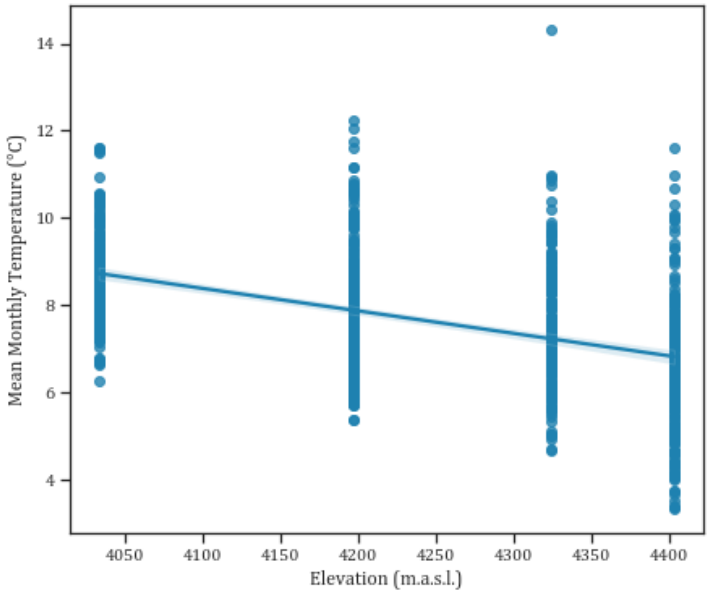

Figure S3. Mean monthly temperature values along the elevation gradient. The data adjust to a linear regression model (y = -0.01x + 29.50; $p < 0.001$; $R^2 = 0.21$) where temperature decreases at higher elevations, but its variability increases.

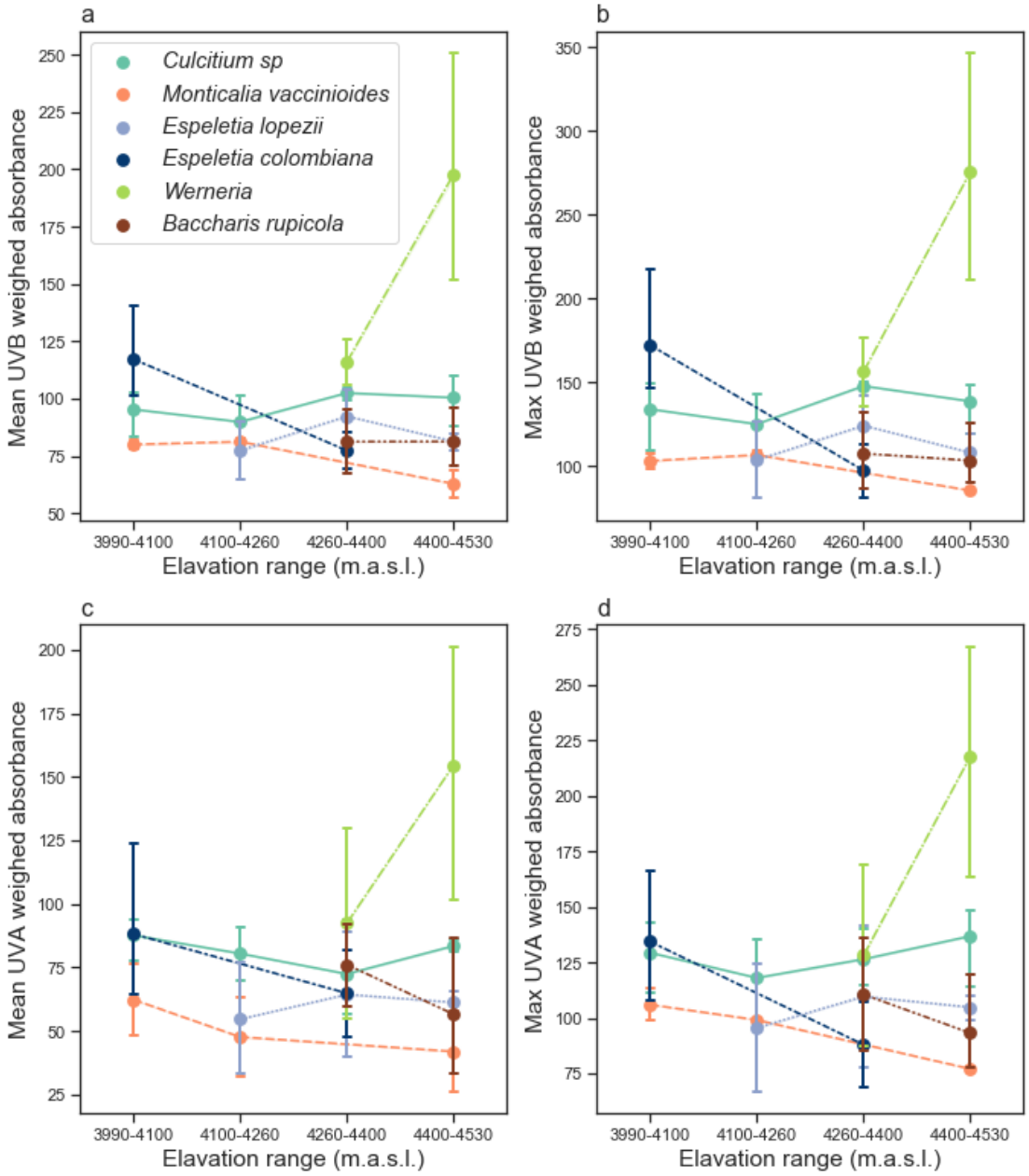

Figure S4. Mean and maximum absorbance of floral extracts in UVB (280 – 315 nm) and UVA (315 – 400 nm) per species, along the elevational gradient. Weighed absorbance values were estimated by dividing absorbance values by the concentration of extract used in the spectrophotometry. Dots correspond to mean values while error bars show standard deviations.