



Morphoanatomy and leaf biomass in *Espeletia standleyana* A.C.Sm. and *Espeletia santanderensis* A.C.Sm. in a northeastern Colombian paramo

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Abstract

The adaptive responses of *Espeletia standleyana* and *E. santanderensis* populations in a paramo in Northeastern Colombia (3350 m a.s.l.), were studied to characterize their spatial segregation. Their anatomical, morphofunctional and leaf biomass characteristics were determined and compared. We found that many traits such as depth of the stomatal crypts, number of vascular bundles, leaf and peduncle xylem vessel diameters, thickness of the mesophyll, leaf area, rosette height and diameter, leaf water content, leaf area index, and leaf biomass were significantly higher in *E. standleyana* ($P < 0.05$). Meanwhile, the diameter of the vascular bundles, width of stomatal crypts, number of leaves, specific leaf area, and percentage of sclerophylly were higher in *E. santanderensis* ($P < 0.05$). Multifactorial segregation indicated highly differential expressions in their morphofunctional and leaf biomass characteristics, evidencing adaptations to their microhabitats. *E. standleyana* showed xeromorphic characters in response to the greater ambient fluctuations typical of the paramo, while *E. santanderensis* responded with scleromorphic traits related to lower soil organic matter and water content, characteristic of the high Andean forest-paramo ecotone. The high spatial heterogeneity of the paramos allows the development of microclimatic and edaphic mosaics that determine population segregation of these growth forms.

Keywords Giant rosettes · Leaf anatomy · Microhabitat adaptations · Morphometric characteristics · Spatial segregation · Tropical alpine

Introduction

Paramo is a Neotropical alpine ecosystem occurring in the Northern Andes, at elevations above the natural upper limit of cloud forest—typically from around 3000 m a.s.l. up to the permanent snow line at approximately 4600 m a.s.l. This

ecosystem plays a key role in the hydrological cycle, providing vital ecosystem services to major Andean cities. It also contributes significantly to the carbon cycle due to their capacity for carbon sequestration. Paramos possess the highest floristic diversity and the greatest number of endemic species among alpine ecosystems worldwide (Luteyn et al. 1999; Sklenář et al. 2014). They are considered some of the world's fastest-evolving biodiversity hotspots and centers of speciation (Myers et al. 2000; Madriñán et al. 2013). The most floristically diverse páramos are found in Colombia, which also represents the center of the ecosystem's geographic distribution.

Paramo vegetation is typically structured in a mosaic of plant communities, shaped by strong environmental gradients and disturbance regimes. Dominant vegetation types include tussock grasslands, shrublands, rosette plants, cushion bogs, and open herbaceous wetlands, with many species exhibiting adaptations to cold temperatures, intense solar radiation, and water-saturated soils. The high spatial heterogeneity of soils and climatic conditions generates complex

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ecological gradients, contributing to this structural and floristic diversity (Llambí and Rada 2019).

The *Espeletia* complex, belonging to the subtribe Espeletiinae, exhibits a remarkable degree of morphological diversity, including branched and unbranched trees, shrubs and sessile, short-branched, or giant caulescent rosettes (Pouchon et al. 2018). The latter ones constitute one of the dominant growth forms of the neotropical high mountains, and more specifically, the Colombian paramos. These giant rosettes, distributed in the paramos of Venezuela, Colombia and Ecuador, represent one of the richest and most dominant genera of these high mountain ecosystems of the northern Andes. Of the approximately 140 described species, Colombia has the largest number (90 spp.) (Mavárez 2021). The *Espeletia* complex is considered one of the most rapidly evolving plant groups (Cortés et al. 2018; Pouchon et al. 2018). Morphological differentiation among species reflects processes of niche specialization, shaped by environmental selection pressures over evolutionary time (Pouchon et al. 2018). The adaptive radiation of the Espeletiinae in the eastern branch of the Colombian Andes has led to extensive diversification and the emergence of numerous species within the genus (Pouchon et al. 2021).

Espeletia stem rosettes are ecologically important in páramo ecosystems due to their capacity to store biomass and, in some species, retain necromass along the stem as standing litter. Their anatomical and morphological traits also contribute to water regulation within the ecosystem. These traits include densely pubescent leaves and stomatal crypts that reduce water loss through transpiration; marcescent leaves surrounding the stem, which help regulate temperature and store biomass; stems with pith tissue capable of storing water; and the parabolic rosette form, which optimizes the interception of photosynthetically active radiation (Roth 1973; Azócar and Rada 1993; Murcia and Mora-Osejo 2003; Roth-Nebelsick et al. 2009). These characteristics allow *Espeletia* plants to regulate water loss to the atmosphere and help maintain soil and microclimatic conditions favorable to ecosystem functioning (Cárdenas et al. 2018; Rada et al. 2019).

Among the many *Espeletia* species described from Colombian páramos, *Espeletia standleyana* A.C. Smith and *E. santanderensis* A.C. Smith are dominant in the páramos of northeastern Colombia. In the Páramo de García, populations of these two species are spatially segregated, while individuals may occur adjacent to one another in some areas, their distributions do not overlap. In this paper, we address the following questions: (1) Do these two *Espeletia* species exhibit differences in their anatomical, morphometric, and leaf biomass traits? (2) If so, can the environmental heterogeneity of the páramo—specifically microclimatic and edaphic conditions—explain their spatial segregation? We hypothesize that morpho-anatomical

traits correspond to soil conditions, with traits associated with sclerophylly occurring in soils with lower organic matter and/or moisture content, and traits linked to hygrophily found in soils richer in organic matter and moisture.

Materials and methods

This research was conducted in the Paramo de García, located near the town of Pamplona, Norte de Santander, Colombia (Fig. 1a), at an elevation of 3350 m a.s.l. (N 7°19'21"W 72°39'30"). The study site lies within the ecotone between the high Andean forest and the paramo *sensu stricto*. The area is characterized by heterogeneous vegetation, including small trees, shrubs, and grasses, with spatially segregated populations of *Espeletia standleyana* and *E. santanderensis* (Fig. 1b). As shown in the figure, both populations are located adjacent to one another on the same east-facing side slope. The total area of this paramo is approximately 1.12 km².

Study species

Espeletia standleyana

A caulescent rosette with a stem 1–2 m in height and pubescent leaves. Leaves and flowering branches densely packed at the top, covered with a tightly tomentose golden or golden-gray indumentum; leaves oblong-spatulate, up to 30 cm long and 3.5 cm wide; flowering branches up to 70 cm long, inflorescence with 10–25 heads in a corymbose-panicle up to 30 cm long; heads at maturity 20–25 mm in diameter, peduncles up to 5 cm long (Smith and Koch 1935). The population under study is located adjacent to the high Andean forest (Fig. 1c), in a community of species belonging to the ecotone forest-paramo. Along with *E. standleyana*, other dominant plants include the grass *Calamagrostis effusa* (Kunth) Steud. and shrub species belonging to the genus *Hypericum*. This site corresponds to the lower elevation in which the first individuals of the species are found in Paramo de García. *E. standleyana* is distributed between 2900 and 3900 m a.s.l. (Bernal et al. 2020) and is also found in nearby paramos from the Paramo de Santurban complex (e.g., Paramo de Tierra Negra and Laguna El Cornal) (Fig. S1).

Espeletia santanderensis

This species has a shorter stem than *E. standleyana* and occurs in the same localities as *E. standleyana*, it has synflorescences forming conglomerates of capitula at the apex and marcescent leaves extending over the soil surface. This species is smaller in size and displays less dense pubescence leaves compared to *E. standleyana* (Fig. 1c).

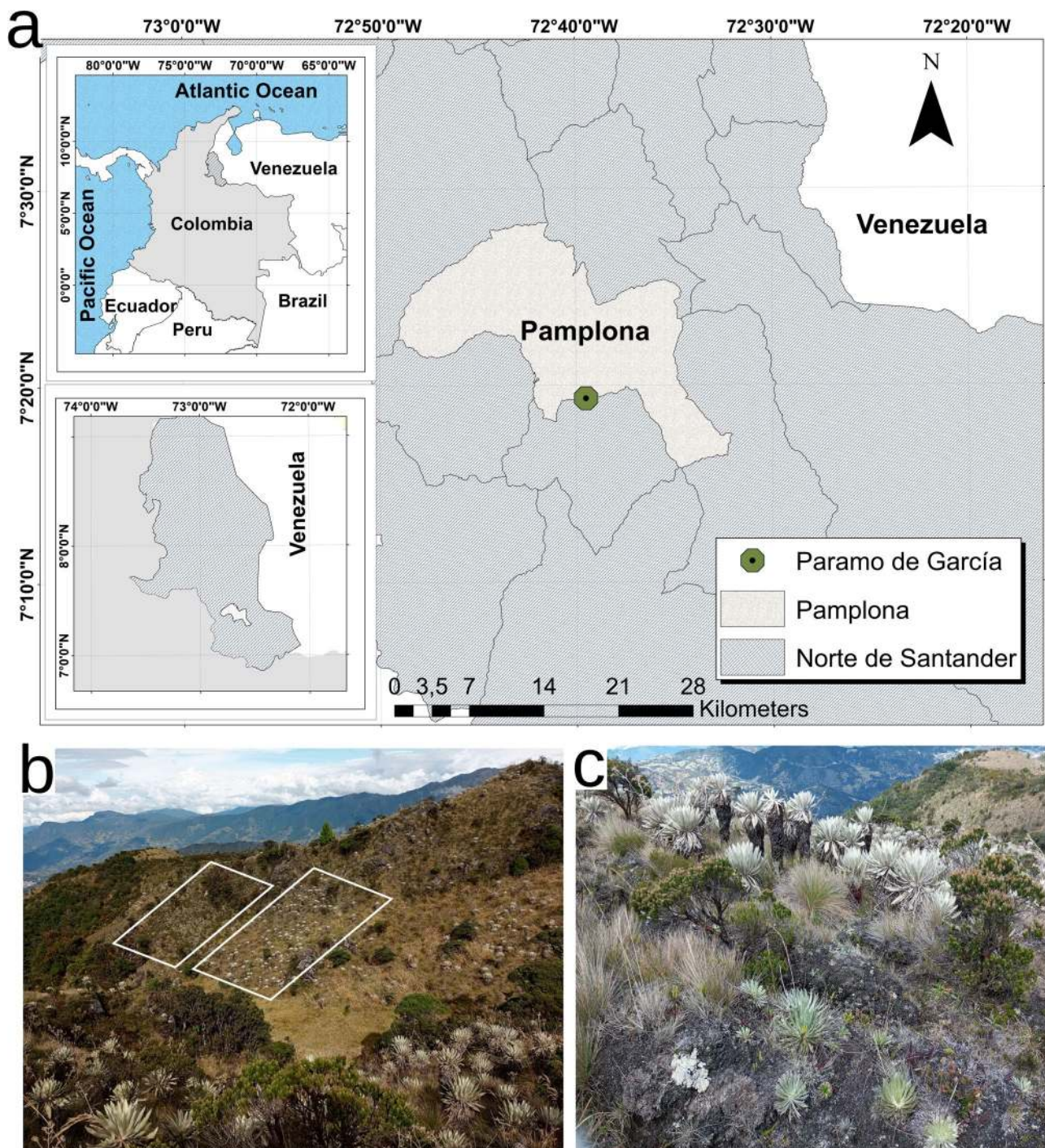


Fig. 1 a. Location map of the Paramo de García. b. Study area, right rectangle corresponds to *E. standleyana*, left rectangle to *E. santanderensis* c. Close up showing adjacent populations of the giant rosettes *E. standleyana* (upper right) and *E. santanderensis* (lower left)

Leaves are oblong measuring 15–25 cm long by 8–15 mm wide and presenting a dense silvery-silky indumentum; it has well-developed corymbose synflorescences, with 20–40 heads measuring 15–20 cm in diameter (Cuatrecasas 1996). In the Paramo de García, the substrate in which individuals of this species are found is generally rocky

with shallow soils no deeper than 10 cm. In the study site, this species ranges from the high Andean forest (ca. 3100 m a.s.l.) up to the forest-paramo ecotone (3400 m a.s.l.) and is associated mainly with grasses (e.g., *C. effusa*) and fewer shrub species. This species is also found in the same neighboring Paramo de Santurban complex from 2500 to

4300 m a.s.l. (Bernal et al. 2020) (e.g., Paramo de Tierra Negra, Laguna El Cornal and Laguna de Jaimes) (Fig. S1).

Edaphic and microclimatic variables

Two 10 x 10 m plots were created, one for each population. From each plot, ten soil samples were randomly collected near the areas of greatest *Espeletia* density. These samples were obtained at a 10 cm soil depth, their wet weight was determined, and dried at 60 °C for 72 h. These samples were then placed in a muffle furnace and were combusted at 550 °C to calculate the following variables (Heiri et al. 2001):

$$\begin{aligned} \% \text{Soil water content} (\% \text{SWC}) \\ = \frac{\text{Soil fresh weight (g)} - \text{soil dry weight (g)}}{\text{soil fresh weight (g)}} \times 100 \end{aligned}$$

$$\begin{aligned} \% \text{Soil organic matter} (\% \text{SOM}) \\ = \frac{\text{Soil dry weight (g)} - \text{soil weight (g) at } 550^\circ \text{C}}{\text{soil dry weight (g)}} \times 100 \end{aligned}$$

The soil pH was measured with a pH meter (PH 7110, MTW inolab®) using a 1:1 sample to distilled water ratio.

To compare the microclimate at each of the plots, where the two species are found, a meteorological microstation (HOBO onset, model U12-012) with relative humidity (RH) and air temperature (T) sensors, was installed around the areas of highest *Espeletia* density at 1 m above the soil surface. Measurements were carried out every 15 minutes from November 6 to 14, 2021 at each sampling site.

Data and sample collection

In each plot, 30 individuals from each species were chosen at random. Rosette height, diameter, and cover; and maximum leaf length and width were measured for all the leaves in each of the rosettes. All measurements and leaf samples taken for the determination of morphoanatomical characteristics were conducted during the same period in which soil samples were collected.

Leaves were transported in hermetically sealed bags to the laboratory to determine fresh weight, dry weight (subjected to 60 °C for 72 h), leaf water content and the morphofunctional indices measured by Berdugo Lattke (2020) and proposed by Veneklaas (1985) and leaf biomass were calculated using the following formulas:

$$\begin{aligned} \text{Leaf water content (LWC)} &= \frac{\text{leaf wet weight (g)}}{\text{leaf dry weight (g)}} \end{aligned}$$

$$\begin{aligned} \% \text{Leaf water content} (\% \text{LWC}) \\ = \frac{\text{leaf water content (g)}}{\text{leaf wet weight (g)}} \times 100 \end{aligned}$$

$$\text{Specific leaf area (SLA)} = \frac{\text{leaf area (cm}^2\text{)}}{\text{leaf dry weight (g)}}$$

$$\% \text{Sclerophilly} (\% \text{Scl}) = \frac{\text{leaf dry weight (g)}}{\text{Leaf wet weight (g)}} \times 100$$

$$\text{Leaf area index (LAI)} = \frac{\text{rosette leaf area (m}^2\text{)}}{\text{rosette cover (m}^2\text{)}}$$

$$\text{Leaf biomass} = \frac{\text{rosette leaf dry weight (g)}}{\text{rosette cover (m}^2\text{)}}$$

We performed a test on how to best quantify leaf area. Using 73 and 68 leaves from different individuals for *E. standleyana* and *E. santanderensis*, respectively; we calculated leaf area using three methods. Firstly, through the formula $2/3 * (\text{maximum leaf length (L)} * \text{maximum leaf width (W)})$ (Montgomery 1911). Then by photocopying leaf silhouettes on graph paper and finally through digital image analysis using ImageJ (scanning of the leaves using a standardized scale). There were no statistically significant differences between the leaf area methods (ANOVA; p-value > 0.05, Fig. S2) tested, which allowed us to use the simplest and non-destructive method in the field, $2/3 (L * A)$, to calculate the leaf area of the rosettes.

Leaf and peduncle anatomy

One leaf and floral peduncle per individual of *Espeletia standleyana* and *Espeletia santanderensis* were collected and brought to the Animal and Plant Histology laboratory of the University of Pamplona. The histological procedures described below are from Johansen (1940) and Roth (1964).

Leaf sections of approximately 1 cm² from the middle region and floral peduncle segments of approximately 1 cm in length were collected and fixed in 20 ml of a FAA solution (formol 40% 90cc, acetic acid 5cc and alcohol 95% 5cc) for 48 h. The samples were then processed using a tissue processor (Microm HPM110) and subjected to different alcohol and xylol concentrations to dehydrate and clarify the sample, respectively. Subsequently, they were impregnated and embedded in molten paraffin, after which 10 µm

cross-sections were obtained using a microtome (H315 MICROM). The sections were placed on a slide treated with a solution of albumin and glycerol to facilitate adhesion. They were then dewaxed and dehydrated through exposure to different concentrations of xylol and alcohols, followed by staining with Ziehl fuchsin and brilliant green. Once air-dried, the samples were sealed with Entellan resin.

Images were captured using an Axiocam ERc5s camera, and anatomical measurements were conducted using the ZEN Core software from ZEISS. The measured variables included the width and length of the peduncle vascular bundle, thickness of the mesophyll, depth and width of the adaxial stomatal crypts, and xylem vessel diameter of the peduncle and central leaf vein.

Statistical analysis

To assess the precision of the leaf area measurement methods, ANOVA hypothesis contrast tests were used. Variables were compared using Student's *T*-test, Student, Welch's *T*-test and Wilcoxon-Mann-Whitney hypothesis contrast tests, following the validation of homogeneity of variances and normality assumptions. All results were visualized using boxplot-violin graphs (Patil 2021), illustrating sample data distribution through a mirror density diagram, which represents the density of points under the curve. These tests examined anatomical, morphophysiological and leaf biomass traits at the univariate level. For species differentiation in a multifactorial context, principal component analysis (PCA) was conducted, with variables standardized to a Z-score distribution, where the variance was set to 1 and the mean to 0. Correlation analyzes were performed to identify relationships between morphophysiological variables and leaf biomass, which was estimated based on the predictors that best explained its variance. Simple regression models were calculated using the linear least squares method, selecting the model that best explained the response variable while

satisfying the assumptions of linearity, homoscedasticity, normality, autocorrelation of residuals and influential values (Cook's distances). To select the models that best explained the response variables, we applied the Akaike Information Criterion (AIC) using the Broom package (Robinson et al. 2024) and employed the stepwise selection method. Additionally, the coefficient of variation (CV) was used to compare temperature and relative humidity across population sites. All analyses were conducted in R (R Core team 2023) using the following packages: tidyverse (Wickham et al. 2023), car (Fox and Wesiberg 2019), lmtest (Zeileis and Hothorn 2002), Facto MineR (Sebastien et al. 2008), ggstatsplot (Patil 2021), factextra (Kassambara and Mundt 2020), among others.

Results

Comparison of edaphic and microenvironmental variables between the two populations

The %SWC and %SOM were between 1.6 ($p = 4.7e^{-10}$) and 1.7 ($p = 2.45e^{-6}$) times significantly higher in *E. standleyana* while the soil pH was 1.15 ($p = 3.21e^{-8}$) higher for *E. santanderensis* (Fig. 2, Table S1).

Temperature and relative humidity daily oscillations were larger in the microsite where *E. standleyana* was present, compared to the *E. santanderensis* site (Fig. 3a, b). The average minimum temperature was 3.2 °C and the average maximum was 27.6 °C at the *E. standleyana* site, in contrast to 3.9 °C and 18.3 °C average minimum and maximum, respectively, for *E. santanderensis*. Absolute temperature and relative humidity had greater oscillations in the *E. standleyana* microsite (1–32 °C, 28–100%) compared to that of *E. santanderensis* (1–22 °C, 47–99%). At night (between 18:00 and 6:00), the relative humidity remained high, above 90%

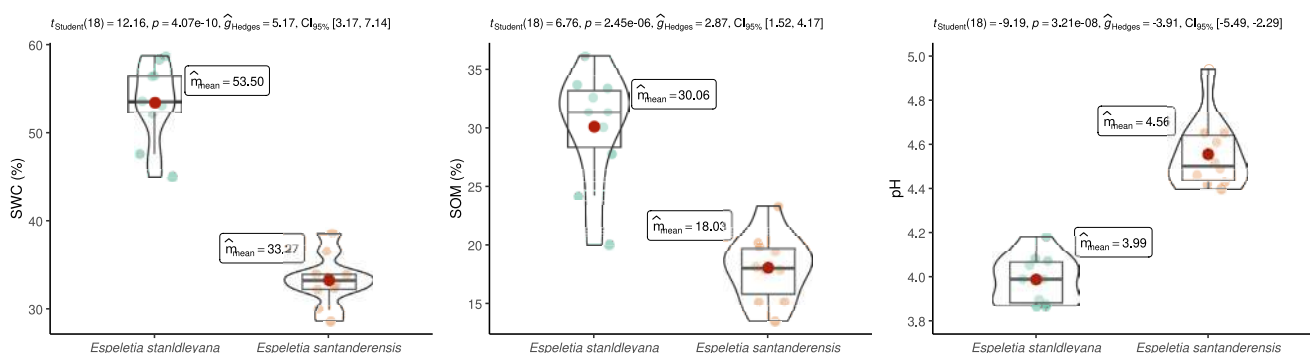


Fig. 2 Comparison of edaphic variables in the *E. standleyana* and *E. santanderensis* microsites (n=10 for each species/site)

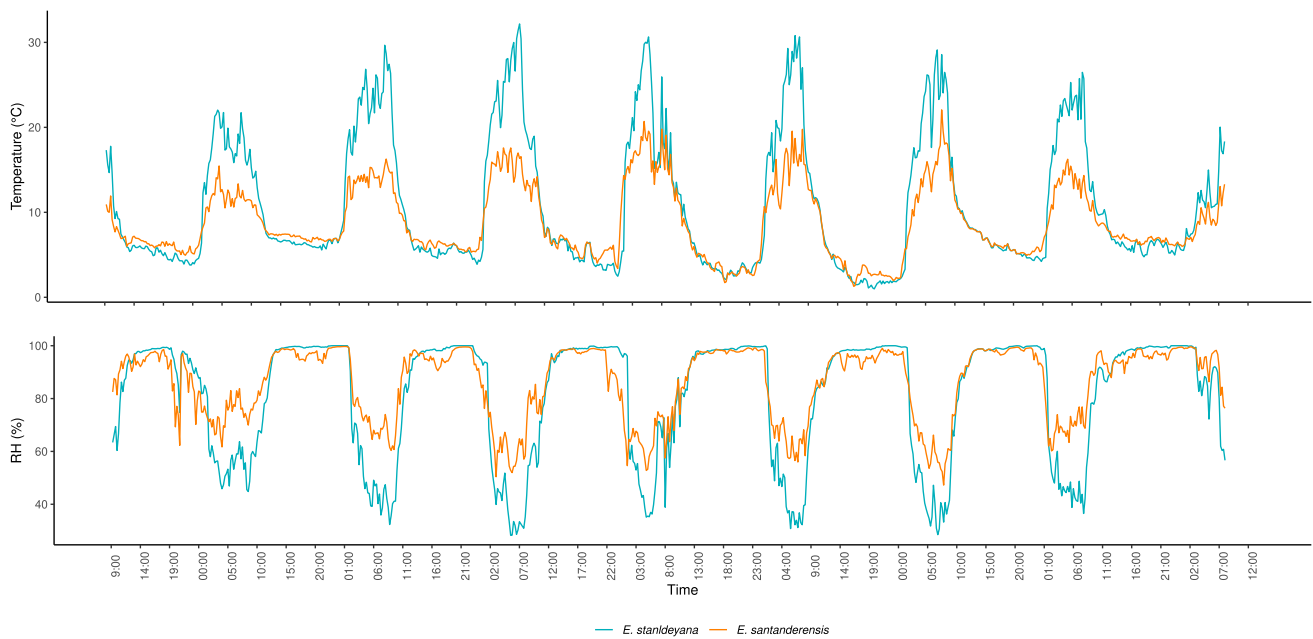


Fig. 3 Daily cycles (November 6–14 2021) of meteorological variables, **a** air temperature and **b** relative humidity (RH), for the *E. standleyana* and *E. santanderensis* microsites

for both species, while the temperature remained between 1 and 10 °C for both species, evidencing relative nocturnal meteorological homogeneity. The coefficients of variation were higher for both temperature (72.04 vs 48.06) and relative humidity (27.9 vs 15.9) in the *E. standleyana* microsite.

Comparison of morphoanatomical variables and indices between the two species

Concerning the morphophysiological traits, we found that *E. standleyana* was proportionally greater in the following variables: Rosette height (5.6:1, $p = 2.14 \times 10^{-11}$), rosette diameter (1.7:1, $p = 6.31 \times 10^{-7}$), leaf length (2.4:1, $p = 4.61 \times 10^{-16}$),

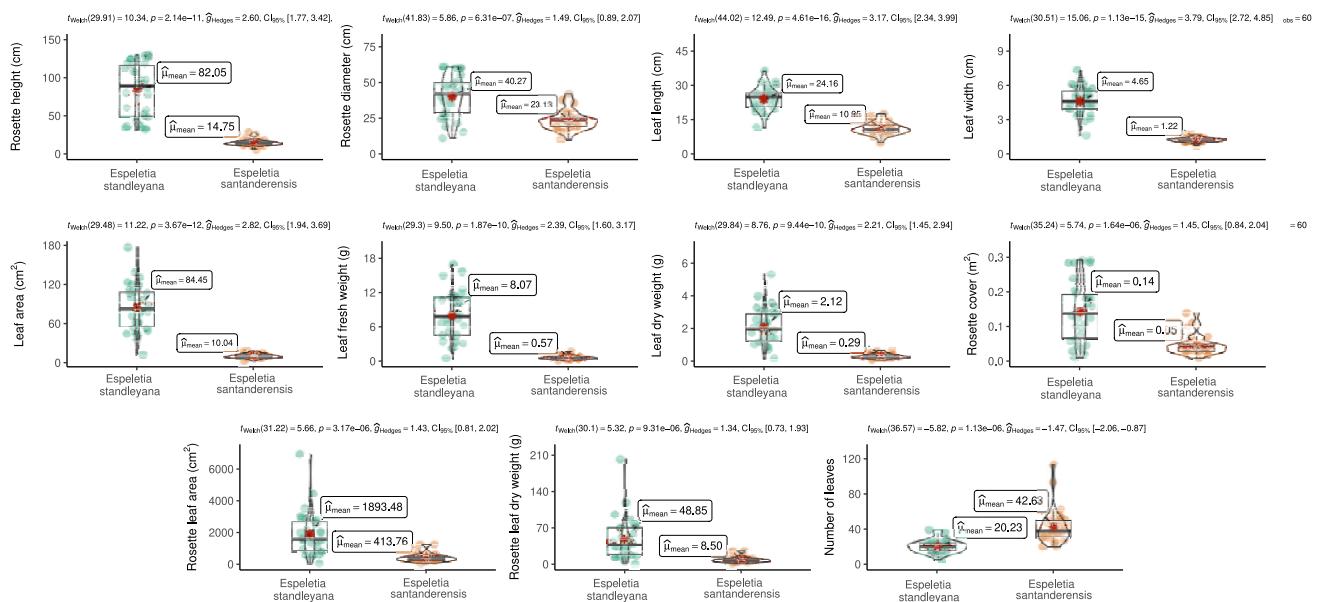


Fig. 4 Comparison of the morphometric variables for *E. standleyana* and *E. santanderensis* ($n = 30$ for each species)

leaf width (4:1, $p = 1.13e^{-15}$), leaf area (10:1, $p = 3.67e^{-12}$), fresh weight (14:1, $p = 1.87e^{-10}$), dry weight (11:1, $p = 9.44e^{-10}$), rosette cover (2.8:1, $p = 1.64e^{-6}$), rosette leaf area (4.6:1, $p = 3.17e^{-6}$), rosette leaf dry weight (5.7:1, $p = 9.31e^{-6}$). In contrast, *E. santanderensis* showed a higher proportion in the number of leaves (1:2.1, $p = 1.13e^{-6}$) (Fig. 4). Percentage sclerophylly (1.26:1, $p = 3.6e^{-8}$) and specific leaf area (1.1:1, $p = 2.92e^{-3}$) were higher in *E. santanderensis*. However, leaf water content (16.4:1, $p = 4.4e^{-10}$), percentage leaf water content (1.16:1, $p = 3.6e^{-8}$), leaf area index (1.3:1, $p = 0.04$) and leaf biomass (1.72:1, $p = 1.61e^{-4}$) were higher in *E. standleyana* (Fig. 5, Table S1)).

Leaf and peduncle anatomy

For anatomical traits, the length ($p = 9.52e^{-4}$) and width ($p = 2.2e^{-4}$) of the vascular bundles were greater in *E. santanderensis*, while the number of vascular bundles of the peduncle were greater in *E. standleyana* (Fig. 6). The stomatal crypts were deeper ($p = 0.02$) in *E. standleyana* and wider ($p = 0.04$) in *E. santanderensis*, and the thickness of the mesophyll was greater ($p = 0.03$) in *E. standleyana*. Furthermore, the diameters of the xylem vessels, both of the peduncle (12.41 ± 0.54 vs 6.89 ± 0.46 μm , $p = 1.097e^{-5}$) and of the midrib of the leaf (12.43 ± 0.42 vs 7.55 ± 0.21 μm , $p = 5.8e^{-5}$), were greater in *E. standleyana*.

Models for estimating leaf dry weight and leaf biomass

The suitable models for estimating leaf dry weight as a function of leaf area were significantly different between the species (ANOVA; p -value < 0.05), finding positive change ratios with a potential trend for *E. standleyana* and

linear for *E. santanderensis* (Fig. 7). These models were chosen based on the AIC (Akaike Information Criterion) and their respective validations (see validation and comparison in Tables S2–S7, Figs. S3–S4). The rate of increase in dry weight with respect to leaf area was greater in *E. standleyana* (at the power of 1.297) than in *E. santanderensis* (at a rate of 0.0207).

Principal components analysis

Principal component analysis (PCA) revealed a clear segregation of the populations within the multifactorial space, explaining 74.5% of the total inertia. Major edaphic variables strongly differentiated the *E. standleyana* population, showing a positive correlation with higher morphometric and morphofunctional trait values. In contrast, *E. santanderensis* was associated with a rocky, low moisture soil, which corresponded to high values of %Scl, SLA, and leaf number, alongside lower morphometric values. The first principal component (PC1) correlated with most morphometric variables, %Scl, and the analyzed edaphic variables. Meanwhile, the second principal component (PC2) exhibited a strong correlation with leaf number, SLA, and edaphic factors (Tables S8–S10; Fig. 8).

Discussion

The anatomical, morphometric and leaf biomass differences between *Espeletia standleyana* and *E. santanderensis* populations at Paramo de García highlight their contrasting adaptations to the specific conditions of their ecological niches. The results also indicate a differentiation between the microenvironments where each species is found, which may explain their spatial segregation at this location.

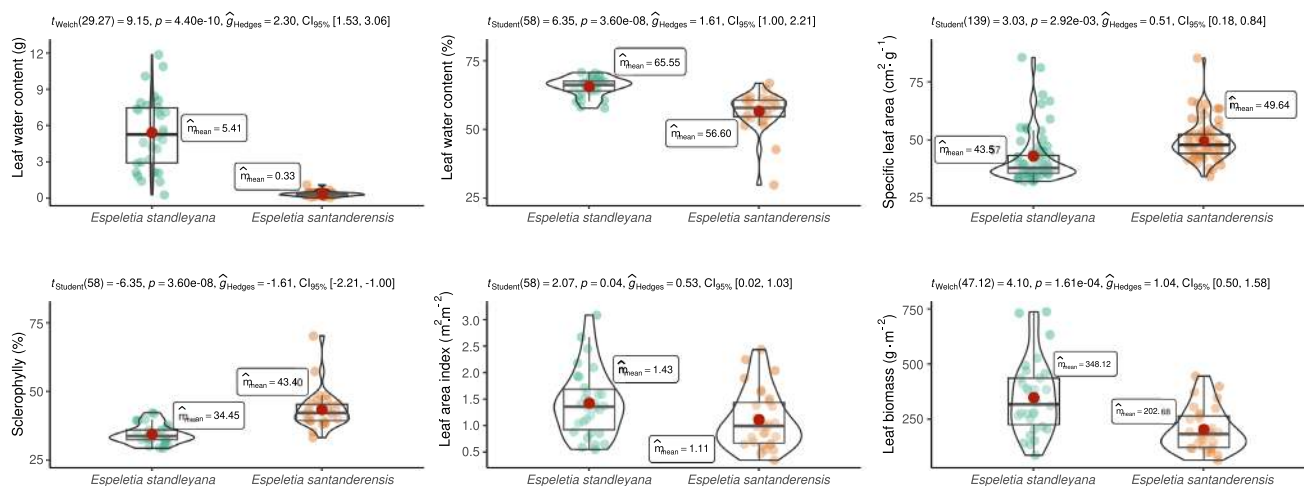


Fig. 5 Comparison of the morphophysiological indices for *E. standleyana* and *E. santanderensis* ($n=30$ for each species)

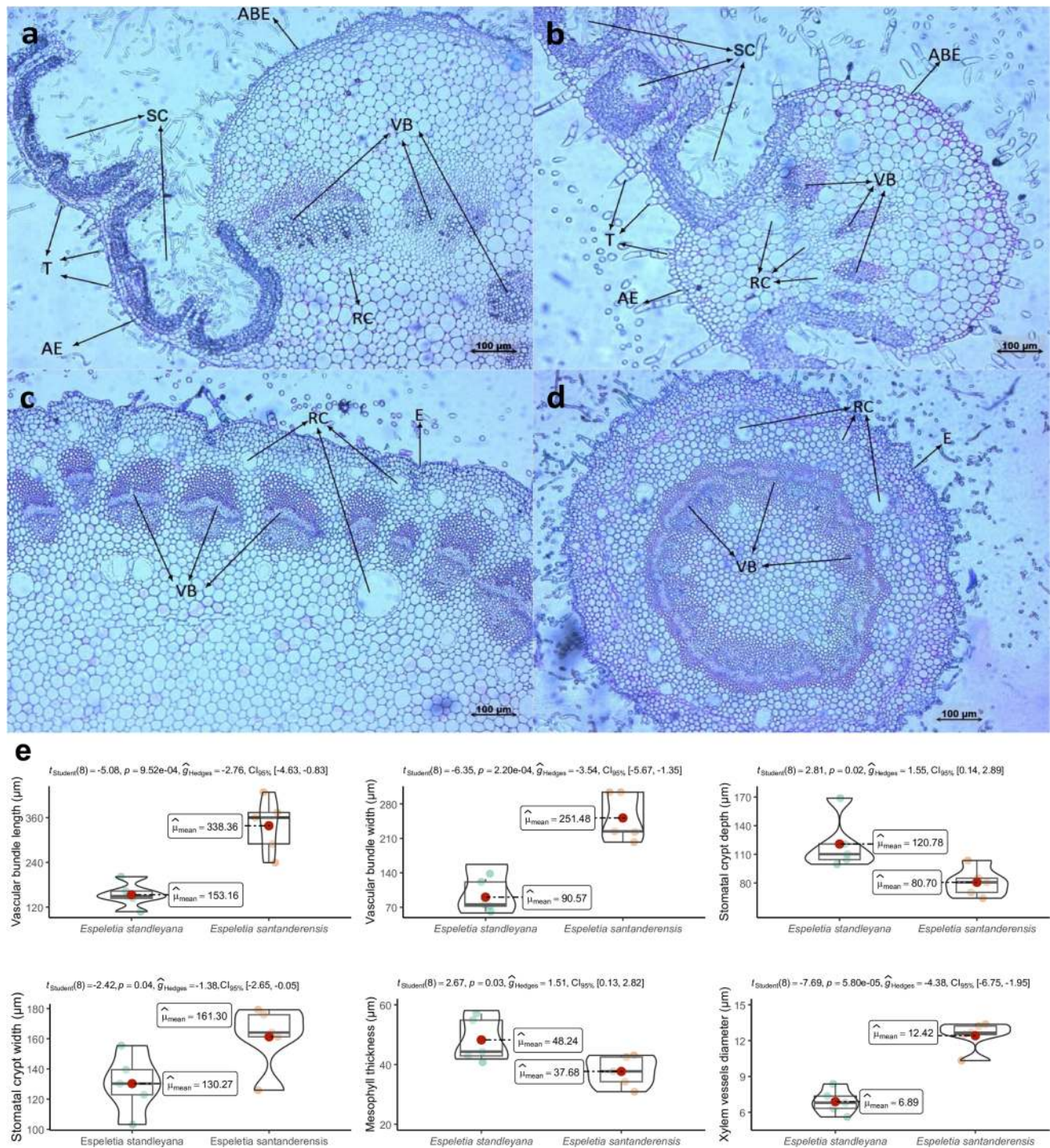


Fig. 6 Anatomical photomicrographs a. leaf *E. standleyana* b. leaf *E. santanderensis* c. peduncle *E. standleyana* d. Peduncle *E. santanderensis* e. comparison of anatomical variables (n=5 for each species).

Microenvironmental characteristics of the two species sites

Paramos are very heterogeneous in both biotic and abiotic characteristics, creating very marked micro and macroscale

environmental gradients, and causing mosaics of species over short distances (Azócar and Rada 1993; Abadín et al. 2002; Llambí and Rada 2019). For example, Cárdenas et al. (2018) found diurnal temperature amplitudes between 10 and 30 °C in the Romerales and Belmira paramos in the

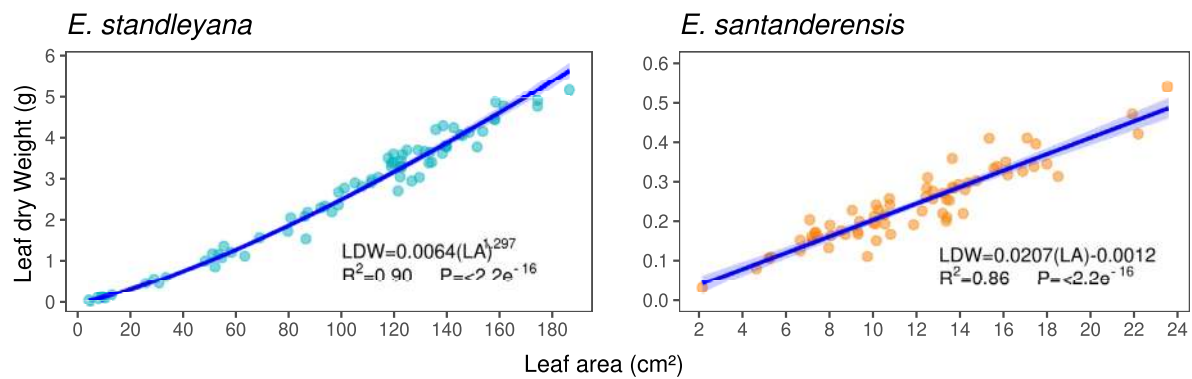


Fig. 7 Dispersion and modeling of dry weight trends as a function of leaf area in *E. standleyana* and *E. santanderensis*

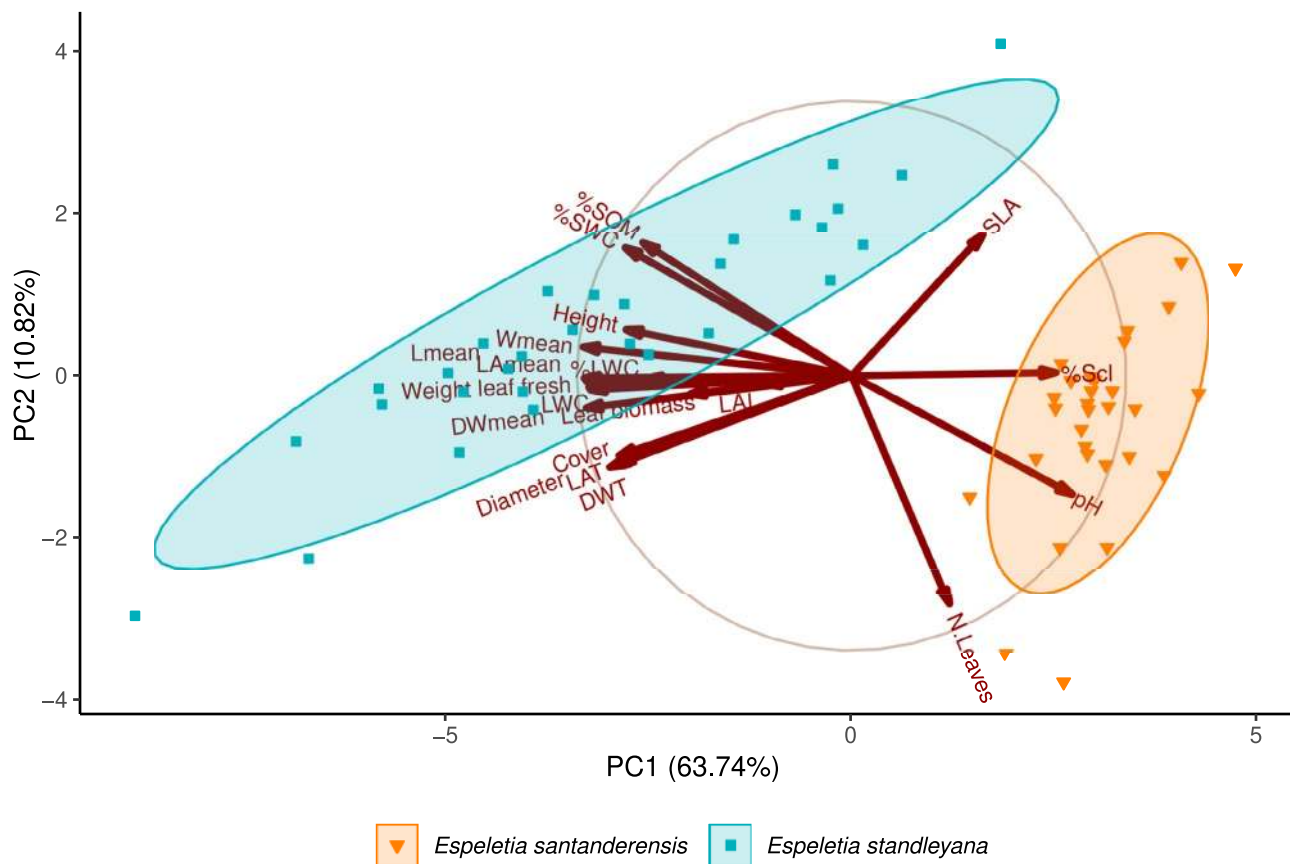


Fig. 8 Principal component analysis of the microsites edaphic and morphofunctional variables of *E. standleyana* and *E. santanderensis*

central mountain range of the Colombian Andes. In the García rural area, air temperature ranges from 1 to 32 °C for *E. standleyana* and between 1 and 22 °C for *E. santanderensis*. The relative humidity also varied within the microsites, broader for *E. standleyana* compared to *E. santanderensis*, which demonstrates that these species are subject to different environmental conditions. It is important to note that these microclimatic characteristics were measured in an extremely

short wet season period, and fluctuation ranges are, most likely, greater during the drier season.

Cárdenas et al. (2018) found water saturation in deeper layers of the soil while in the most superficial ones the volumetric water contents were high in the dry season. In our work, the gravimetric humidity values remained above 50% and organic matter was high for the *E. standleyana* microsite at a depth of 10 cm. However, for *E. santanderensis*, lower

values were found for both gravimetric soil moisture and organic matter. Llambí et al. (2012) report organic matter values ranging from 3 to 44% of volume in paramo ecosystems and highlight its strong relationship with soil humidity. As organic matter increases, the substrate becomes more porous, allowing for greater water retention. As reported by Zhao et al. (2023) for an alpine grassland on the Tibetan Plateau, soil organic matter alleviates drought stress by positively affecting the water holding capacity through the improvement of soil structure and cation exchange capacity. The substrate of *E. santanderensis* is more inorganic and rockier, allowing greater water infiltration and potential loss from the topsoil. The soil pH of both species is acidic, though *E. santanderensis* exhibits a higher pH. According to Moreno et al. (1994) and Estupiñán et al. (2009), paramo soils tend to be acidic due to the high presence of aluminum compounds, exchangeable aluminum and organic matter. At the *E. standleyana* site, the pH is lower, likely due to the high organic matter content, which includes significant amounts of humic acids. Denney et al. (2020) suggest that the heterogeneity can be observed at distances below 2 m, influenced by factors such as topography, nutrient levels and available water, which create contrasting microhabitats over short spatial scales. This variation leads to differences in morpho-physiological traits and plant responses, i.e., water stress can reduce stomatal conductance and limit photosynthesis.

Leaf and peduncle anatomy

The sunken stomata in *E. standleyana* may help mitigate the negative impact of microclimatic variables (Roth 1973; Roth-Nebelsick et al. 2009), particularly fluctuations in temperature and relative humidity, which influence water loss through transpiration (López Rhenals 2021; FAO 2022). These two variables showed greater fluctuations in the microsite where *E. standleyana* was present. Stomatal crypts may represent a scleromorphic feature linked to mesophyll thickness, enhancing gaseous diffusion efficiency (Hill 1998; Jordan et al. 2008). The findings of this study align with previous reports, as *E. standleyana*, characterized by a thicker mesophyll, exhibited more sunken stomata. The greater mesophyll thickness in *E. standleyana* suggests increased mesophyll conductance, which may contribute to higher photosynthetic capacity compared to *E. santanderensis*, a pattern also observed in *Rhododendron* (Cai et al. 2014).

According to Alemán-Sancheschultz (2019), the number of conductive elements per unit area influences hydraulic conductivity efficiency. As a taller species, *E. standleyana* requires conducting large amounts of water to support its larger structures. Consequently, its greater number of vascular bundles and the greater diameter of the xylem vessels facilitate efficient water distribution to all organs. In

contrast, *E. santanderensis*, which is shorter in height and inhabits drier soils, exhibits narrower xylem vessel that may enhance water conduction safety by reducing the risk of embolisms. Similar adaptations have been observed in woody grasses of the genus *Chusquea* (Ely et al. 2019) and the perennial herb *Senecio formosus* (Araujo et al. 2023), both characteristic of the paramo. The efficiency of water transport in *E. santanderensis* is likely driven by its ability to ensure safer conduction under conditions of low soil moisture. Meanwhile, *E. standleyana* depends on rapid water movement through its vascular system, enabled by a higher soil water content.

During the dry season and under freezing temperatures, taller plants are more vulnerable to cavitation related damage (Olson et al. 2018). In contrast, smaller xylem vessel diameters prioritize safety over efficiency in water conduction. The larger size of *E. standleyana* may be linked to more favorable soil water conditions at the study site. Additionally, during periods of limited water availability during the dry season, its substantial water reservoir in the stem pith could help it withstand drought stress, as observed in other *Espeletia* species in the Venezuelan paramos (Goldstein et al. 1984). Meanwhile, *E. santanderensis*, an acaulescent and smaller sized rosette, benefits from shorter water transport distances, enhancing conduction safety through its narrow xylem vessels.

The greater number of leaves and the smaller leaf area in *E. santanderensis* may serve as an adaptation for thermal energy dissipation, water loss regulation and the thermal insulation of the juvenile leaf bank, thereby protecting the apical meristem (Murcia and Mora-Osejo 2003). According to Leigh et al. (2017), smaller leaves exhibit a higher rate of heat convection per unit area, facilitating efficient heat dissipation between the leaf and the surrounding air. This adaptation allows them to maintain a relatively large total leaf area within a compact space, maximizing the capture of photosynthetically active radiation. In contrast, *E. standleyana* has fewer leaves with a greater leaf area. This species regulates leaf temperature by increasing boundary layer thickness through enhanced pubescence on the leaf surface, which improves light reflectance, an adaptation reported in other caulescent rosettes (Baruch and Smith 1979; Meinzer and Goldstein 1985).

Morphophysiological and leaf biomass traits

E. standleyana thrives in a moist soil and possesses leaves with high water content, reaching up to 70%, which suggests a lower %Scl. In contrast, *E. santanderensis* has higher %Scl, lower gravimetric soil humidity and lower leaf water percentages. This could indicate a conservative strategy for resource retention in plants, potentially due to lower resource availability of organic matter in the substrate.

Berdugo Lattke (2020) describe strong positive relationships between %Scl and relative air humidity, and negative relationships with succulence and water content.

Specific leaf area is a key functional trait for understanding plant responses, as it influences growth strategies, photosynthetic efficiency and water use dynamics. Differences in SLA may contribute to niche partitioning among species, with lower SLA values often associated with adaptations for slow growth rates in nutrient poor soils, particularly those deficient in nitrogen (Poorter et al. 2009; He et al. 2018). In a study of 207 woody species, He et al. (2018) identified soil nitrogen and organic carbon as the primary factors influencing variations in SLA. We may expect lower SLA under conditions of lower nitrogen and organic carbon in the soil. Other species of Espeletiinae such as *Ruilopezia atropurpurea* and *Espeletia schultzii*, with glabrous and pubescent leaves respectively, showed low SLA in a Venezuelan paramo (Rada and Navarro 2022). In Colombian paramos, Cabrera and Duivenvoorden (2020) indicate that plant communities are dominated by species with low SLA values, supporting the idea that plants in these environments adopt resource-conserving strategies to cope with limited nutrient availability (Wright et al. 2004). In our study, both species exhibited low SLA values; however, the lower SLA observed in *E. standleyana* does not align with previous findings that associate lower SLA with poorer soils. This discrepancy could be explained by the species' higher pubescence and/or greater mesophyll thickness, which contribute to increased leaf dry mass (Molina-Montenegro 2008; Villar et al. 2013; Pompelli et al. 2019; Rada et al. 2021).

The low LAI for our two studied species agrees with what was reported for *E. schultzii* and *R. atropurpurea* (Baruch and Smith 1979). These authors positively related LAI to the availability of water in their microenvironments. According to Bucci et al. (2008), LAI decreases when water availability becomes limited. The soil water availability was greater at *E. standleyana*'s site. That species also presented the highest LAI, which is consistent with these studies. Despite the marked differences in terms of sizes, some individuals of *E. santanderensis* can reach leaf biomasses as high as that of some individuals of *E. standleyana*. This could be explained by a compensation for these individuals due to the greater number of leaves in the acaulescent rosette.

The PCA showed that species mostly segregated due to the differential expression of morphometry, the percentage of sclerophylly, and the number of leaves, which were related to edaphic variables. The distinct microenvironmental characteristic influence the responses of the two species. The traits of *E. standleyana* appear to have evolved primarily to address water regulation challenges at the leaf-air interface. These adaptations include increased pubescence, deeper stomatal crypts, a rosette with a large stem pith, a higher number of vascular bundles and a smaller diameter.

Together, these features enhance its ability to withstand more extreme and fluctuating conditions, such as increased evaporative demand driven by higher air temperature and lower relative humidity. While *E. santanderensis* experiences a more stable leaf-air interface, its soil conditions are more constrained compared to the microhabitat of *E. standleyana*. Consequently, the species may adapt by developing smaller leaves, fewer but larger diameter vascular bundles, and a compact size that facilitates efficient water distribution to all organs. Additionally, its tougher leaves reinforce the idea that sclerophylly tends to occur in environments with nutrient limitations.

Conclusions

E. standleyana and *E. santanderensis* populations in Paramo de García exhibit highly contrasting adaptive responses, driven by the microclimatic and edaphic conditions of their ecological niches. The microenvironment of *E. standleyana* is subject to greater fluctuations in aboveground temperature and relative humidity, shaping its morphology and anatomy to efficiently regulate gas exchange and water relations. In contrast, *E. santanderensis* faces challenges associated with lower water and organic matter availability in the soil. To adapt, it exhibits increased sclerophylly and reduced size allowing it to cope with rockier, drier, and nutrient poor conditions. The spatial segregation of these species appears to be driven by the microscale heterogeneity of the Paramo de García, resulting in highly contrasting anatomical, morphological and biomass traits that help each species withstand the specific types of environmental stress present in their respective microsites. This adds support for adaptive processes acting at a microclimatic scale during the diversification of *Espeletia*, as suggested and shown for the Venezuelan clades at a macroevolutionary scale (Pouchon et al. 2021). Future work needs to be focused on other *E. standleyana* and *E. santanderensis* populations, and other species of the *Espeletia* complex, to further support our findings on the relation between adaptive traits and environmental conditions. Finally, as suggested by Cortés et al. (2018), understanding the adaptations of the giant rosettes in the paramos to local heterogeneous environments will help establish how climate change may affect vegetation in this highly diverse and fast-evolving ecosystem.

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Data Availability All raw data supporting our findings and conclusions of this article will be available by the corresponding author, without undue reservation.

Declarations

Conflict of interest The authors declare no competing interests.

Informed Consent All authors read and approved the final manuscript. We hereby provide consent for the publication of the above manuscript.

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