

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

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Abstract

To characterize the spatial segregation of two *Espeletia standleyana* and *E. santanderensis* populations, in a paramo in Northeastern Colombia (3350 m), the adaptive responses in each of their corresponding microclimates were studied. Their anatomical, morphofunctional and leaf biomass characteristics were determined and compared. The 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, diameter of the vascular bundles, width of stomatal crypts, number of leaves, specific leaf area and percentage of sclerophylly were higher in *E. santanderensis* ($P < 0.05$). Leaf biomass was estimated from the leaf area index for the two species, suggesting the latter as an efficient measure of productivity. 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.

INTRODUCTION

The paramos, alpine ecosystems of the humid tropical Andes, present unique conditions associated with low latitude cold climates. They possess the highest floristic diversity and the largest number of endemic species of alpine ecosystems worldwide (Luteyn et al. 1999; Sklenář et al. 2014). These ecosystems are considered the world's fastest evolving biodiversity hotspots and speciation centers (Myers et al. 2000; Madriñan et al. 2013). Additionally, the high spatial heterogeneity at regional and local scales is one of the main environmental drivers and determinant factors of the vegetation composition of the paramos. Different soil and climate conditions at short distances generate complex environmental gradients that result in intricate mosaics of vegetation types (Llambí & Rada, 2019).

The differentiation in morphology is an expression of a process by which species occupy certain niches, therefore, their characteristics are modified by selection forces exerted by environmental factors during their evolutionary history (Pouchon et al. al. 2018). The 'Espeletia complex', giant rosettes belonging to the subtribe Espeletiinae, belong to one of the dominant growth forms of the neotropical high mountains, and more specifically, the Colombian paramos. The morphological evolution of this 'complex', considered one of the most rapidly evolving plant groups (Cortés et al. 2018) mediated by the process of adaptive radiation of the Espeletiinae in the eastern branch of the Andean Cordillera in Colombia, allowed the diversification and emergence of many species within the genus (Sánchez Andrade 2005).

Andean giant rosettes are of great importance due to their characteristic structures that allow them to accumulate water: the presence of a stem pith that serves as a water reservoir (Azócar & Rada 1993; Rada 2016; Rada et al. 2019), leaves that maintain 70–80% of the fresh weight stored in water (Reyes et

al. 2019), and stomatal crypts that buffer microclimatic oscillations in the leaf-air interface, allowing a decrease in transpiration (Roth 1973; Roth-Nebelsik et al. 2009), among others. These characteristics allow them to regulate water losses to the atmosphere and, thus, maintain the ecosystem and the soil under favorable conditions (Cárdenas et al. 2018).

Among the many *Espeletia* species described for the Colombian paramos, *Espeletia standleyana* A.C.Sm. and *E. santanderensis* A.C.Sm. are dominant in the paramos of the Northeastern Colombia. Populations of these two species converge in the locality in the Paramo de García and are spatially segregated; there are places where some individuals of the two species are adjacent to each other, but they do not usually overlap. In this research we address the following questions: Do the two rosette species exhibit differences in their anatomical, morphometric, and leaf biomass traits? If there are differences in these traits, can the environmental heterogeneity of the paramo (microclimatic and edaphic characteristics) explain such segregation?

MATERIALS AND METHODS

This research was carried out in the Paramo de García in Pamplona (Fig. 1a), North of Santander-Colombia (3350 m a.s.l. N 7°19'21" W 72°39'30"), in the ecotone between the high Andean forest and the paramo. This study site is characterized by a heterogeneous vegetation, with small trees, shrubs and grasses and populations of the two studied species segregated in space (Fig. 1b). This paramo has an area of 1.12 km².

Study species

Espeletia standleyana

A caulescent rosette with a stem 1–2 m in height and pubescent leaves. Associated with a graminoid matrix in the Paramo de García, and a soil with an apparently high organic matter content. The population under study is located adjacent to the high Andean forest in an intermix with species belonging to the ecotone forest-paramo, at 3350 m a.s.l. This site corresponds to the lower elevation in which the first individuals of the species are found (Fig. 1b).

Espeletia santanderensis

This rosette has a shorter stem, with synflorescences forming conglomerates of chapters at the apex and marcescent leaves extending over the soil surface. This species is smaller in size and displays less dense leaf pubescence compared to *E. standleyana* (Fig. 1b). In the Paramo de García, the substrate in which individuals of this species are found is generally rocky with soil depths no greater than 10 cm. In addition, 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 closely related to grasses and shrubs (Díaz Piedrahita & Rodríguez Cabeza 2010).

Edaphic and microclimatic variables

Soil samples (10 cm deep) for both populations were taken, their fresh weight determined and dried at 60°C for 72 h. These samples were then placed in a muffle at 550°C to calculate the following variables (Heiri et al. 2001):

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

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

The soil pH was measured with a pHmeter (PH 7110, MTW inolab®) in samples with a 1:1 sample to distilled water proportion.

To compare the microclimate of the sites where the two species are found, meteorological microstations (HOBO onset, U12-012 model) with relative humidity (RH) and air temperature (T) sensors were installed. Measurements were carried out every 15 minutes from November 6 to 14, 2021 at each sampling site.

Data and sample collection

A 10x10 plot was delimited for each species and 30 individuals 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. The leaf area of a leaf per individual was measured by 3 methods: a non-destructive method through the formula $2/3 * (\text{maximum leaf length} * \text{maximum leaf width})$ (Montgomery 1911) and the destructive methods of photocopy (simple rule of 3 between the weight of the silhouette of the photocopy of the leaves and the weight of one cm^2), and ImageJ (Scanning of the leaves using a standardized scale). There were no statistically significant differences between the leaf area methods (Anova > 0.05) tested, which allowed us to use the simplest and non-destructive method, $2/3 (L * A)$, to calculate the leaf area of the rosette. To model leaf dry weight, the average of the three methods was used.

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:

$$\text{Leaf water content (LWC)} = \text{leaf wet weight (g)} - \text{leaf dry weight (g)}$$

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

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

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

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

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

Leaf and peduncle anatomy

Five samples of leaves and floral peduncles were collected and brought to the Animal and Plant Histology laboratory of the University of Pamplona. These samples were treated using standardized methods (Johansen 1940; Roth 1964) for all anatomical determinations.

Sections of approximately 1 cm² were taken from the middle region of the leaves and approximately 1 cm in length from the floral peduncle, then fixed with 20 ml of a FAA solution (formol 40% 90cc, acetic acid 5cc and alcohol 95% 5cc) for 48 h. Subsequently, these samples were introduced into a tissue processor (Microm HPM110), and subjected to different alcohol concentrations to dehydrate, with xylol to clarify the sample. Finally, they were impregnated and embedded with molten paraffin and 10 µm cross-sections were obtained with the help of a microtome (H315 MICROM). The samples were then placed on a slide with a solution of albumin and glycerol to facilitate adhesion and were subjected to different concentrations of xylol and alcohols to dewax and rehydrate the samples and proceeded to staining with Ziehl fuchsin and brilliant green. Finally, after the samples were air dried, they were sealed with Entellan resin.

Images were captured with a camera Axiocam ERc5s and the following anatomical variables were measured with the software package for connected microscopy ZEN core from the ZEISS company: 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 determine the precision of the leaf area measurement methods, ANOVA hypothesis contrast tests were used. The variables were compared using Student's T, Student Welch's T and Wilcoxon-Mann-Whitney hypothesis contrast tests, according to the validation of the assumptions of homogeneity of variances and normality. All these tests were represented with boxplot-violin graphs (Patil, 2021), evidencing the distribution of the data of the samples with a mirror density diagram, which corresponds to the density of points under the curve. These tests compared anatomical, morphophysiological and leaf

biomass traits at the univariate level. To discriminate the species in the multifactorial scope, a principal component analysis (PCA) was used, with the variables rescaled to the Z distribution, with variance 1 and mean 0. Correlation analyzes were done to find relationships between morphophysiological variables and leaf biomass. Leaf biomass was estimated based on the predictors that best explained its variance. The simple and multiple regression models were calculated using the linear least squares method, choosing the models that best explain the response variable and that meet the assumptions of linearity, homoscedasticity, normality, autocorrelation of residuals and influential values (Cook's distances). We verified that the variance inflation factor (VIF) and the condition index did not exceed the allowed threshold for the multiple models. The Akaike Information Criterion (AIC) and the Step-by-Step method were used to choose the ideal models that best explained the response variables. All analyzes and graphs were done with the statistical analysis programming software R (R Core team 2023) and its integrated development environment Rstudio, with the help of the packages tidyverse, car, lmtest, factominer, ggstatsplot, factoextra, among others.

RESULTS

Comparison of edaphic and microenvironmental variables between the two populations

The %SWC and %SOM were between 1.6 and 1.7 times significantly higher in *E. standleyana* while the soil pH was 1.15 higher for *E. santanderensis* (Fig. 2).

Clearly, 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% for both species, while the temperature remained between 1–10°C for both species, evidencing relative nocturnal meteorological homogeneity.

Comparison between anatomical and morphophysiological variables and indices of the two species

E. standleyana, on average, was proportionally greater in the following variables: Rosette height 5.6:1, rosette diameter 1.7:1, leaf length 2.4:1, leaf width 4:1, leaf area 10:1, fresh weight 14:1, dry weight 11:1, rosette cover 2.8:1, rosette leaf area 4.6:1, rosette leaf dry weight 5.7:1. However, *E. santanderensis* showed a higher proportion in the number of leaves 1:2.1 (Fig. 4). %Scl (1.26:1) and SLA (1.1:1) were higher in *E. santanderensis*. However, LWC (16.4:1), %LWC (1.16:1), LAI (1.3:1) and leaf biomass (1.72:1) were higher in *E. standleyana* (Fig. 5).

Leaf and peduncle anatomy

The length and width 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 in *E. standleyana* and wider in *E. santanderensis*, and the thickness of the mesophyll was greater in *E. standleyana*. Furthermore, the diameters of the xylem vessels, both of the peduncle (12.47 ± 0.16 vs 6.22 ± 0.13 μm) and of the midrib of the leaf (12.43 ± 0.42 vs 7.55 ± 0.21 μm) 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 Pvalue < 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 with other models in the annexes). 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).

The leaf biomass of *E. standleyana* was modeled with the leaf area index and the average leaf dry weight of the leaves (Fig. 8). However, with a simple model with only the leaf area it was possible to explain 92% of the biomass variability, but the intersection with the Y axis was not significant and the validations were rejected, so it was decided to model with the leaf dry weight. The trend of the leaf area index agreed with the variation of the values observed and estimated by the model.

The LAI for *E. santanderensis* was also the best predictor of leaf biomass. This alone explained 99.8% of the variability of the data, in contrast to *E. standleyana*, it did not require other predictors. However, the adjustment indicating a value close to the probability of 1 was affected by the elimination of an influential value calculated by Cook's distance statistic (Individual number 26), so the elimination of the individual affected the percentage of explanation. However, the estimate was obtained based on the leaf indexes of the 30 individuals.

Principal component analysis

The principal components analysis segregated the populations of the two species at a multifactorial level, both in location and variability, explaining 78% of the total inertia (Fig. 9). Unfavorable soil variables were correlated with highest values of %Scl, SLA and N.Leaves were the most representative for *E. santanderensis*. However, the rest of the morphometric and edaphics variables were pulled towards the *E. standleyana* group, thus demonstrating the difference in sizes in all the variables that measure shape. Principal component number 1 was correlated with morphometry and %Scl; while principal component number 2 showed a strong correlation between leaf biomass and leaf area index, variables previously related in previous models.

DISCUSSION

The anatomical, morphometric and leaf biomass differences between these two species allow us to infer contrasting adaptations due to exposure to the particular conditions of their ecological niches. On the other hand, the results also show that there is a differentiation between the microenvironments where each of these species are found, which could explain the spatial segregation between *E. standleyana* and *E. santanderensis*.

Microenvironmental characteristics of the two species sites

The paramos are very heterogeneous in both biotic and abiotic characteristics, creating very marked micro and macroscale gradients, causing mosaics of species with differential characteristics over short distances (Azócar & Rada, 1993; Abadín et al. 2002; Llambí & Rada 2019). Cárdenas et al. (2018) found diurnal temperature amplitudes between 10 and 30°C in the Romerales and Belmira paramos in the central mountain range of the Colombian Andes. In the García rural area, air temperature oscillations remained in the range of 1–32°C for *E. standleyana* and between 1–22°C for *E. santanderensis*. The RH also had different ranges for the microsites of the two species, 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 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) state that paramo soils can have from 3 to 44% of the volume in organic matter and this has a strong relationship with soil humidity. As organic matter increases, the substrate becomes more porous, allowing a large amount of water to be stored. As reported by Zhao et al. (2023) for an alpine grassland, soil organic matter alleviates drought stress by positively affecting the water holding capacity through the improvement of soil structure and cation exchange capacity. Because the substrate of *E. santanderensis* is more inorganic and rockier, more water may infiltrate and be lost from the topsoil. The soil pH of both species is acidic, although higher for *E. santanderensis*. According to Moreno et al. (1994) and Estupiñán et al. (2009), the pH of paramo soils is usually acidic due to the high amount of aluminum compounds, exchangeable aluminum and organic matter. For the *E. standleyana* site the pH is lower due to a high content of organic matter with high amounts of humic acids. According to Denney et al. (2020), the heterogeneity of a site can be observed at distances below 2 m due to topography, nutrient levels and available water, creating contrasting microhabitats in very short distances. This in turn results in differential morpho-physiological traits/responses in plants, i.e., water stress can reduce stomatal conductance and limit photosynthesis.

Leaf and peduncle anatomy

The sunken stomatal crypts in *E. standleyana* correspond to a more suitable anatomical character that allows it to buffer the adverse effect of microclimatic variables (Roth 1973; Roth-Nebelsick et al. 2009),

especially temperature and relative humidity, variables that determine water loss through transpiration (López Rhenals 2021; FAO 2022). These two variables showed greater fluctuations in this species' microsite, which could indicate that it is a response to the greater variation and more extreme conditions. Other authors mention that it may be a scleromorphic character, given by an increase in the thickness of the mesophyll, so that the stomatal crypts increase in depth, with the aim of increasing the efficiency of gaseous diffusion, as has been described in species of the genus *Banksia* (Hill 1998; Jordan et al. 2008; Hassiotou et al. 2009). This study agrees with these reports, since *E. standleyana*, having greater thickness of the mesophyll, was the species with much more sunken stomata.

The greater thickness of the mesophyll for *E. standleyana* would indicate greater photosynthetic tissue, mesophyll conductance and therefore greater photosynthetic capacity, as has been reported in species of the genus *Rhododendron* (Cai et al. 2014).

According to Alemán Sancheschultz (2019), the number of conductive elements per unit area is related to the efficiency of hydraulic conductivity. *E. standleyana*, being a taller species, requires conducting large amounts of water towards larger structures. Therefore, the greater number of vascular bundles and the greater diameter of the xylem vessels favors water distribution to all organs. However, *E. santanderensis*, having a smaller size and being in a drier soil environment, increases the safety of water conduction with a smaller diameter of the xylem vessels to avoid embolisms. This has been recorded in woody grass species in the genus *Chusquea* (Ely et al. 2019) and the perennial herb *Senecio formosus* (Araujo et al. 2023), characteristic species of the paramo. Furthermore, the diameters of the small xylem vessels indicate that both *E. standleyana* and *E. santanderensis* aim for safety in water conduction, avoiding the risk of embolisms (Davis et al. 1999; Pittermann and Sperry 2003). The efficiency of water conduction in *E. santanderensis* would be determined by greater safety in the conduction of water from the soil to all organs due to a low water content of the soil; while *E. standleyana* would rely on a quicker movement of water to all compartments of the plant, due to a higher soil water content.

Morphometry

Taller plants are subject to suffering from embolisms, because they tend to have larger xylem vessels to transport large amounts of water to higher structures. Therefore, in the dry season and under freezing temperatures, these are more vulnerable to injury by cavitation (Olson et al. 2018). While smaller xylem vessel diameters prioritize safety over efficiency in the conduction of water. The larger size of *E. standleyana* could be associated to more favorable water conditions in its microsite. Furthermore, during the dry season, where the availability of water would be affected, its large water reservoir in the stem pith, would allow it to withstand conditions of water stress (Goldstein et al. 1984). On the other hand, *E. santanderensis*, an acaulescent rosette, does not have a pith to store water, but at the same time, water travel shorter distances, increasing the safety of conduction to all organs by their small xylem vessel diameters.

The greater number of leaves and the smaller leaf area in *E. santanderensis* could be an adaptation for the dissipation of thermal energy, regulation of water loss and thermal insulation of the juvenile leaf bank

and, therefore, the apical meristem (Murcia & Mora 2003). According to Leigh et al. (2017), smaller leaves have a higher rate of heat convection per unit area between the leaf and the air, which allows them to maintain a high leaf area in a small coverage to capture photosynthetically absorbable light. However, these characters are opposite in *E. standleyana*, fewer leaves and greater leaf area. *E. standleyana* regulates leaf temperature through an increase in the boundary layer caused by greater pubescence on the leaf surface, which also allows greater light reflectance, as has been reported in other caulescent rosettes (Baruch & Smith 1979; Meinzer & Goldstein 1985).

Morphophysiological and leaf biomass traits

Strong positive relationships were found between %Scl and relative air humidity, and negative relationships with succulence and water content (Berdugo Lattke 2020). Here we find consistency with our results, *E. standleyana* presents a substrate richer in water and leaves with large water percentages of up to 70%, it presents a lower %Scl. In contrast, *E. santanderensis* has higher %Scl, lower gravimetric soil humidity and lower leaf water percentages. Furthermore, a conservative use of resources could be indicated, due to the lower availability of organic matter in the substrate.

Specific leaf area is a key functional trait in the study of functional responses in plants. Differences in SLA have been linked to niche separation between species, e.g., low SLA values are associated with strategies related to slow growth rates and nutrient poor soils, especially nitrogen (Poorter et al. 2009; He et al. 2018). He et al. (2018) find that soil nitrogen and organic carbon are the factors that best explain changes in SLA in a study of 207 woody species, i.e., lower SLA under conditions of lower nitrogen and organic carbon in the soil. Other species of Espeletiinae such as *Ruilopezia atropurpurea* and *Espeletia schultzii*, the first with glabrous leaves and the second very pubescent, showed low SLA, 36 and 67 cm²/g, respectively, in a Venezuelan paramo (Rada & Navarro 2022). Cabrera & Duivenvoorden (2020) indicate that, in the paramos of Colombia, plant communities are dominated by species with low SLA values, reinforcing the idea that in these environments, plants rely on strategies for the conservative use of nutrient resources (Wright et al. 2004). In our case, low values were also reported for the two species, however, the lower SLA in *E. standleyana* does not coincide with what was expected in terms of its relationship with poorer soils as suggested by previous authors. This could be due to the fact that this species shows greater pubescence and/or a greater thickness of the mesophyll, which would increase the dry mass of the leaf (Molina Montenegro 2008; Villar et al. 2013; Pompelli et al. 2019; Rada et al. 2021).

The low LAI for the two species agrees with what was reported for *E. schultzii* and *R. atropurpurea*; furthermore, for these species it was positively related to the availability of water in their microenvironments (Baruch & Smith 1979). According to Bucci et al. (2008), LAI decreases when water availability becomes limited. The highest availability of water found in our study was obtained by *E. standleyana*, likewise, it is the species that presented the highest LAI, which is consistent with these studies. On the other hand, LAI has been proposed as a good predictor of leaf biomass (Parker 2020). In this work, a strong correlation between these two variables was found for both species, supporting Parker's results. Despite the marked differences in terms of sizes, there are certain individuals of *E.*

santanderensis that can reach leaf biomasses as high as that of some individuals of *E. standleyana*, which proposes a compensation for these individuals due to the greater number of leaves in the acaulescent rosette.

The ellipsoids shown in the PCA agree with a segregation of all the measured characters, determining completely contrasting expressions of the traits for the two species. The differential characteristics in the microenvironment correspond to the responses of the two species, the features of *E. standleyana* mainly aim to solve problems of water aspects related to the leaf-air interface, greater pubescence, deeper stomatal crypts, rosette with a large stem pith, a greater number of vascular bundles and a smaller diameter, which allows it to withstand these more fluctuating and extreme conditions. While *E. santanderensis* faces a more favorable environment, but the edaphic conditions are more extreme compared to the microhabitat of *E. standleyana*. For this reason, the species responds to these conditions with smaller leaves, fewer and larger diameter vascular bundles, small size allowing water to reach all organs more quickly, and tougher leaves, which supports the idea that sclerophylly occurs in environments where nutrients are limiting.

CONCLUSIONS

E. standleyana and *E. santanderensis* exhibit highly contrasting adaptive responses, given by the microclimatic and edaphic conditions of their ecological niches. A more extreme microenvironment was evident at the air level for *E. standleyana*, directing its morphology and anatomy to solve problems of gas exchange and water regulation; while *E. santanderensis* tends to solve problems caused by nutrient and water stress, increasing its sclerophylly and decreasing its size, in response to a rockier, drier and nutrient-poor soils. Spatial segregation of the two species is due to factors determined by the microscale heterogeneity of the García paramo, which causes the anatomical, morphological and biomass characters to be highly contrasting to counteract the type of stress to which they are subjected in their respective microsite locations. 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.

Declarations

Competing Interests

The authors declare no competing interests

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Author Contribution

Conceptualization: MM, FR, JR, PO

Methodology: All authors

Field data collection: JR, MM

Laboratory analysis: EM, JR, MM, PO

Data analysis: JR, MM

Writing – original draft: JR, MM, FR

Writing – reviewing and editing: All authors

Data Availability Statement

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

Research Involving Human and/or Animals

Not Applicable

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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Figures

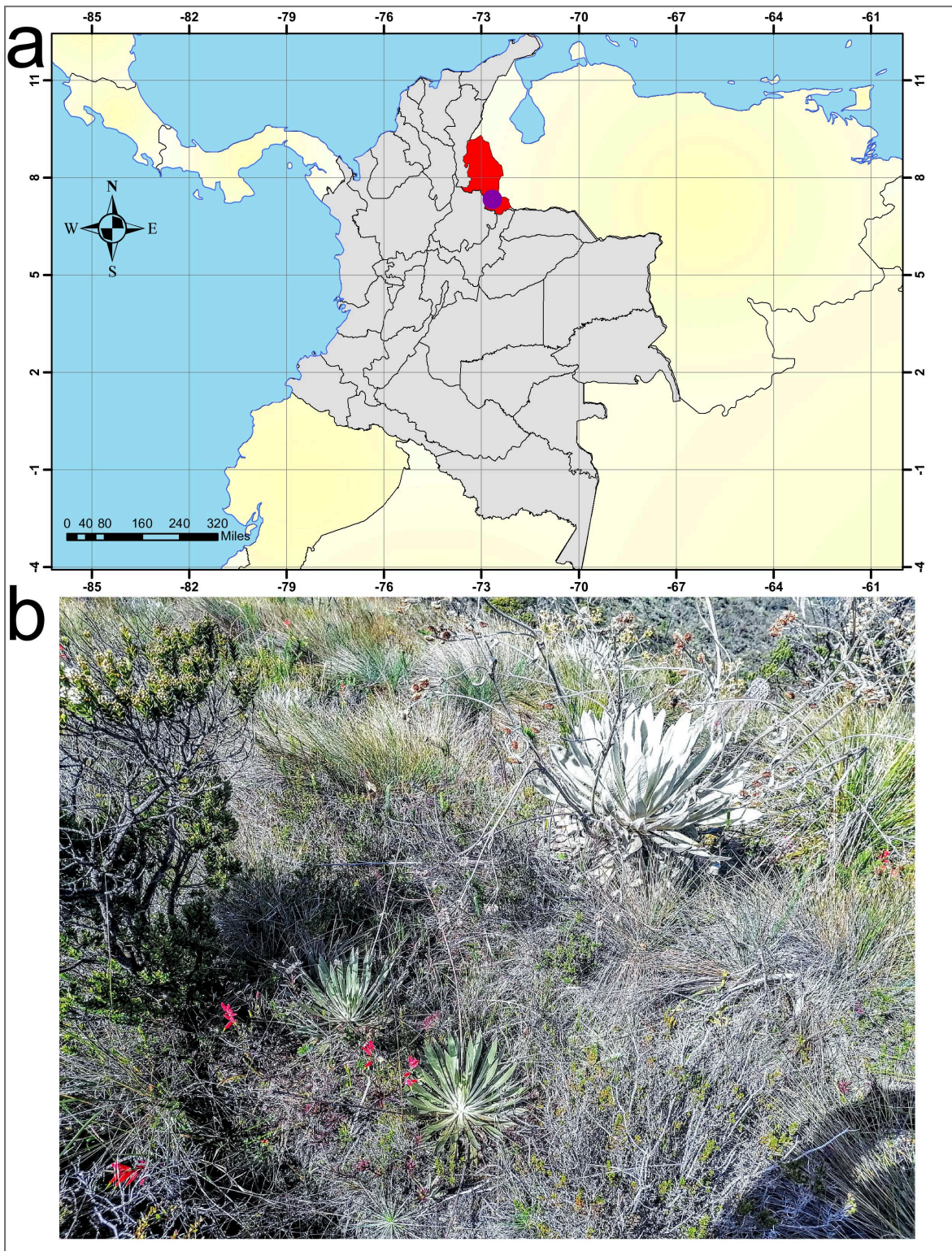


Figure 1

a. Location map of the Paramo de García. The red region corresponds to the department of Norte de Santander and the violet dot to the study area. b. Area of adjacent populations of the giant rosettes *E. standleyana* (upper right) and *E. santanderensis* (lower left)

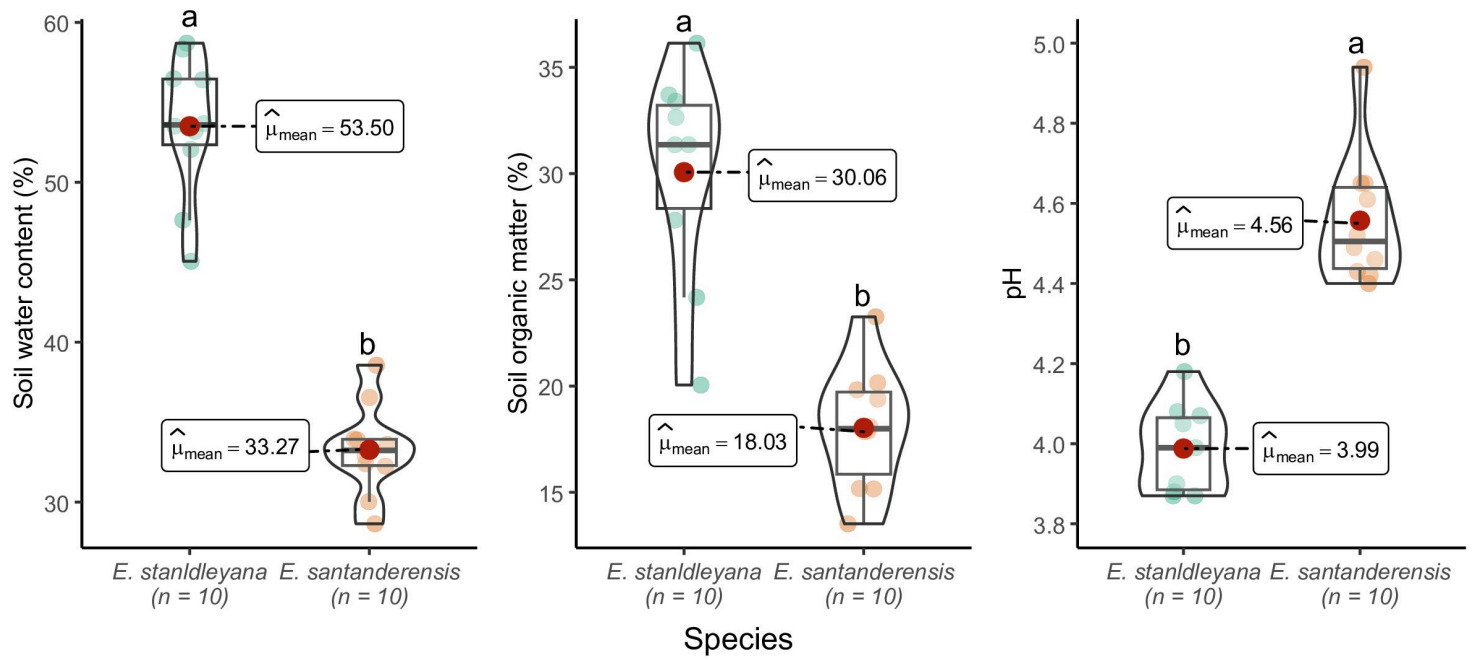


Figure 2

Comparison of edaphic variables in the *E. standleyana* and *E. santanderensis* microsites

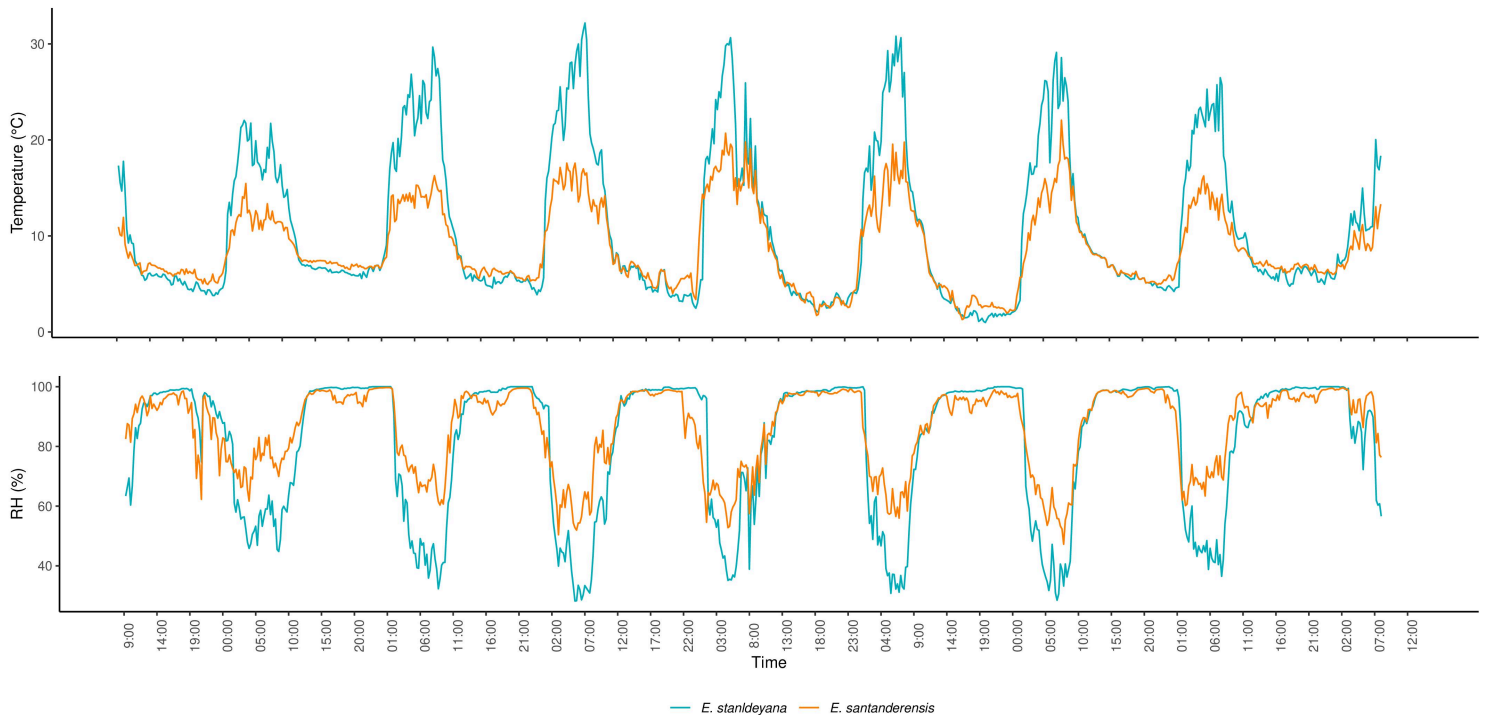


Figure 3

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

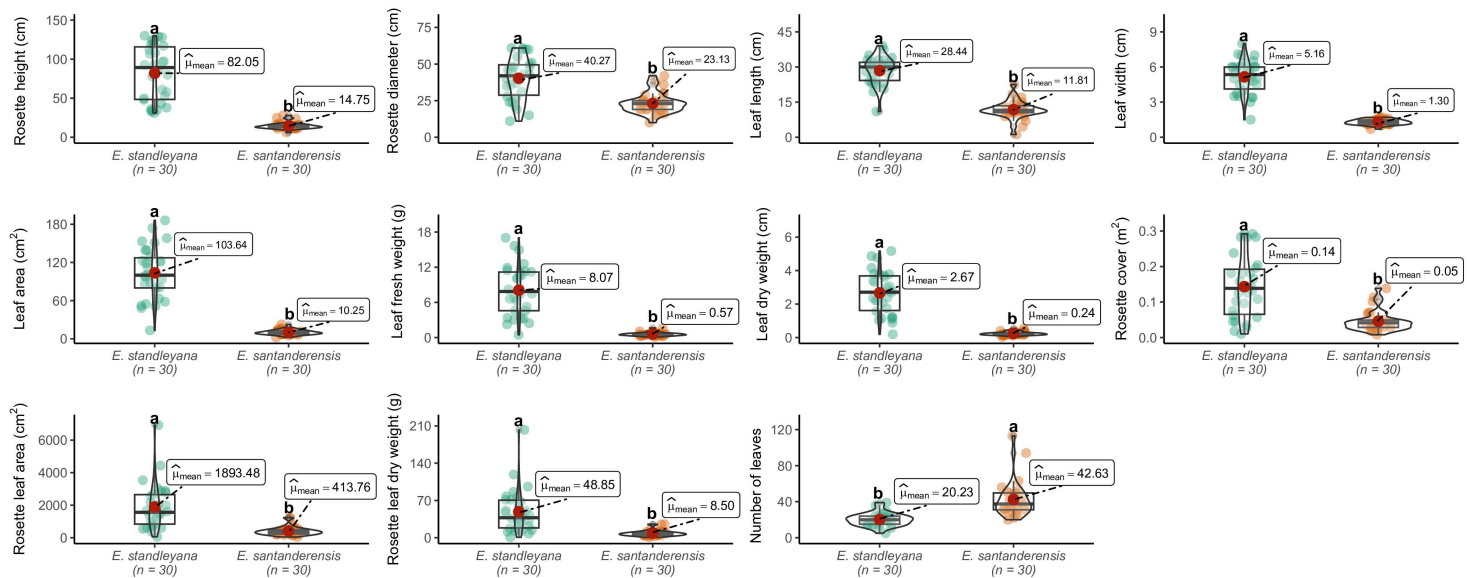


Figure 4

Comparison of the morphometric variables for *E. standleyana* and *E. santanderensis*

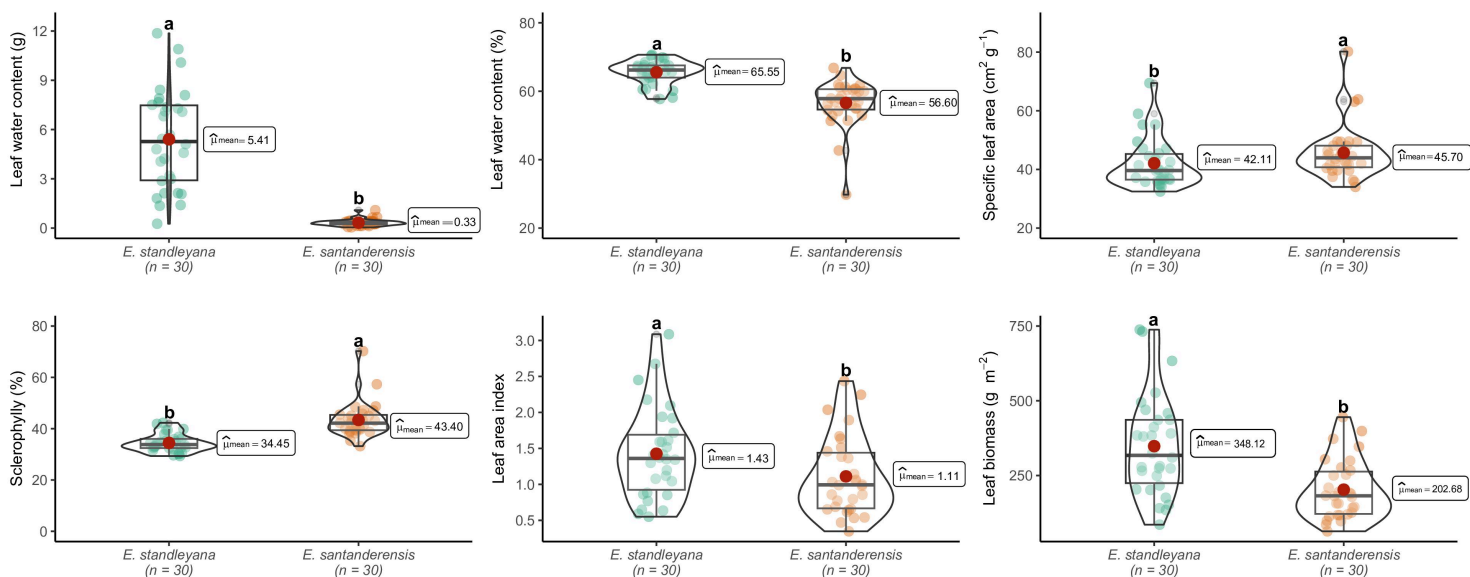


Figure 5

Comparison of the morphophysiological indices for *E. standleyana* and *E. santanderensis*

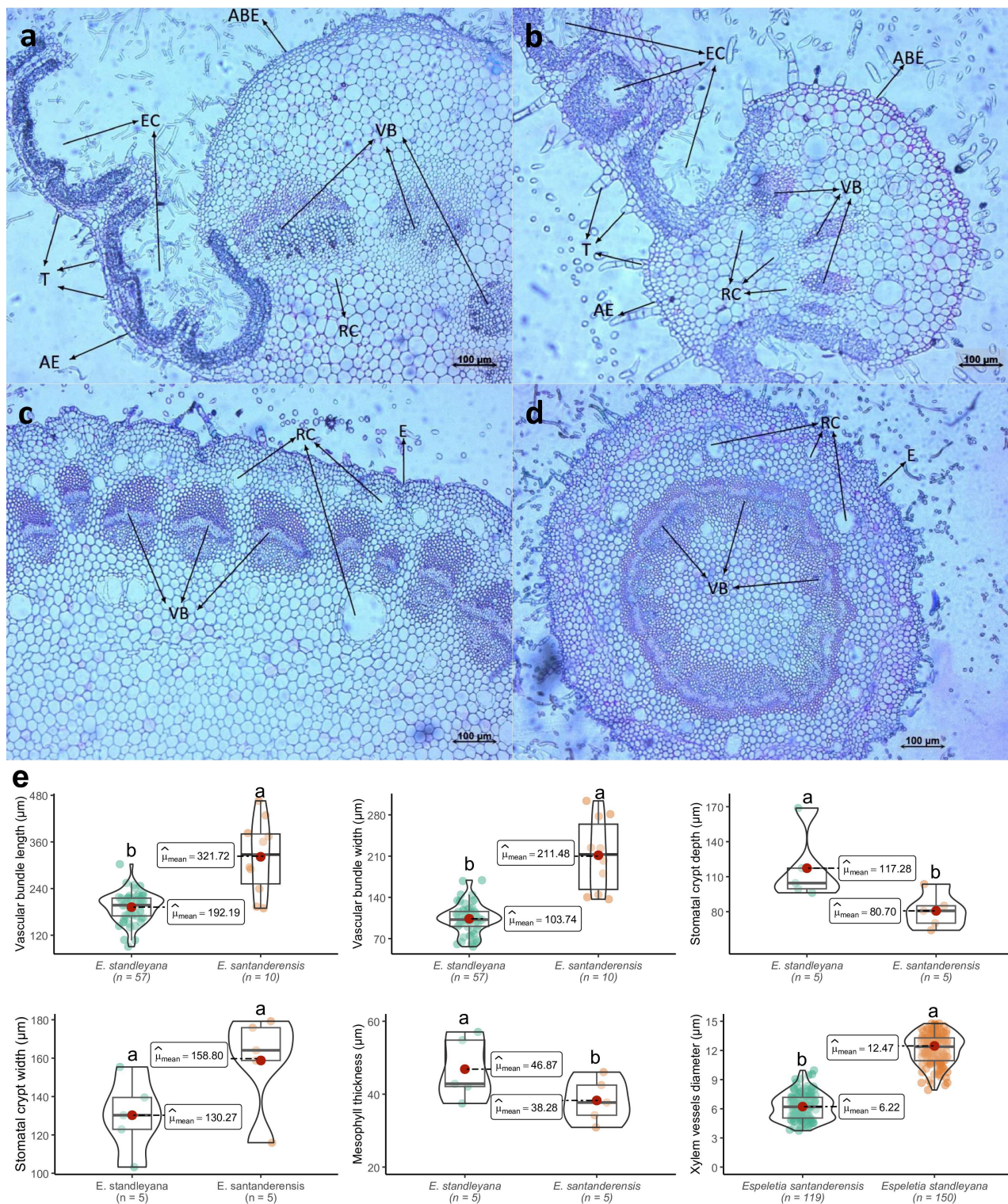


Figure 6

Anatomical photomicrographs a. leaf *E. standleyana* b. leaf *E. santanderensis* c. peduncle *E. standleyana* d. Peduncle *E. santanderensis* e. comparison of anatomical variables

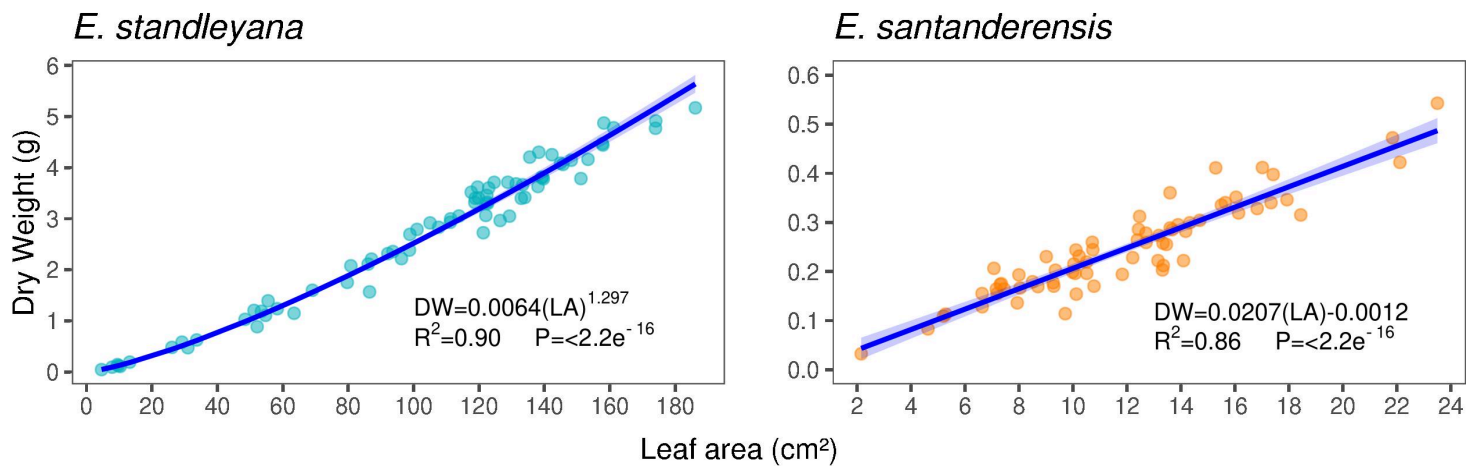


Figure 7

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

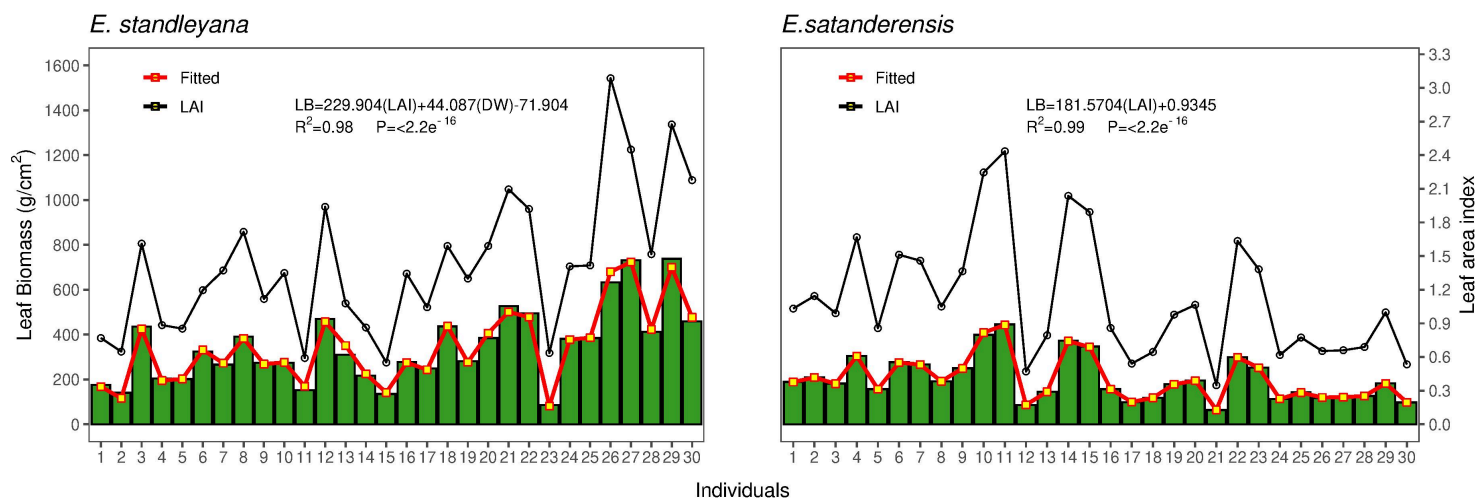


Figure 8

Modeling of the leaf biomass of *E. standleyana* and *E. santanderensis*

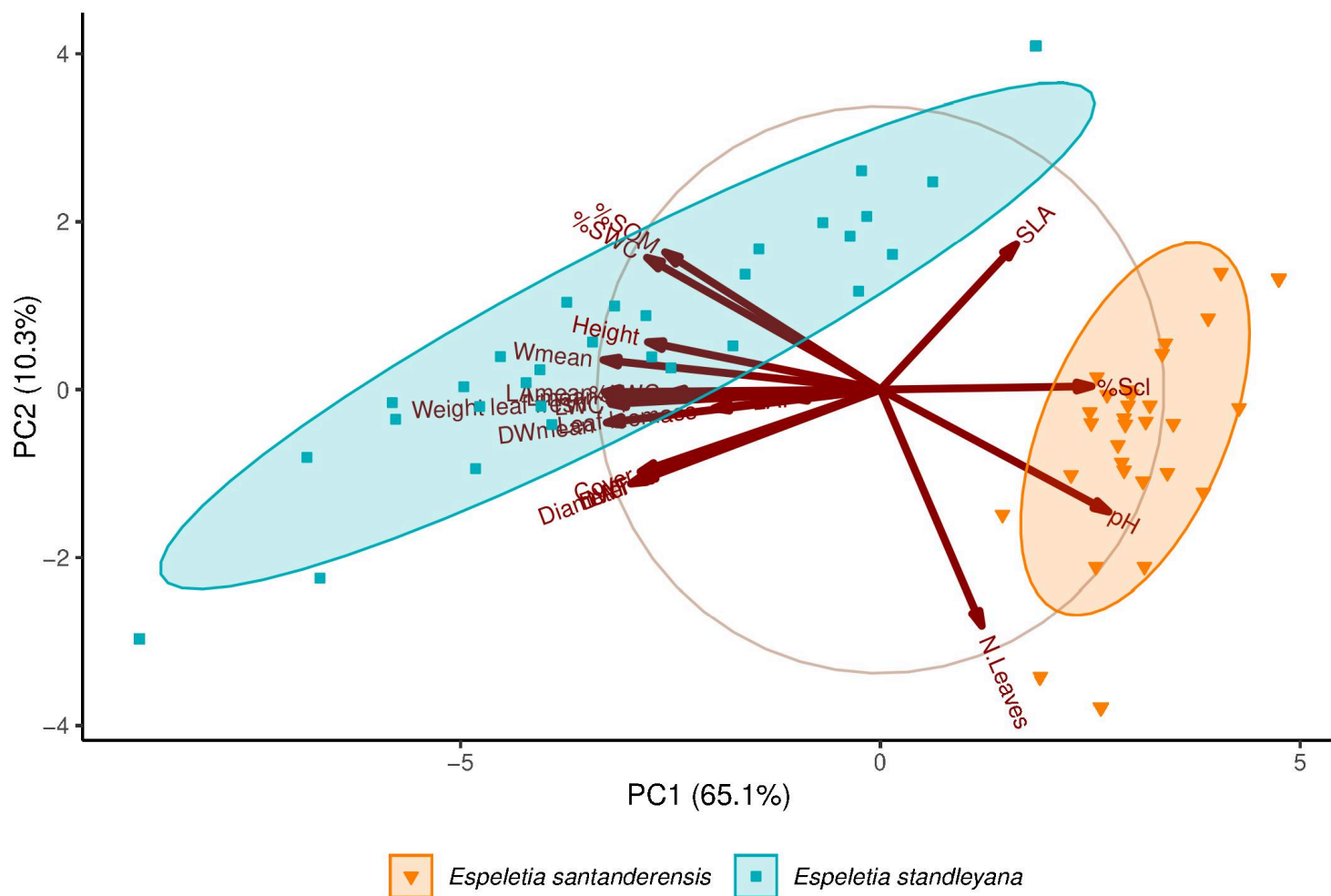


Figure 9

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

Supplementary Files

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