Using functional traits to explore species diversity of *frailejones* (Espeletiinae Cuatrec., Asteraceae)

Paula Valeria Vargas Martínez

Universidad del Rosario
Facultad de Ciencias Naturales
Bogotá, Colombia
2022

Using functional traits to explore species diversity of *frailejones* (Espeletiinae Cuatrec., Asteraceae)

Paula Valeria Vargas Martínez

Artículo presentado como requisito para obtener el título de:

Bióloga

Adriana Sanchez

Facultad de Ciencias Naturales
Biología
Universidad del Rosario
Bogotá, Colombia
2022

Using functional traits to explore species diversity of *frailejones* (Espeletiinae Cuatrec., Asteraceae)

Valeria Vargas[1] and Adriana Sanchez[1]

[1] Programa de Biología, Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, D.C., Colombia

Abstract

The use of phenotypic data to delimit species is one of the least explored approaches in systematics. Normal Mixture Models (NMM) provide a useful framework to test for the existence of distinct species based on multivariate phenotypic information. In consequence, functional traits can be used as input data for these models, since they reflect species adaptations to certain environment. *Frailejones* (Espeletiinae Cuatrec., Asteraceae), which are the most emblematic plant group of the páramo ecosystem, represent an opportunity to apply this approach because they are an example of a recent ongoing adaptive radiation and have high morphological variation. Moreover, as functional traits are inherited characteristics, including them in NMM analyses may provide new insights into group delimitation with phenotypic information. As traits can also be affected by external and/or internal filters, they give additional information on the community assembly process. In this study we aim to determine functional units for *frailejones* based on functional traits and to understand the filter influence on these units. Four functional traits were measured in five individuals of each morphotype found throughout seven páramo complexes in the departments of Cundinamarca and Boyacá in Colombia. NMM were used to test the existence of different functional groups or units, in these seven páramo complexes. Bayesian Information Criterion (BIC) was used to find the number of functional units with the best empirical support. To evaluate the influence of external and internal filters on the functional units, we used T-statistics. An estimated 29 morphospecies were collected in the seven complexes. However, the model with the highest BIC supported nine functional units. The nine functional units occurred in multiple complexes and were sympatric with other units. Guantiva–La Rusia was the complex with the largest number of coexisting functional units (eight). In most complexes internal filters (e.g., competition, facilitation, microhabitat heterogeneity) had a greater influence on the assembly of functional units than the external filters (e.g., climate, dispersal). The nine functional units did not reflect the currently recognized species, although most of these species are also not supported phylogenetically. Internal and external filters can influence the convergence or degree of niche separation between functional units. Therefore, it is important to understand their contribution in the assembly of functional units and their distribution. There is a need for further studies that address species limits in the Espeletiinae as well as to explore this method and its usefulness within the group.

Keywords. Páramo, normal mixture models, leaf traits, species limits, external filters, internal filters

Introduction

There has been a long controversy around the species concept and the methods used for their delimitation (De Queiroz, 2007; Wiens, 2007). Some biologists, especially botanists, regard species as arbitrary divisions of biological diversity that do not necessarily correspond to discrete natural groups of organisms (Levin, 1979; Raven, 1986). Furthermore, even if species occur in nature as discrete sympatric groups of individual organisms, man-made taxonomic classification at the species level may not reflect these groups (Pineda Torres, 2019). Thus, although the literature on the theory of species concepts is extensive (e.g., Simpson, 1951; Wiley, 1978;

Wheeler & Meier, 2000; Christenhusz, 2020), there are fewer studies addressing the methods used for their delimitation (e.g., Sites Jr. & Marshall, 2003; Cadena et al., 2018).

Species delimitation has gained importance within modern systematics and the development of new methods for establishing boundaries has increased (e.g., Luo et al., 2018; Ahrens et al., 2021). However, one of the least explored approaches has been the use of phenotypic data for the delimitation (e.g., Ezard et al., 2010; Solís-Lemus et al., 2015; Cadena et al., 2018). Although the use of objective criteria for species diagnosis based on phenotypic characters has been widely explored in taxonomy, the way in which analytical approaches are used is often inadequate (Cadena et al., 2018). According to the basic evolutionary model, phenotypic distributions for individuals of the same sex and age, belonging to the same species, would tend to be normal (Fisher, 1918; Templeton, 2006). Thus, different phenotypic distributions can represent the boundaries of a species. This can be clarified by using multiple traits to determine specimens form distinct morphological groupings, with different morphological spacing, and without having *a priori* knowledge (Ezard et al., 2010; McKay & Zink, 2015; Cadena et al., 2018). Normal Mixture Models (NMM) provide an analytical framework to test for the existence of distinct species based on multivariate phenotypic information. These models assume that phenotypic variation is a combination of different normal distributions (Cadena et al., 2018).

An additional source of data that could inform species delimitation are functional traits. These are well-defined, heritable and measurable morphological, physiological or phenological characteristics that influence the performance of an individual in a given environment and community (Violle et al., 2007). Functional traits may also provide a better mechanistic understanding of species-environment relationships (Wilkes et al., 2020). In addition, their expression within the community assemblage influences ecosystem functioning (Wilkes et al., 2020; Cruz & Lasso, 2021). Therefore, functional traits can constitute a useful source of information to infer species limits, considering that they may reflect species adaptations to environmental conditions and to interaction within the community.

Páramos constitute a unique ecosystem located in altitudes of more than 3200 m in the Andes. This ecosystem, present only in Colombia, Ecuador, Peru and Venezuela (Diazgranados & Barber, 2017), is known for its extreme environmental conditions (e.g., Luteyn, 1999; Cruz & Lasso, 2021). These conditions, along with their geographic isolation (páramos are known as sky islands), has favored a high diversity, making it one of the most rapidly evolving ecosystems (Madriñan et al., 2013; Diazgranados & Barber, 2017). The most representative and emblematic plant group of the páramos are the *frailejones* (Subtribe Espeletiinae Cuatrec., Asteraceae) (Diazgranados & Barber, 2017). Besides being essential for the regulation of the water cycle of the páramo, *frailejones* represent one of the main examples of recent ongoing adaptive radiations (Diazgranados, 2015, Diazgranados & Barber, 2017; Pouchon et al., 2018), with more than 144 species described (e.g., Diazgranados, 2012; Diazgranados, 2015; Diazgranados & Barber, 2017; Mavarez, 2021), many occurring in sympatry. This radiation has been favored by a high hybridization within the group and for an incomplete separation of lineages, which is consistent with the high morphological variation (Diazgranados & Barber, 2017). This has hindered the delimitation and classification of species within the subtribe, which varies depending on the author, representing an opportunity to apply species delimitation methods based on phenotypic information.

In addition, the theories of individual variation propose intraspecific variation as the main driver of local diversity, so that a high variation in traits within species favors the maintenance of diversity (Violle et al., 2012). Thus, including the high morphological diversity of *frailejones* in the analysis of community structuring allows new perspectives on the filters acting on the diversity and coexistence of these species within this ecosystem. Violle et al. (2012) proposed that two types of filters, external and internal, operate at the individual instead of the species level. Internal filters are related to processes that occur within the community, such as competition, facilitation, microhabitat heterogeneity, among others. External filters operate on a larger scale than the community, and some examples are climate, environmental filtering and dispersal factors. The influence of external filters is expected to be greater under harder environmental conditions, leading to convergent distribution of trait values in a given community. In environments with less abiotic restrictions, internal filters are expected to be predominant. Since trait values can respond to these two types of filters, it is also important to address what type of filter may be structuring the community assembly and thus, influence the delimitation of groups using phenotypic data.

In this study, we aim to (i) determine functional units based on functional traits, which could serve as an approximation to a species delimitation based on phenotypic information; and (ii) understand the possible influence of internal and external filters on these functional units. With respect to the filters, due to the abiotic stressful conditions that characterize the páramos, we expect that external filters will have a higher influence in the functional units, leading to trait distribution convergence within the *frailejon* communities in each páramo complex.

Materials & Methods

Study site

The study was carried out in seven páramo complexes of Colombia (Fig. 1). The páramos of Guantiva–La Rusia, Pisba (in the outer limits of the National Park), Tota–Bijagual–Mamapacha and Iguaque–Merchán (specifically Arcabuco), are located on the western side of the Eastern Andean Cordillera, in the Department of Boyacá, with an average annual precipitation of 1950 mm (Morales et al., 2007), 979-3878 mm (Pérez et al., 2017), 965-2078 mm (Corpochivor, 2016) and 1853 mm (Villarreal et al., 2017), respectively. The páramos of Rabanal–Río Bogotá (specifically in Guacheneque), Chingaza and Cruz Verde–Sumapaz (in the outer limits of the National Parks) are in the eastern side of the Eastern cordillera, in the Department of Cundinamarca, with an average annual precipitation of 700 - 1000 mm (Forero-Gómez, Gil-Leguizamón & Morales-Puentes, 2020), 3000 mm (Torres & Aragón, 2021) and 757.7 mm (Peñalosa, Uribe & Murcia, 2018), respectively. The climatic conditions of the Colombian páramos are very variable in terms of rainfall distribution, temperature variations, luminosity, daylight duration, relative humidity and winds (Morales et al, 2007). Some of the wettest páramos are located on the eastern slopes of the eastern cordillera, and the driest in certain areas of the interior of this cordillera (Morales et al, 2007) (Fig. S1). The sampling was carried out in the months of November, early December and March, which are still part of the wet season.

The páramos of Boyacá are known for their Espeletiinae diversity (i.e., *frailejones*) with ca. 21 species registered for Guantiva–La Rusia, ca. 18 in Pisba, ca. 15 in Tota–Bijagual–Mamapacha (where we specifically sampled the páramo of Curíes, Sarna, El Guachal and El Alto) and 12 in Iguaque–Merchán (Diazgranados, 2012; Mavarez, 2021). The páramos of Cundinamarca are less speciose with ca. five species reported in Chingaza, ca. nine in Cruz Verde–Sumapaz and ca. seven in Rabanal–Río Bogotá (Diazgranados, 2012; Mavarez, 2021 Species of *frailejones* grow in sympatry alongside grasses and shrubs (Luteyn, 1999; Peyre et al., 2015). At each sampling site we used keys (e.g., Cuatrecasas, 1976, 1996; Diazgranados, 2012; Mavarez, 2021) and aids (provided by Diazgranados comm. pers.) to identify species. However, given that this is not a taxonomic study and that errors in the identification of species are likely, we grouped them into morphotypes. We therefore collected individuals that had the same morphological characteristics (such as floral and leaf morphology, see below) and were growing in sympatry.

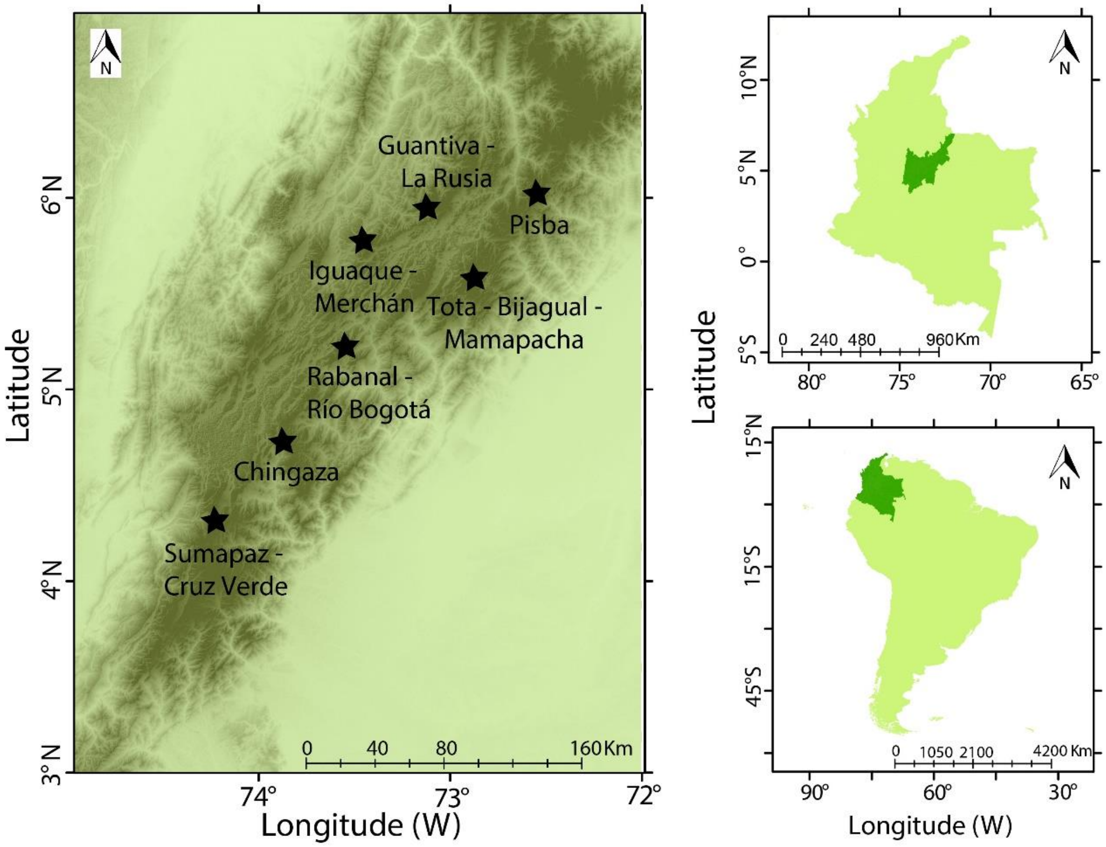

Figure 1: Location of sampled páramo complexes in Colombia. Black stars represent the location sampled at each complex in the Departments of Cundinamarca and Boyacá.

Measurement of functional traits

Approximately five individuals per morphotype were collected at each sampling site. We defined a morphotype based on morphological characteristics of the inflorescence such as number of capitula, bracts, branching patterns and pubescence, as well as the size, shape (of the leaf and the insertion in the stem), pubescence of the leaf, and the ratio between the leaf and inflorescence size. Individuals with the same characteristics were thus considered from the same morphospecies. Four leaf functional traits associated with plant growth and survival were measured for each individual: leaf area (LA (cm^2)), specific leaf area (SLA (cm^2 g^{-1})), leaf dry matter content (LDMC (mg g^{-1})), and leaf thickness (LT (mm)). Although we only sampled leaf functional traits, these traits provide information on plant growth and leaf investment strategies (Wright et al., 2004).

Functional traits were measured following standardized protocols (Pérez-Harguindeguy et al., 2013; Negret, 2016) and leaf traits were measured on three healthy leaves exposed to light per individual. Leaves were photographed and ImageJ digital image processing program (Gao et al., 2011; Schneider et al., 2012) was used to measure LA. Leaf dry weight was obtained after drying leaves in an oven at 60 °C for five days. Specific leaf area (SLA) was obtained based on LA and dry weight, while LDMC was obtained using the fresh weight and leaf dry weight. For LT, three measurements per leaf were averaged (always consistent in the different individuals and morphotypes: base, tip and middle areas of the vein) with a digital Vernier caliper at the sampling site.

Data analysis

Normal Mixture Models (NMM) were used to test for the existence of different functional units in the seven páramo complexes sampled (following Cadena et al., 2018). To define the foliar functional space a PCA on the covariance matrix of log-transformed data was used (McKay & Zink, 2015). The R package clustvarsel (Scrucca & Raftery, 2004) was used to reduce the dimensionality of the data by selecting the principal components most useful for discriminating groups in NMMs (Cadena et al., 2018). We averaged the data of the leaf traits for each individual, but they were not assigned *a priori* to a morphospecies for the NMMs analysis. The R package mclust 5.0 (Scrucca et al., 2016) was used to fit a wide range of NMMs. Using the Bayesian Information Criterion (BCI; Schwarz, 1978) we tested for the empirical support between NMMs models (Fraley & Raftery, 2002) to find the number of functional units with the highest support (Cadena et al., 2018).

Additionally, to understand the influence of external and internal filters on the functional units, we used the "Trait statistics" (T statistics) (Violle et al., 2012). This approach is based on the comparison of intraspecific and interspecific variation of functional traits at different organizational levels. The three T statistics (T_IP.IC, T_IC.IR, T_PC.PR, described in detail in Bonilla et al., 2020) were calculated per functional trait for all páramo complexes and for each páramo complex separately. The groupings used for this test were based on the best NMM model. Therefore, all the individuals of the same group were taken as a population; all individuals belonging to a páramo complex were taken as a community; and all individuals sampled in the seven páramo complexes were taken as the regional pool. The observed values of the T-statistics were compared to random values by calculating the standardized effect size (SES) (Taudiere & Violle, 2016). SES allows to measure the magnitude of deviation of the observed values from the null models . Two sets of null models were implemented, at the local and at the regional level. The local null model randomizes trait values for all individuals within a community. For the regional null models, there are three submodels: the first randomizes the trait values for all individuals within the regional pool while maintaining the number of individuals; the second model assigns an average population-level trait value to each individual in a given population and takes into account species abundance; while the third, does not consider species abundance (Taudiere & Violle, 2016). This was performed with R package cati (Taudiere & Violle, 2016). The significance is tested using one-tailed permutation tests.

Results

In our sampling we recognized 11 morphospecies in the complex of Guantiva–La Rusia, five in the Pisba complex, one in Iguaque–Merchán, 11 in Tota–Bijagual–Mamapacha, two in Chingaza, four in Rabanal–Río Bogotá and two in Sumapaz–Cruz Verde. In the seven páramo complexes, we estimated the collection of about 29 morphospecies in total. Among the morphospecies collected, they could be assigned to the following species: *Espeletia argentea* Bonpl., *Espeletia boyacensis* Cuatrec., *Espeletia episcopalis* S. Díaz & Rodríguez-Cabeza, *Espeletia jaramilloi* S. Díaz, *Espeletia lopezii* Cuatrec., *Espeletia ramosa* J. Mavárez & M.T. Becerra, *Espeletia pisbana* S. Díaz & Rodríguez-Cabeza, *Espeletia pleiochasia* Cuatrec., *Espeletia congestiflora* Cuatrec., *Paramiflos glandulosus* (Cuatrec.) Cuatrec., *Espeletia garciae* Cuatrec., *Espeletia guacharaca* S. Díaz, *Espeletia corymbosa* Bonpl., *Espeletia muiska* Cuatrec., *Espeletia killipi* Cuatrec., *Espeletia summapacis* Cuatrec., *Espeletia uribei* Cuatrec., *Espeletia tunjana* Cuatrec. *and Espeletia murilloi* Cuatrec.

Are there distinct groups of *frailejones* based on functional traits?

Four principal components were used for the delimitation of the functional units (Fig. 2) and ignoring one of these resulted in less empirical support (ΔBIC > 20). The use of the four components had the highest empirical support when implementing a forward algorithmic search of principal components, same as when using a backward algorithmic search. We additionally found that the model specifying nine functional units, based on the analysis of the four functional traits, obtained the highest empirical support (Fig. 3). The model with the lowest empirical support (ΔBIC > 400) was the one representing a single functional unit present in all seven páramo complexes (Fig. 3). Likewise, the model supporting 29 units (based on what we had identified as different morphospecies) had a much lower support value (ΔBIC > 200; Fig. 3), compared to the nine functional units (Table S1).

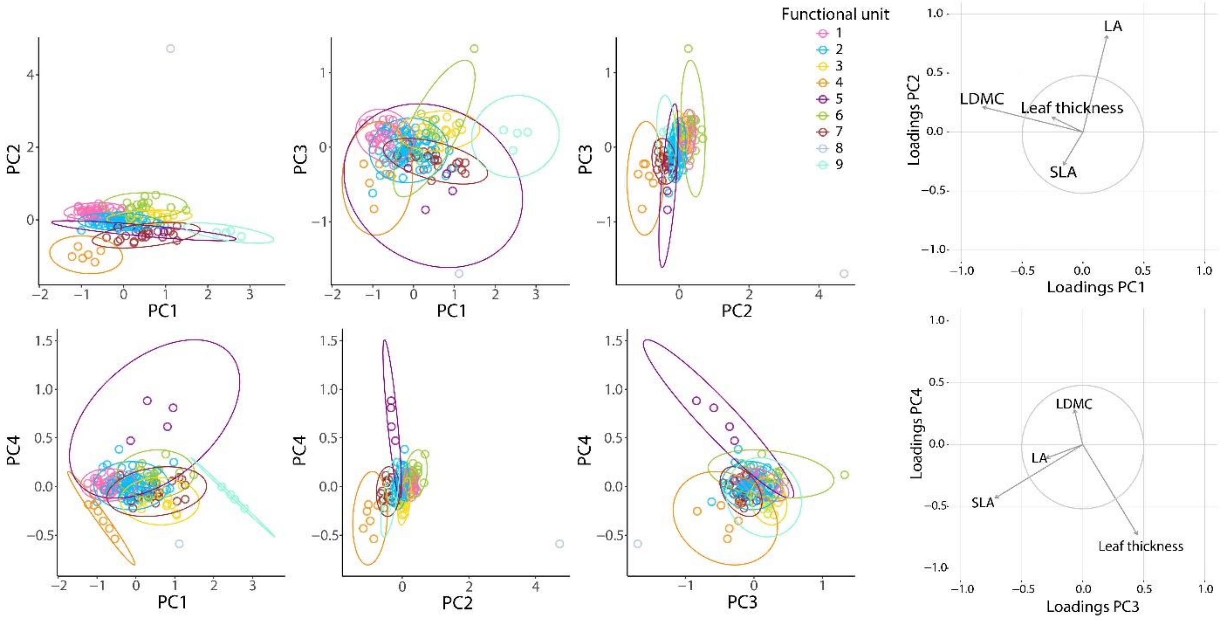

Figure 2: Nine functional units of *Espeletiinae* identified by the best Normal Mixture Model (NMM). The figure shows the functional units in the space defined by four principal components used for the group discrimination. Colored circles represent specimens assigned to different functional units and ellipses represent 95% high- density regions for normal distributions, for each functional unit. The arrows in the loadings show the contribution of measured functional traits to each principal component, and the circles show the length of arrows expected if all four traits contributed equally to the principal component spaces (G - H). The arrows exceeding the circle represent the traits that contribute most significantly.

In the foliar functional space (Fig. 2), PC1 and PC2 are mainly determined by LDMC and LA. The functional units four and one were characterized by leaves with high values of LDMC, while the functional unit two had leaves with lower LDMC. The PC3 and PC4 are mainly determined by LT and SLA, with the functional unit five having leaves with relative low LT (Fig. 2). The nine functional units (Fig. 2) supported occur in multiple complexes and are sympatric with other units (Fig. 4, Table S1). The only unit present in all complexes and co-occurring with all other units was unit two, composed of 83 individuals (Fig. 4; Table S2). The complex with the highest number of co-occurring units was Guantiva–La Rusia (Fig. 4). The complex with the least number of units was Iguaque–Merchán, given that only two localities were sampled. However, most complexes harbored several functional units (Fig. 4). In all páramo complexes the number of functional units differed from the number of morphospecies collected, except for the Iguaque–Merchán complex (Table S3).

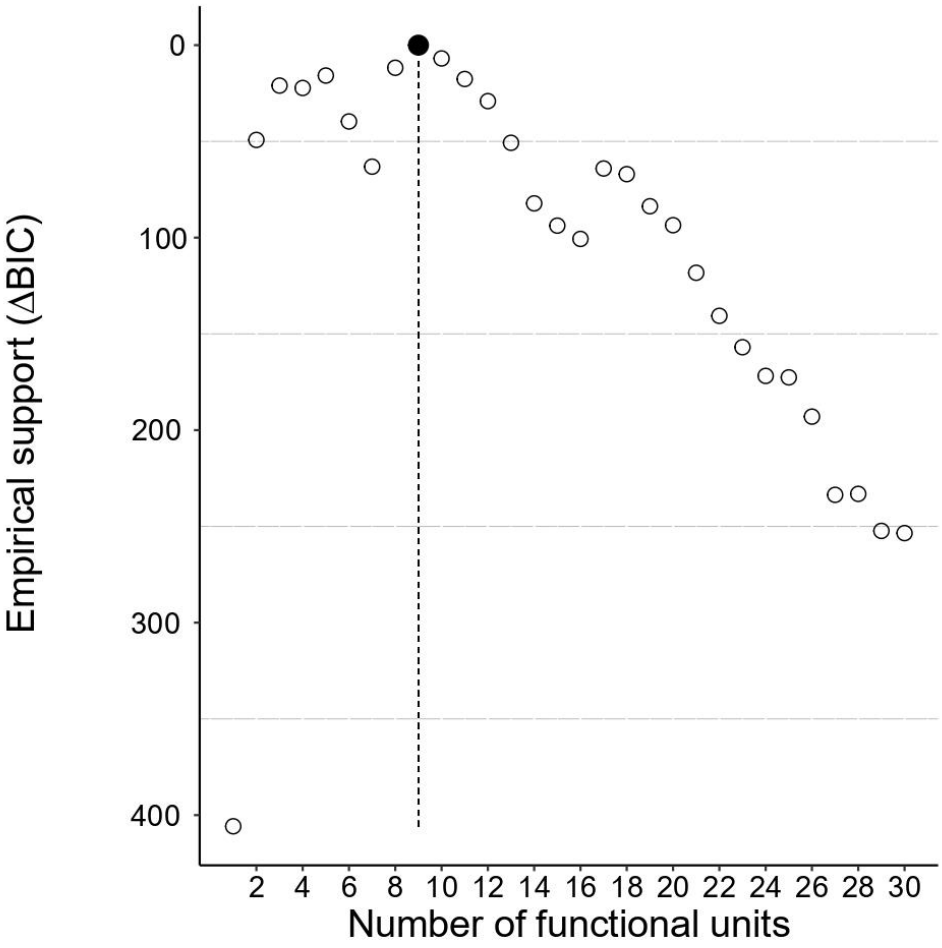

Figure 3: Number of functional units based on the Normal Mixture Models (NMM). Analysis of functional units based on leaf functional traits strongly supported hypotheses that there are nine distinct groups of *frailejones* in the seven páramo complexes. Empirical support is presented as a difference in BIC relative to the best model (ΔBIC). The graphic shows the empirical support for the NMM, without assuming any *a priori* classification of the 29 morphospecies sampled. The existence of only one functional unit was the least supported hypothesis (ΔBIC > 400).

Internal and External Filters Influence on the Community Assembly of Espeletiinae

Leaf area, LT and SLA were the functional traits that had significantly lower values than those found for the null models for T_IP.IC (Fig. 5). For T_IC.IR and T_PC.PR there were no significant differences from the null models for any trait (Fig. 5). P-values for the tests performed are reported in Table S4. At the complex level, traits tended to have significantly lower SES values than the null models for T_IP.IC (Figs. S2-S8, Table S4). In Guantiva–La Rusia (p = 0.01, p = 0.02; Fig S1; Table S4) and Tota–Bijagual–Mamapacha all traits showed this trend (p = 0.01; Fig. S2, Table S4). In Pisba, LDMC was the only trait that did not exhibit the pattern (p = 0.08; Fig. S3, Table S4).

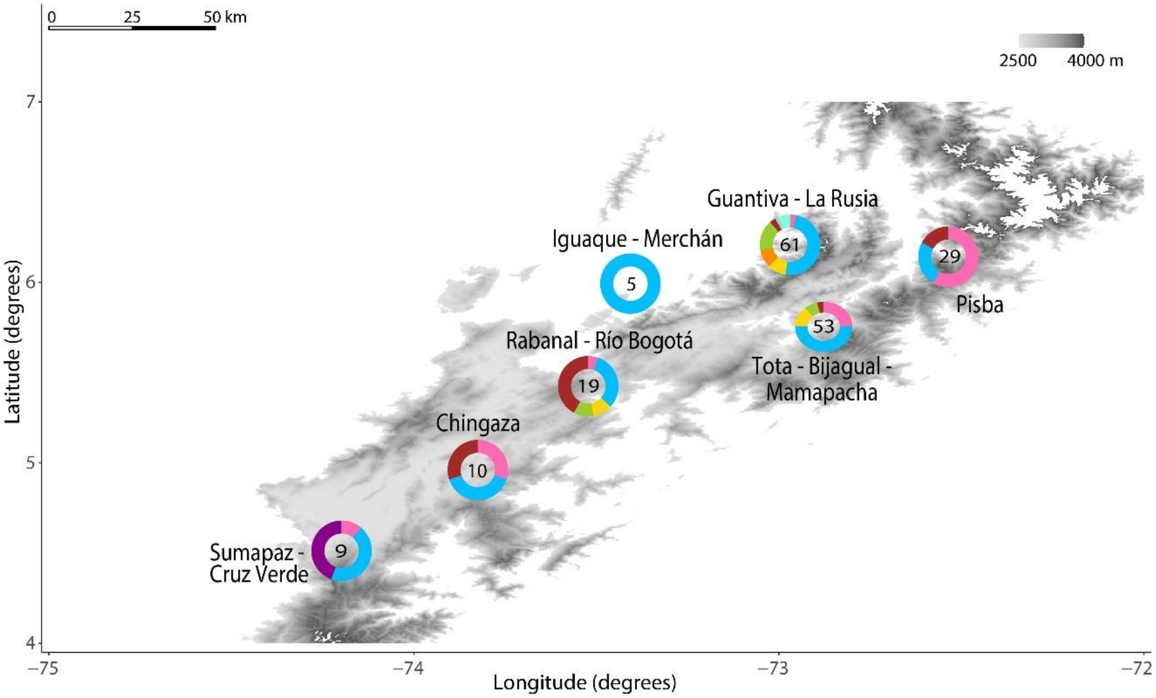

Figure 4: Distribution of the nine functional units identified by the best normal mixture model (NMM) in the seven páramo complexes. For each complex, the number indicates the individuals included in the analysis and the ring-plots show the fraction of those individuals assigned to each functional unit. Each color represents a different functional unit. Functional unit one is represented in pink, two in blue, three in yellow, four in orange, five in purple, six in green, seven in brown, eight in grey (Guantiva-La Rusia) and nine in teal. Color codes are the same as in Fig. 2.

Leaf thickness and SLA also had significant lower SES values in Chingaza (p = 0.01, p = 0.02, respectively; Table S4). For Sumapaz–Cruz Verde, only LT (p = 0.01, Table S4), and in

Rabanal–Río Bogotá, only SLA (p = 0.04; Figs. S4, S5, S6; Table S4). T_IC.IR presents significant lower SES values in Rabanal–Río Bogotá for LA (p= 0.01; Table S4), Guantiva–La Rusia for LT (p = 0,01, Table S4), Pisba for SLA and LDMC (p = 0.02, p= 0.01, respectively; Table S4), and in Tota–Bijagual–Mamapacha LT was the only trait with no significant differences (p = 0.22; Figs. S2-S8; Table S4). In Iguaque–Merchán, SLA was the only trait without significant differences (p = 0.16, Table S4). T_PC.PR showed significant differences for LDMC in Pisba, Tota–Bijagual–Mamapacha and Chingaza (Figs. S3, S4, S5, Table S4).

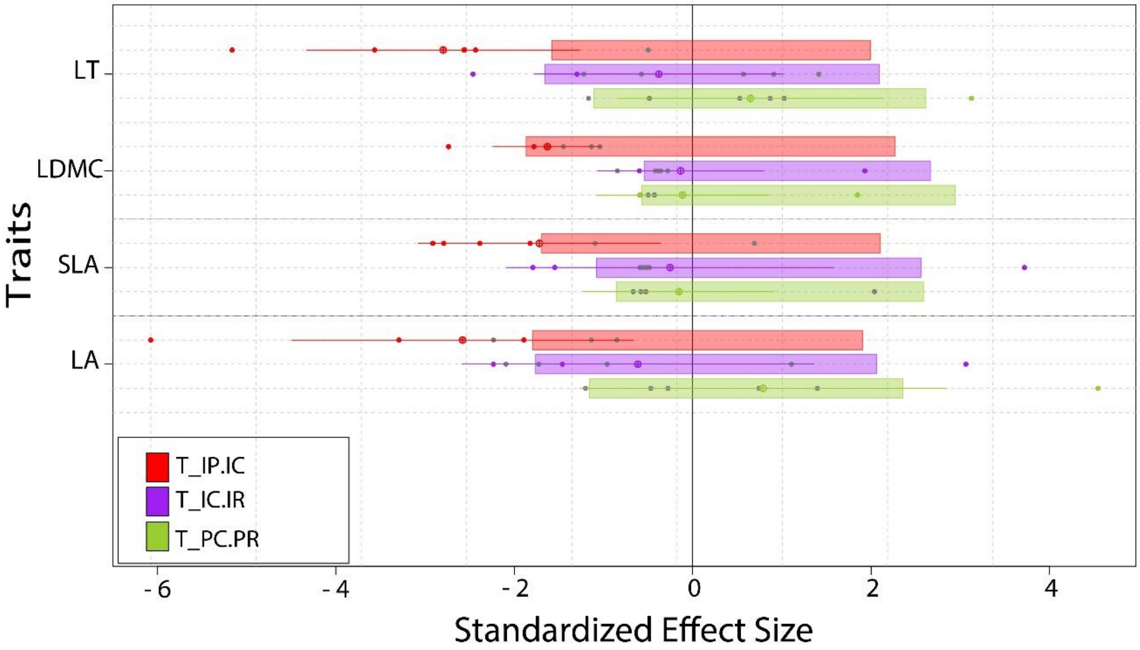

Figure 5: Standardized effect size (SES) for three T-statistics evaluated on the four functional traits. The traits used are: (1) Leaf thickness (LT); (2) Leaf dry matter content (LDMC); (3) Specific leaf area (SLA); (4) Leaf area (LA). T_IP.IC (red) refers to the ratio observed between the variance of the population and the total variance of the community at the individual level. T_IC.IR (purple) refers to the ratio between the variance of each community and the total variance of the regional pool at the individual level. T_PC.PR (green) refers to the ratio between the variance of each community and the total variance of the regional pool but at the population level, ergo, it is calculated with the average trait value for each

population. The filled circles represent the SES values for each páramo complex accordant with each statistic and trait. The boxes delimit the confidence intervals of each null model by trait: when the mean of the SES values (crossed circle) is out of the box, this value is significantly different from the random distribution represented by the null models ($p < 0.05$).

Discussion

Of the 75 species, hybrids and subspecies of *frailejon* reported for the páramo complexes in Cundinamarca and Boyacá (Diazgranados, 2012), we collected about 29 morphospecies. The analysis based on four foliar traits revealed nine distinct and most of the time, sympatric functional units, differing from what was expected based on the 29 morphospecies identified (Figs. 3, 4). Regarding the filters acting on the functional units, contrary to what we expected, in most complexes the internal filters were more important than the external, except in Iguaque–Merchán, where only one unit was collected (Fig. 5).

Are there distinct groups of *frailejones* based on functional traits?

In the seven páramo complexes nine functional units were found. Although we did not collect all the recognized species in each páramo complex, the number of units is still different from the number of *frailejon* species reported for these areas (Table S1). In Guantiva–La Rusia ca. 21 species have been reported, in Tota–Bijagual–Mamapacha ca. 15, in Pisba ca. 18, ca. 12 in Iguaque–Merchán, ca. nine in Sumapaz–Cruz Verde, in Chingaza ca. five, and ca. seven in Rabanal–Río Bogotá (e.g., Cuatrecasas, 2013; Diazgranados, 2012; Mavarez, 2021). Although there are discrepancies between the number of functional units and the number of species reported, the complex with the highest number of sympatric functional units and species is Guantiva–La Rusia, which is located in one of the three radiation centers recognized for this group (Diazgranados & Barber, 2017). Thus, this complex has a greater diversity of species and a greater variability in traits, resulting in a greater number of sympatric functional units. This higher number of functional units in Guantiva–La Rusia could be explained by its large area (Morales et al, 2007). Considering that páramos are known as sky islands, a larger area may provide opportunities for more habitats and can therefore have a larger number of functional units than other complexes.

Pineda Torres (2019) reported that for Sumapaz páramo, seven morphological groups of Espeletiinae are found with the highest empirical support (using 13 morphological traits), while with genome-wide allele frequency analysis, three groups are supported by the best model. Although the sampling proposed by Pineda Torres (2019) covered more area than the one in this study, the number of groups defined with genomic information agree with the functional units found for that complex (Fig. 4). Consequently, as more functional trait samples from this complex are available, we would be able to test if the number of functional units is maintained, or if it is closer to the number of morphological groups reported by Pineda Torres (2019). Also, so far, we have tested four foliar traits. As more traits are included, we could test if functional units change and how closely they resemble other morphological and phylogenetic studies. Within the nine functional units recognized, the morphospecies identified as *P. glandulosus* was assigned to a functional unit that did not contain other morphospecies. This agrees with what was reported by Cuatrecasas (2013), who proposed the genus *Paramiflos* as a separate group. However, the placement of *Paramiflos* is not supported by phylogenetic studies, which places it as sister to *E. guacharaca* (Fig. 4 in Diazgranados & Barber, 2017). Most functional units were composed of multiple different morphospecies, which in some cases are congruent to an extent with phylogenetic studies. For instance, a functional unit composed by all individuals collected from morphospecies identified as *E. pisbana* and *E. epicopalis* (functional unit 1; Table S5), would be partially in accordance with Diazgranados & Barber (2017). The presence of multiple morphospecies per functional unit could be explained by different factors, such as the high degree of hybridization within the Espeletiinae, even between distantly related species (Diazgranados & Barber, 2017). Another potential factor is incomplete lineage sorting, which could cause trait values to differ within what has been stipulated as a morphospecies. Likewise, convergence or divergence in traits may be found between different morphospecies (Diazgranados & Barber, 2017). It will also be interesting to understand if these functional units are maintained as more traits, reflecting the several acquisitive dimensions of plant form and function, are included.

There are some interesting patterns within the functional units. Unit one and two are present in all or almost all complexes (Fig. 4) and they are characterized by high LDMC, as well as low SLA and LT values (Fig. 2). Having a high LDMC would allow these functional units to maintain leaf integrity and low water potentials under conditions of high irradiance and water stress (Lebrija-Trejos et al., 2010), which are usually present in the páramos (Luteyn, 1999; Sanchez et al., 2018; Cruz & De Paulis, 2020). Similarly, high LDMC would increase the resistance to herbivory, and low SLA is associated with higher leaf longevity and a lower growth rate (Cruz & Lasso, 2021). Therefore, these units would be able to invest more resources in maintaining and protecting leaves, a strategy that may be advantageous, especially in the dry season (when environmental conditions are more extreme; Sanchez et al., 2018). This is line with the category of the CSR ecological scheme triangle reported for this group, given that it is between the stress tolerator and competitor (Cruz & Lasso, 2021). Similar values of these traits have also been reported in other studies for species belonging to the Espeletiinae (Cruz & Lasso, 2021). Functional unit five (only found in Sumapaz-Cruz Verde; Fig. 4) has lower LT values compared to the rest of the units (Fig

2). Low LT values are associated with a decrease in herbivore resistance (Lebrija et al., 2010), which could restrict this unit to a specific area.

The separation of functional units based on the analysis of functional traits is interesting because it offers a new perspective on how individuals are grouped due to their function (in a given ecosystem and community), in this case, reflected by foliar traits. However, it would be interesting to include other traits such as reproductive traits, given that they have been widely used for the taxonomic delimitation of *frailejones*, as well as their importance in fitness and dispersal.

Internal and External Filters Influence on the Community Assembly of Espeletiinae

External filters did not have a strong influence on the functional units when considering the seven páramo complexes (Fig.5). Páramos function as an archipelago, with separate continental islands where species have little or no contact with species in other complexes. Groups with limited seed dispersal and long-distance pollination are restricted to a given island because of this (Padilla et al., 2017). However, according to palynological records, several glaciation and interglaciation events occurred during the Quaternary, resulting in short periods of páramo expansion during glaciations, allowing colonization of other mountain systems and a continuous flow between species (Diazgranados & Barber, 2017; Padilla et al., 2017; Flantua & Hooghiemstra, 2018). Thus, during these glaciations, groups with a high dispersal capacity and with functional characteristics such as high LDMC and low SLA, were able to colonize a greater number of mountain complexes (which would be the case of functional units 1 and 2); especially in an ecosystem where dispersal poses several challenges (Peyre et al., 2020). These functional characteristics could represent an advantage when facing the climatic conditions of the páramo and favor the greater dispersion of functional units 1 and 2. As a result, the influence of external filters (Fig. 5) at the regional level may not have been as strong as we expected for the *frailejon* communities.

On the other hand, internal filters had a strong influence at the regional level with respect to SLA, LA and LT (Fig. 5). This may be driven by factors such as competition, which could be limiting the ecological similarity of coexisting species and creating greater niche packing (Lebrija et al, 2010). In addition, microhabitat heterogeneity may also be exerting a strong internal filter on these traits, because microclimatic and soil properties may influence the expression of functional traits (Morales et al., 2007; Dantas de Paula et al., 2021). Thus, microhabitat may influence the distribution of functional units in páramo complexes, creating greater niche separation between coexisting units (Fig. 4). In most of the páramos the internal filters were more important than the external ones, except in Iguaque–Merchán (Figs. S2-S8, Table S4). The pattern in Iguaque–Merchán may be due to only one morphospecies was sampled, which corresponded to a single functional unit, so there was no difference in variance between the population and the community in this complex.

The greater internal filtering in most of the complexes is reflected in the functional leaf space separation (Fig. 2). In Guantiva–La Rusia, Pisba, Tota–Bijagual–Mamapacha and Rabanal–Río Bogotá this internal filtering may be favoring the presence of a greater number of functional units (Fig. 4), with a greater niche packing (Taudiere & Violle, 2016) in páramos with high climatic variability (Fig. S1). Likewise, most of the complexes present significant influence of external filters on certain traits that are associated with resistance to mechanical or herbivory damage (LT and LDMC) and nutrient acquisition and growth rate (SLA and LA) (Figs. S2-S8, Table S4). This suggests that in these complexes there may be climatic conditions that lead to convergence in the trait values but, that internal filters also increase niche separation favoring the coexistence of more functional units. Within these climatic conditions is precipitation, which is lower in some of these complexes (Morales et al., 2007) (Fig. S1) and favors a convergence in relatively high values of LDMC. These values are related to maintaining leaf integrity and low water potentials in conditions of low water availability (Lebrijas et al, 2010). Since most complexes present significant differences at the level of external filters, the number of functional units could be underestimated, since they generate a convergence in the traits within each complex. Thus, it is important to have a wider selection of traits and to complement this type of studies with genomic and phylogenetic analyses that give a broader view of the evolution of the group. On the other hand, the importance of T_IC.IR in several complexes (Table S4) demonstrates the importance of including individual-level information in NMMs without prior taxonomic classification.

Conclusions

In this study, we explore a different way to establish groups within Espeletiinae based on functional traits. Functional traits defined nine functional units in the seven páramo complexes using NMM. The nine functional units did not reflect the number of species proposed by several authors, which indicates that it is important to review the species boundaries within Espeletiinae. However, it will be interesting to test if functional units remain stable as more traits are included, representing other acquisitive dimensions of the plants. Internal filters can affect trait values, generating a greater separation in the leaf functional space, allowing the coexistence of a greater number of functional units in certain complexes, while external filters can generate convergence in certain trait values. Thus, understanding the influence of these two types of filters is of great importance to understand the assembly of the functional units and their distribution along the seven páramo complexes. As species are the basic units of study in areas such as ecology and conservation, we highlight the importance of addressing the methods used for their delimitation, especially in groups as emblematic as the Espeletiinae. Likewise, we emphasize the need for other studies that address this issue in order to further explore NMM methods and its usefulness within the *frailejones*.

Acknowledgements

We thank V Castañeda, G Castaño, J Fonseca, Y Hoyos, C Marquez, A Ortiz, M Salamanca and JD Sanchez for their help in the field. We also thank C Lora, M Diazgranados and M Baruffol for their help in defining field collection sites. We are also grateful to A Corrales, F Zapata and AM Aldana for their ideas on the data analysis. The authors also thank the Laboratory of Functional and Ecosystemic Ecology of the Universidad del Rosario. All field trips and collections are under the ANLA permits 0530-2014 and 0061-2016.

Additional information and declarations

Funding

This work is part of the undergraduate thesis of V Vargas and was supported by Universidad del Rosario (UR) through the scholarships offered by Facultad de Ciencias Naturales and Jóvenes Talento (provided by the Research and Innovation Department at UR). The Research and Innovation Department also supported fieldwork through the internal funds of A Sanchez. The funders did not participate in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

Valeria Vargas performed the field sampling, processed the samples in the laboratory, analyzed the data, and authored and reviewed drafts of the article.

Adriana Sanchez performed the field sampling, processed the samples in the laboratory, analyzed the data, authored and reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw data is available in the Supplemental Files and available on request from the contact author.

Supplemental Information

Supplemental information for this article can be found online at **agregar link**.

References

Ahrens D, Ahyong ST, Ballerio A, Barclay MVL, Eberle J, Espeland M, Huber BA, Mengual X, Pacheco TL, Peters RS, Rulik B, Vaz-De-Mello F, Wesener T, Krell F-T. 2021. Is it time to describe new species without diagnoses?—A comment on Sharkey et al. (2021). *Zootaxa* 5027:151–159. DOI: 10.11646/zootaxa.5027.2.1.

Bonilla D, Aldana AM, Cárdenas S, Sanchez A. 2020. Functional Divergence between Várzea and Igapó Forests: A Study of Functional Trait Diversity in the Colombian Orinoco Basin. *Forests* 11:1172. DOI: 10.3390/f11111172.

Cadena CD, Zapata F, Jiménez I. 2018. Issues and Perspectives in Species Delimitation using Phenotypic Data: Atlantean Evolution in Darwin's Finches. *Systematic Biology* 67:181–194. DOI: 10.1093/sysbio/syx071.

Christenhusz MJM. 2020. On species concepts, phylogenetics and the science of natural history three current issues facing taxonomy. *Megataxa* 1:67–72. DOI: 10.11646/megataxa.1.1.14.

Corpochivor. 2016. Documento sintesis para la declaratoria del distrito regional de manejo integrado (DRMI) paramo mamapacha y bijagual, jurisdicciòn de corpochivor.Available at: http://fauna.corpochivor.gov.co/wp-content/uploads/2016/04/S%C3%ADntesis-Declaratoria-DRMI-Mamapacha-Bijagual.pdf (accessed 25 April 2022)

Cruz M, Lasso E. 2021. Insights into the functional ecology of páramo plants in Colombia. *Biotropica* 53:1415–1431. DOI: 10.1111/btp.12992.

Cuatrecasas J. 1976. A new subtribe in the Heliantheae (Compositae): Espeletiinae. *Phytologia, 35:* 43-61.

Cuatrecasas J. 1996. Clave provisional de las especies del género Espeletiopsis Cuatrec.(Espeletiinae, Compositae). *Anales del Jardín Botánico de Madrid* 54:370-377. Real Jardín Botánico.

Cuatrecasas J. 2013. A Systematic Study of the Subtribe Espeletiinae (Heliantheae, Asteraceae) The New York Botanical Garden Press 107.

Dantas de Paula M, Forrest M, Langan L, Bendix J, Homeier J, Velescu A, Wilcke W, Hickler T. 2021. Nutrient cycling drives plant community trait assembly and ecosystem functioning in a tropical mountain biodiversity hotspot. *New Phytologist* 232:551–566. DOI: 10.1111/nph.17600.

De Queiroz K. 2007. Species Concepts and Species Delimitation. *Systematic Biology* 56:879–886. DOI: 10.1080/10635150701701083.

Diazgranados M. 2012. A nomenclator for the frailejones (Espeletiinae Cuatrec., Asteraceae). *PhytoKeys*:1–52. DOI: 10.3897/phytokeys.16.3186.

Diazgranados M. 2015. Una mirada biológica a los páramos circundantes a la Sabana de Bogotá. In: 175–205.

Diazgranados M, Barber JC. 2017. Geography shapes the phylogeny of frailejones (Espeletiinae Cuatrec., Asteraceae): a remarkable example of recent rapid radiation in sky islands. *PeerJ* 5:e2968. DOI: 10.7717/peerj.2968.

Ezard TH, Pearson PN, Purvis A. 2010. Algorithmic approaches to aid species' delimitation in multidimensional morphospace. *BMC Evolutionary Biology* 10:175. DOI: 10.1186/1471-2148-10-175.

Fick SE, Hijmans RJ, 2017. WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. Fick, S.E. and R.J. Hijmans, 2017. WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* 37: 4302-4315.

Fisher RA. 1919. XV.—The Correlation between Relatives on the Supposition of Mendelian Inheritance. *Earth and Environmental Science Transactions of The Royal Society of Edinburgh* 52:399–433. DOI: 10.1017/S0080456800012163.

Flantua S., & Hooghiemstra H. 2018. Historical connectivity and mountain biodiversity. In Hoorn C, Perrigo A, Antonelli A. (Eds.), *Mountains, climate and biodiversity*. 171–185. Hoboken, NJ, USA: John Wiley & Sons Ltd.

Forero-Gómez YK, Gil-Leguizamón PA, Morales-Puentes ME. 2020. Conectividad estructural entre los Páramos de Guacheneque y Los Cristales, complejo Rabanal-río Bogotá, Colombia. *Revista de Teledetección*:65. DOI: 10.4995/raet.2020.13946.

JianChang G, GuangJun G, YanMei G, XiaoXuan W, YongChen D. 2011. Measuring plant leaf area by scanner and ImageJ software. *China Vegetables*: 73–77.

Lebrija-Trejos E, Pérez-García EA, Meave JA, Bongers F, Poorter L. 2010. Functional traits and environmental filtering drive community assembly in a species-rich tropical system. *Ecology* 91:386–398.

Levin DA. 1979. The nature of plant species. *Science*, 204:381–384. DOI: 10.1126/science.204.4391.381

Luo A, Ling C, Ho SYW, Zhu C-D. 2018. Comparison of Methods for Molecular Species Delimitation Across a Range of Speciation Scenarios. *Systematic Biology* 67:830–846. DOI: 10.1093/sysbio/syy011.

Luteyn JL. 1999. Páramos: A Checklist of Plant Diversity, Geographical Distribution, and Botanical Literature. *New York Botanical Garden Press*, Brooklyn

Madriñán S, Cortés A, Richardson J. 2013. Páramo is the world's fastest evolving and coolest biodiversity hotspot. *Frontiers in Genetics* 4.

Mavárez J. 2021. A Taxonomic Revision of Espeletia (Asteraceae). II. Updated List of Taxa, Nomenclature, and Conservation Status in the Colombian Radiation. *Harvard Papers in Botany* 26:131–157. DOI: 10.3100/hpib.v26iss1.2021.n.

McKay BD, Zink RM. 2015. Sisyphean evolution in Darwin's finches. *Biological Reviews* 90:689–698. DOI: 10.1111/brv.12127.

Morales M, Otero J, Van Der Hammen T, Torres A, Cadena CE, Pedraza CA, Rodriguez N, Franco CA, Betancourth JC, Olaya E, Posada E, Cardenas L. 2007. Atlas de páramos de Colombia. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt. Bogotá, D. C. Available at http://www.humboldt.org.co/es/estado-de-los-recursos-naturales/item/1291-atlas-paramos-colombia. (accessed 19 July 2022)

Padilla-González GF, Diazgranados M, Da Costa FB. 2017. Biogeography shaped the metabolome of the genus Espeletia: a phytochemical perspective on an Andean adaptive radiation. *Scientific Reports* 7:8835. DOI: 10.1038/s41598-017-09431-7.

Peñalosa E, Uribe M, Murcia FL. 2018. Identificación y priorización escenario riesgo. Consejo local de gestión del riesgo y cambio climático. Available at https://www.idiger.gov.co/documents/220605/329657/Identificaci%C3%B3n+y+Priorizaci%C3%B3n.pdf/c0c0d444-dcce-4c14-9f06-a40e0f9f7777 (accessed 19 July 2022)

Pérez KE, Rico CA, Moreno HP, Bermúdez NT, Cabra D, Pineda MC, Tovar DR, Zea DV, Sanabria O, Alba S, Granados D, Cruz FP, Cruz PT, Becerra L, Peña W. 2018. Estudio Técnico, Económico, Social y ambiental para la identificación y delimitación de complejos de páramo a escala 1:25.000. Available at http://corporinoquia.gov.co/images/2021/Educacion_Ambiental/ETESA_PISBA%20CORPORINOQUIA%20(1)_compressed.pdf (accessed 19 July 2022)

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L, Wright IJ, Ray P, Enrico L, Pausas JG, de Vos AC, Buchmann N, Funes G, Quétier F, Hodgson JG, Thompson K, Morgan HD, ter Steege H, Sack L, Blonder B, Poschlod P, Vaieretti MV, Conti G, Staver AC, Aquino S, Cornelissen JHC. 2013. New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* 61:167. DOI: 10.1071/BT12225.

Peyre G, Balslev H, Martí D, Sklenář P, Ramsay P, Lozano P, Cuello N, Bussmann R, Cabrera O, Font X. 2015. VegPáramo, a flora and vegetation database for the Andean páramo. *Phytocoenologia* 45:195–201. DOI: 10.1127/phyto/2015/0045.

Peyre G, Lenoir J, Karger DN, Gomez M, Gonzalez A, Broennimann O, Guisan A. 2020. The fate of páramo plant assemblages in the sky islands of the northern Andes. *Journal of Vegetation Science* 31:967–980. DOI: 10.1111/jvs.12898.

Pineda Torres Y. 2019. The nature of *Espeletia* species. *Universidad de los Andes*. Available at https://repositorio.uniandes.edu.co/handle/1992/44108 (accessed 19 July 2022)

Pouchon C, Fernández A, Nassar JM, Boyer F, Aubert S, Lavergne S, Mavárez J. 2018. Phylogenomic Analysis of the Explosive Adaptive Radiation of the Espeletia Complex (Asteraceae) in the Tropical Andes. *Systematic Biology* 67:1041–1060. DOI: 10.1093/sysbio/syy022

Raven PH. 1986. Modern aspects of the biological species in plants. *Modern Aspects of Species*.11–29

Sanchez A, Rey-Sánchez AC, Posada JM, Smith WK. 2018. Interplay of seasonal sunlight, air and leaf temperature in two alpine páramo species, Colombian Andes. *Agricultural and Forest Meteorology* 253–254:38–47. DOI: 10.1016/j.agrformet.2018.01.033.

Simpson GG. 1951. The Species Concept. *Evolution* 5:285–298. DOI: 10.2307/2405675.

Sites JW, Marshall JC. 2003. Delimiting species: a Renaissance issue in systematic biology. *Trends in Ecology & Evolution* 18:462–470. DOI: 10.1016/S0169-5347(03)00184-8.

Solís-Lemus C, Knowles LL, Ané C. 2015. Bayesian species delimitation combining multiple genes and traits in a unified framework. *Evolution* 69:492–507. DOI: 10.1111/evo.12582.

Taudiere A, Violle C. 2016. cati: an R package using functional traits to detect and quantify multi-level community assembly processes. *Ecography* 39:699–708. DOI: 10.1111/ecog.01433.

Templeton AR. 2006. *Population Genetics and Microevolutionary Theory*. Hoboken (NJ): John Wiley & Sons.

Torres P, Aragón J. 2021. Estudio de registros extremos de precipitación asociados al cambio climático en el Parque Nacional Natural Chingaza durante el periodo de 1981 - 2010 | Ingenierías USBMed.

Villarreal H, Núñez M, Zorro W, Pacheco C. 2017. Plan de Manejo del Santuario de Fauna y Flora Iguaque. Parques Nacionales Naturales de Colombia. Available at https://www.parquesnacionales.gov.co/portal/wp-content/uploads/2018/06/PM-SFF-IGUAQUE-adoptado.pdf (accessed 19 July 2022)

Violle C, Navas M-L, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E. 2007. Let the concept of trait be functional! *Oikos* 116:882–892. DOI: 10.1111/j.0030-1299.2007.15559.x.

Violle C, Enquist BJ, McGill BJ, Jiang L, Albert CH, Hulshof C, Jung V, Messier J. 2012. The return of the variance: intraspecific variability in community ecology. *Trends in Ecology & Evolution* 27:244–252. DOI: 10.1016/j.tree.2011.11.014.

Wheeler QD, Meier R. 2000. *Species Concepts and Phylogenetic Theory: A Debate*. Columbia University Press.

Wiens JJ. 2007. Species Delimitation: New Approaches for Discovering Diversity. *Systematic Biology* 56:875–878. DOI: 10.1080/10635150701748506.

Wiley EO. 1978. The Evolutionary Species Concept Reconsidered. *Systematic Biology* 27:17–26. DOI: 10.2307/2412809.

Wilkes MA, Edwards F, Jones JI, Murphy JF, England J, Friberg N, Hering D, Poff NL, Usseglio-Polatera P, Verberk WCEP, Webb J, Brown LE. 2020. Trait-based ecology at large scales: Assessing functional trait correlations, phylogenetic constraints and spatial variability using open data. *Global Change Biology* 26:7255–7267. DOI: 10.1111/gcb.15344.

**Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J,

Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R. 2004. The worldwide leaf economics spectrum. *Nature* 428:821–827. DOI: 10.1038/nature02403