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RESEARCH ARTICLE

Conserved leaf traits and phytochemical plasticity of *Polylepis sericea* Wedd. (Rosaceae) across an altitudinal gradient in the Venezuelan Andes

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ABSTRACT

This study examined the foliar anatomical plasticity, based on standard leaf traits, as well as the phytochemical plasticity, based on the accumulation and composition of polyphenolic compounds in *Polylepis sericea* along an altitudinal gradient (3,549–4,223 m.a.s.l.) in the Venezuelan Andes. Morphoanatomical analyses showed no variations in specific leaf area (SLA), cuticle thickness, epidermal cells height in general, nor in the proportion of palisade chlorenchyma respective spongy chlorenchyma, indicating a highly conserved anatomical structure. Phytochemical profiling revealed the accumulation of polyphenolic chelating agents—mainly dihydroxy-substituted flavonoids and phenylpropanoids within chlorenchyma tissues, especially in the two upper layers of palisade parenchyma. Variations in total phenolic content did not follow a linear altitudinal trend, since the highest concentration corresponded to the sample collected at the lowest elevation. However, the highest concentrations of oxidized flavonoids were observed in the chlorenchyma tissues of the samples collected at the highest elevation of the gradient. These differences, and presence of glycosylated or oxidized flavonols in the samples collected at 3,459 m a.s.l. indicate that the synthesis and type of polyphenolic compounds in *P. sericea* are influenced by site-specific environmental factors, and suggest that these secondary metabolites play a key role in the mitigation of the photooxidative stress generated in extreme high montane environments. Our findings reveal conservative anatomical traits coupled with phytochemical plasticity that may help us understand adaptations developed by high-elevation woody plants.

Keywords:
flavonoids, leaf morpho-anatomical traits, phenylpropanoids, polyphenols, tropical Andes, UV stress.

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INTRODUCTION

Polylepis sericea Wedd. (Rosaceae, Rosales) is one of the woody plants that grows at the highest elevations on the planet. In the Northern Andes, it forms islands of discontinuous forests on steep slopes and rocky debris. According to Arnal (1983), these forests typically occupy a 500 m elevation belt above the treeline, ranging between 3,800–4,300 m a.s.l. However, in the Cordillera de Mérida, Venezuela, they can descend as low as 3,300 m a.s.l. with optimal development occurring between 3,500-4,000 m a.s.l. (Rada *et al.*, 2011). Although the physiognomy of *P. sericea* forests is strongly influenced by the frequency and volume of precipitations, the trees maintain their twisted trunks, covered with a scaly reddish bark (Simpson, 1979), from which their common name ”Coloradito” in the region is derived. *Polylepis sericea* forests are evergreen and form closed to semi-open canopies, depending on their orientation and elevation. These forests have been frequently regarded as monospecific; yet the tree stratum may frequently be composed of woody species of the genera *Hesperomeles*, *Gynoxys* and *Libanothammus* (Delgado, 2015). The floristic diversity of *Polylepis* forests is apparently low compared to the Andean Cloud Forests of the same mountain range; nevertheless, when compared to the open Andean páramo, they outstand for the variety of growth-forms and the richness of non-vascular species, especially bryophytes, due to co-occurrence of species from the high-montane Cloud Forest and the Páramos (Delgado & León-Vargas, 2017). These forests provide unique microenvironments that foster the development of other growth-forms, as they buffer the adverse effects of the high-Andean ecosystem and the adjacent periglacial desert (Rada *et al.*, 2011).

The colonization of the entire Andes Mountain range by different species of the genus *Polylepis* occurred thanks to the development of functional traits, that include resistance to low temperatures and highly efficient carbon assimilation rates (Azócar *et al.*, 2007). In the case of *P. sericea*, its establishment combines both sexual reproduction by anemochorous seeds and clonal reproduction by resprouts. The survival of seedlings and juvenile individuals is enabled by the protection provided by adult canopy individuals, who play an important role as nurse plants, creating a less harsh microclimate for seedlings and juveniles compared to the surrounding open páramo (Rada *et al.*, 2011). The mechanisms of resistance to freezing temperatures in the genus *Polylepis* vary depending on the species and the elevation at which they grow, and accordingly, they can be frost avoiders or frost tolerant (Azócar *et al.*, 2007). In the specific case of *P. sericea*, resistance to sub-zero temperatures is achieved through tissue supercooling of -9 °C in the parenchymal tissues of branches and leaves, and of -12°C in meristematic tissues (Rada *et al.*, 1985, 2009). This supercooling occurs due to the accumulation of osmotically active solutes, mainly sugars and proline during the night, which causes a decrease in the tissues' osmotic potential, and consequently, a decrease in the freezing temperature of the parenchymatic extracellular fluids (Rada *et al.*, 1985; Azócar & Rada, 2006). The energy cost required to maintain the transport and accumulation of osmotically active solutes is compensated by elevated respiratory rates, coupled with a highly efficient C3 photosynthetic metabolism under conditions of high radiation and abrupt temperature fluctuations (Rada *et al.*, 1996), whose effect in both cases intensifies across the high-Andean páramo-periglacial desert altitudinal gradient (Azócar *et al.*, 2007; Goldstein *et al.*, 1994).

Anatomical studies in the genus *Polylepis* conducted by Simpson (1979) suggest a potential foliar plasticity that has facilitated its wide latitudinal and altitudinal distribution. In the case of *P. sericea*, Colmenares-Arteaga *et al.* (2005) describes this species as a typical heliophyte; characterized by compound leaves with thick folioles protected by a dense silvery indumentum on the abaxial surface, thick cuticles on both surfaces, small epidermal and hypodermal cells with thickened walls, and a multilayered palisade parenchyma, that occupies over two thirds of the mesophyll.

The adaptation of *P. sericea* to high mountain ecosystems has likely involved, aside from the physiological and anatomical traits; the biosynthesis of photoprotective compounds such as secondary metabolites, given their ability to protect plants from excessive radiation, particularly in the UV spectrum (Morales *et al.*, 2009; Del Valle *et al.*, 2020; Wang *et al.*, 2025). The intensity and duration of the UV radiation in mountain ecosystems increases significantly due to the thinning of the atmospheric ozone at high elevations, particularly above 4,000 m a.s.l. (Cabrol *et al.*, 2014; Liu *et al.*, 2024). Foliar anatomical traits can be very useful for interpreting the structural and physiological adaptations of plants to extreme ecosystems (Zhou *et al.*, 2019; Bertel *et al.*, 2022; Soheili *et al.*, 2023) and to microclimatic changes across altitudinal gradients (Ma *et al.*, 2012; He *et al.*, 2017; Pfennigwerth *et al.*, 2017; Bertel *et al.*, 2022; Li *et al.*, 2022; Soheili *et al.*, 2023; Yang *et al.*, 2022; Liu *et al.*, 2021, 2024). Foliar tissues in both herbaceous and woody plants, particularly epidermal and chlorenchyma tissues are known for their remarkable plasticity, and the same occurs with the ratio of palisade parenchyma/spongy parenchyma in the mesophyll in response to abiotic conditions such as incident radiation. For these reasons, anatomical traits have proven useful to understand adaptations developed by plants in response to environmental conditions across latitudinal and altitudinal distribution ranges (Kuster *et al.*, 2016; He *et al.*, 2017; Pfennigwerth *et al.*, 2017; Liu *et al.*, 2021, 2024; Bertel *et al.*, 2022).

The adaptations of plants to extreme environments are also measured based on the presence, location, and accumulation of secondary metabolites (Agati *et al.*, 2012; Mierziak *et al.*, 2014; Deng & Lu, 2017). In the genus *Polylepis*, phytochemical studies conducted to date have focused mainly on the isolation and identification of secondary metabolites stored in the bark, young branches, and to a lesser extent, leaves (Simpson, 1979; Catalano *et al.*, 1995; Vélez *et al.*, 1998; Lampasona *et al.*, 1998; Neto *et al.*, 2000; Caceda, 2012). These studies have been conducted with the purpose of identifying secondary metabolites with medicinal uses in the case of *Polylepis sericea* (Simpson, 1979), *P. racemosa* (Neto *et al.*, 2000), and *P. rugulosa* (Caceda, 2012), and of taxonomic use, in the case of *P. incana*. So far, the only phytochemical study in this genus that linked the accumulation of secondary metabolites isolated from foliar tissues with adaptations to extreme environmental conditions was conducted in *P. quadrijuga*, a species from the Colombian Andes; that experiences an increase in the synthesis of polyphenols, specifically, flavones and catechins during the coldest months of the year (Vélez *et al.*, 1998).

The accumulation of secondary metabolites, specifically polyphenols in this genus could be related to its tolerance to conditions of high UV-B (290–315 nm) radiation, combined with low temperatures. The in situ effect of UV-B radiation and low temperatures on the synthesis and accumulation of polyphenols has been little studied in plants from Andean ecosystems; nevertheless, previous studies suggest that under these conditions, there is an increase in the synthesis of phenolic compounds including flavonoids (Carrasco-Ríos, 2009). Thus, it is feasible to assume that *P. sericea* accumulates higher concentrations of polyphenols across its altitudinal distribution range in the tropical Andes, due to the increase in the intensity and duration of UV-B radiation with increasing elevation (Cabrol *et al.*, 2014).

The synthesis of polyphenols in the leaves occurs in the chlorenchyma tissues, from which they are later transported to the epidermis, where they accumulate and contribute to reduce the amount of UV radiation that penetrates foliar tissues. Polyphenols also play an important role as non-enzymatic antioxidants in metabolically active tissues, acting as scavengers of free radicals that may affect the photosynthetic complex (Morales *et al.*, 2009; Zhou *et al.*, 2019; Del Valle *et al.*, 2020; Kim *et al.*, 2021; Cesarino *et al.*, 2022). Broadly speaking, secondary metabolites synthesized by plants are divided into four chemically distinct groups: phenylpropanoids, terpenes, glycosides, and alkaloids (Ávalos & Pérez-Urria, 2009; Wang *et al.*, 2025). Phenylpropanoids encompass the largest group of secondary metabolites and include: chalcones, flavones, flavonols, flavandiols, flavonoids, anthocyanins and proanthocyanidins (Williams *et al.*, 2004; Falcone Ferreyra *et al.*, 2012; Kim *et al.*, 2021; Salam *et al.*, 2023). Most phenylpropanoids are products of phenylalanine ammonia-lyase (PAL). The latter catalyzes the deamination of phenylalanine to originate cinnamic acid, which is a precursor of various phenolic compounds including coumarin; which, in turn, is the precursor of lignin and of flavonoids (Kim *et al.*, 2021). PAL plays a key role in the transition from primary to secondary metabolites, and its activity increases in response to low nutrient levels, light exposure (through its effect on phytochrome), and infections caused by pathogens (Mierziak *et al.*, 2014; Deng & Lu, 2017; Misra *et al.*, 2023).

Under conditions of prolonged exposure to high concentrations of UV-B radiation (greater than 1 $\mu mol \cdot m^{-2} \cdot s^{-2}$), the synthesis and accumulation of phenolic compounds in the cells increases (Takahashi & Badger, 2011; Del Valle *et al.*, 2020); where they play an important role as non-enzymatic antioxidants mitigating the deleterious effects caused by reactive oxygen species (ROS) that are generated under conditions of high radiation (Hu *et al.*, 1995). Among the most important damage caused by oxidative stress from ROS at the intracellular level are the oxidation of nucleic acids and the denaturation of proteins and membrane lipids (Takahashi & Badger, 2011; Chen *et al.*, 2022). The production of ROS in chloroplasts induces photooxidation and photoinhibition of the components of the photosynthetic complex and can affect the production and stability of the Rubisco enzyme (Morales *et al.*, 2009; Mierziak *et al.*, 2014; Zhou *et al.*, 2019; Del Valle *et al.*, 2020; Kim *et al.*, 2021; Cesarino *et al.*, 2022; Misra *et al.*, 2023).

The presence of phenolic compounds in all the groups of terrestrial plants suggests that their synthesis and accumulation were key in the colonization of terrestrial ecosystems (Tohge *et al.*, 2013; Mierziak *et al.*, 2014; Mouradov & Spangenberg, 2014). In addition to these important benefits, flavonoids also compensate for soil nutrient deficiencies, by interacting with beneficial rhizosphere organisms that can increase plant defenses against pathogen attacks through the synthesis of phytoalexins (Mierziak *et al.*, 2014; Misra *et al.*, 2023; Wang *et al.*, 2025).

Flavonoids represent one of the most abundant and diverse groups of secondary metabolites in plants (de Rijke *et al.*, 2006). Both flavonoids and triterpenoids have been isolated from aerial organs of *Polylepis incana* (Catalano *et al.*, 1995); yet in the case of *P. australis*, the main secondary metabolites isolated were oleanane and ursane-type of triterpenoids (Lampasona *et al.*, 1998). In the case of *P. sericea*, no phytochemical studies have been conducted to date; however, we are convinced that both the synthesis and accumulation of phenolic compounds have played an important role in its tolerance to the high radiation coupled with low-temperature stress of the high elevation Andean páramo conditions.

In mountain ecosystems radiation levels increase as air temperatures decrease along elevation gradients (Cabrol *et al.*, 2014). Therefore, it is feasible to assume that these variations induce anatomical and phytochemical changes in the leaf tissues of *Polylepis sericea* along its altitudinal distribution gradient in the Venezuelan Andes, due a phenotypic and phytochemical plasticity that has facilitated its occurrence across a broad elevation gradient above the continuous forest line.

To test this hypothesis, we propose the following objectives:

1. Asses the foliar plasticity of *Polylepis sericea* along its altitudinal distribution gradient in the Sierra Nevada National Park.
2. Determine the location, composition, and concentration of phenolic compounds in mature leaves of *P. sericea*, collected from populations located at the lower, middle, and upper limits of its altitudinal distribution gradient in this National Park.
3. Establish whether variations in the foliar structure of this species are related to changes in the composition and concentration of the isolated phenolic compounds.

METHODS

| Study Site Location and Climate

Polylepis forests typically grow on rocky substrates formed by large stone blocks on steep mid-to-lower mountain slopes (Arnal, 1983). This study was conducted in the northern area of the Sierra Nevada National Park, located within the Cordillera de Mérida, in the Venezuelan Andes. Here, we established an elevation gradient following the route of the Mukumbarí Cable Car system in the State of Mérida, parallel to the stations La Aguada (3,320 m a.s.l.) and Loma Redonda (4,050 m a.s.l.), and selected four forests along this gradient, based on the species distribution map by Arnal *et al.* (2014) in the Cordillera de Mérida. The first forest, was situated at the lowest limit of this gradient, at 3,549 m a.s.l., approximately 30 m from La Fría Lake, the second was situated at 3,836 m a.s.l, facing Los Anteojos Lake, the third at 4,087 m a.s.l on the slope facing Loma Redonda Cable Car Station, and the fourth at 4,223 m, located on the site known as El Alto de La Cruz, behind the Loma Redonda Station at the base of Pico El Toro (Fig. 1). Geographic coordinates and elevation data were recorded using a Garmin GPSmap 60CSx device (Table 1).

The Venezuelan Andes are marked by annual isotherms and sharp daily temperature fluctuations, ranging from 10–20°C in a 24-hour cycle. These diurnal variations exceed monthly averages, with an estimated temperature lapse rate of 0.6°C per 100 m (Sarmiento, 1986). At La Aguada, mean annual temperatures range between 7–10°C (Sarmiento, 1986; Ely *et al.*, 2011),

TABLE 1 Location of the *Polylepis sericea* forests selected for the collection of plant samples along the Mukumbarí Cable Car System, in the State of Mérida, Venezuela. UTM Zone: 19 N(*). Data taken from Arnal et al. (2014).

Reference point	Location	W	N	Altitude (m.a.s.l.)	Perimeter* (m)
VEN_Sie_083	La Fría	269867	945520	3,549	5,110
VEN_Sie_081	Los Anteojos	271832	944834	3,836	8,046
VEN_Sie_077	Loma Redonda	271180	944275	4,087	565
VEN_Sie_076	Alto de La Cruz	271293	943627	4,223	1,012

but on clear days temperatures can easily rise above 22°C and drop to -0.68°C at night (Ely *et al.*, 2011). At 3,550 m, monthly temperature variations are usually below 2°C (Sarmiento, 1986), although daily oscillations may reach 30°C (Monasterio & Reyes, 1980). At 4,050 m (Loma Redonda), mean annual temperatures range from 5.8°C to 1.4°C; above this, the annual average may fall to 2°C, with minimums close to -2.5°C (Monasterio & Reyes, 1980).

At the lower limit of this gradient, the climate at La Aguada (3,320 m a.s.l.) is humid year-round, with an annual precipitation of 1,550 mm. Above 4,000 m, annual precipitations diminish from 1,200 mm on humid slopes, to 200 mm on dry ones like Pico El Toro (4,223 m a.s.l.) (Ataroff & Sarmiento, 2004). Nevertheless, we assume these values may have increased in recent years due to greater overall rainfall and the absence of a distinct dry season during the past seven years (Ely, personal communication).

| Vegetation

According to Delgado (2015), these forests may harbor as many as 127 species, distributed across 53 families and 97 genera. The main families in the Sierra Nevada *Polylepis* forests are Asteraceae (22 species), followed by Rosaceae (12 species), and both Scrophulariaceae and Poaceae with 6 species each, (León, 1991). The *P. sericea* forests near the Loma Redonda station (4,087 m a.s.l.) exhibit three distinct vegetation strata: a tree stratum (3–5 m height) dominated by *Polylepis sericea* and *Gynoxys moritziana*; a sparse shrub stratum, composed mostly by *Libanothamnus neerifolia*, *Ribes canescens*, *Senecio greenmanianus* and prostrate shrubs such as *Hesperomeles pernettioides*, and a herbaceous stratum composed by *Senecio formosus*, *Castilleja fissifolia*, *Senecio longepenicillatus* and *Polypodium rigescens*. Surrounding vegetation corresponds to the desert páramo, typically observed from 4,200 m a.s.l. onward, composed mainly by giant rosettes from the genus *Espeletia*, acaulescent, miniature rosettes (*Hypochoeris*, *Calandrinia*, *Acaulemalva*), and dwarf sclerophyllous shrubs (*Hypericum*, *Senecio*, *Draba*). The lower stratum (0–5 cm) comprises cushion-forming herbaceous species of the genera *Arenaria*, *Acaena*, *Azorella*, and *Aciachne* (Ataroff & Sarmiento, 2004).

| Morphological Traits of *Polylepis sericea*

Trees of *P. sericea* range from 1.5 to 4.0 m high and are easily recognizable due to their twisted trunks and branches (Fig. 1A, B), which are covered by a multi-layered, exfoliating bark (rhytidome) of reddish-brown tones. Leaves are alternate, stipulate, compound, and imparipinnate, with 7 leaflets of obtuse-rounded apices and asymmetrically cordate bases (Fig. 1C-E). Leaflet textures range from chartaceous to coriaceous. The adaxial surface is glabrescent and matte green, while the abaxial surface is dull green and covered with a dense silvery-gray indumentum (Fig. 1E).

| Sampling and laboratory studies

Leaves used to determine Specific Leaf Area (SLA) and used for anatomical preparations consisted of fully expanded, healthy, mature leaves. These were collected from n=3-5 trees randomly sampled in each of the four forests selected across the elevation gradient (3,549, 3,836, 4,087 and 4,223 m a.s.l.). In each case, we collected n=3 leaves per tree, per elevation, which generated a sample of n=9-15 leaflets per elevation. Both studies were conducted at the Plant Anatomy Laboratory of the Botanical Institute of Mérida, Faculty of Sciences, University of the Andes.

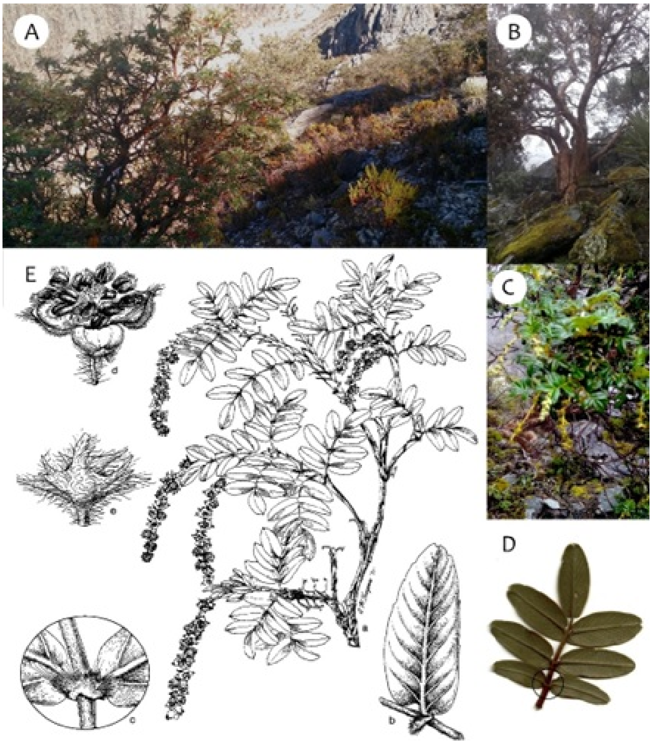

FIGURE 1 General features of Polylepis sericea, based on individuals growing in the National Park Sierra Nevada de Mérida. (A) *P. sericea* forest located at 4,223 m a.s.l. (B) *P. sericea* tree of the forest located near the Loma Redonda cable car station at 4,087 m a.s.l. The reddish, scaly bark (rhytidome), characteristic of the genus *Polylepis* may be observed. (C) Expanded, compound leaf of a *P. sericea* tree in bloom. (D) Leaves of *P. sericea* preserved in F.A.A. (E) Botanical illustration of *P. sericea* in which taxonomic characters may be appreciated. The ring of glandular trichomes, proper of *P. sericea* are here noticeable, as well as the leaflet insertion point. Picture modified from the original drawing made by Simpson (1979).

| Specific Leaf Area estimates

Leaf samples used to determine SLA were immediately wrapped in moistened absorbent paper as soon as they were removed from the branches, stored in sealed, labeled plastic bags and refrigerated at 12°C for no longer than 48 hours. For each SLA calculation, we measured 5–7 randomly selected from the 9-5 leaf sample collected at each elevation. SLA was determined as:

$$SLA = \frac{\textit{Fresh leaf area}(cm^2)}{\textit{Dry mass}(g)}$$

Leaves were scanned using an Epson 1000XL scanner at 300 dpi resolution and processed using IrfanView 4.2. Leaf areas (in pixels) were converted to cm² using ImageJ 1.52a (Schneider *et al.*, 2012). Samples were oven-dried at 50°C for 96 hours in a Standard Memmert electric oven until they reached constant weight. Dry weights were recorded using a Mettler PN-163 analytical balance. Due to the limited sample size, each leaf was measured 30 times, yielding 30 pseudo-replicates per leaf, analyzed using Primer v.6.1.15 and its PERMANOVA+ 1.0.5 extension.

| Anatomical Characterization and Phenolic Compound Detection

Anatomical specimens also consisted of hydrated, fully expanded leaves fixed in FAA (70% ethanol: glacial acetic acid: formalin), following the fixation solution proposed by Johansen (1940), taking care of removing the leaflets from the main axis before immersing them in the fixating solution. After 48 hours, leaves were removed from the solution and rinsed under running water and left for 24 hours soaking in water. During this period, three changes of water were made and the leaves were rinsed thoroughly to ensure the removal of the fixating solutions. Free-hand transverse sections of 5–7 leaflets per location were used for anatomical characterization and phenolic compound detection. Anatomical sections were cleared with 5% sodium hypochlorite for 15 minutes, rinsed thoroughly in distilled water (7–10 min) and immersed during 1 min in 10% acetic acid to neutralize residual bleach, and rinsed again with abundant water before placing them in 50% ethanol for 5 minutes to remove the air of the leaf tissues.

Leaflet sections used to study leaflet structure and tissue characteristics were stained with the differential double staining of alcian blue/safranin 7:3 (Luque *et al.*, 1996). The anatomical traits examined in this comparative study were: lamina thickness (measured at the leaflets' mid-region), cuticle thickness, height and width of the adaxial epidermal cells, adaxial hypodermal cells, palisade parenchyma cells, and the total percentage of palisade vs spongy parenchyma.

Detection, localization, and qualitative estimation (visual) of polyphenolic compounds (tannins and flavonoids) accumulated in foliar tissues were conducted using iron-based stains: ferric chloride ($FeCl_3$) and ferrous sulfate ($FeSO_4$), according to protocols of Johansen (1940). These stains evidence the presence of polyhydroxylated phenolic compounds in foliar tissues (Kennedy & Powell, 1985), The intensity of the coloration is proportional to the content of phenolic compounds stored in foliar tissues. Prior to staining, freehand leaf sections were immersed in 50% ethanol for 5 minutes. Samples were divided equally: half stained with 10% $FeCl_3$ for 10 minutes, and the remainder with Schreder's reagent (2% $FeCl_4$, 0.25% acetic acid, 10% formaldehyde) during a period of 2 hours. Following staining, all sections were rinsed in 5% hydrochloric acid for 5 minutes to remove excess iron and reduce chelating interference, then washed thoroughly 2-3 times with distilled water.

Stained sections were observed using a Leica EZ4 binocular stereoscope. Selected high-quality preparations were mounted in 50% aqueous glycerin and examined under a Leica M822 compound microscope. Micrographs were captured using a Ken-A-Vision Pupil Cam camera. For each slide, 15 fields were examined, and 30 measurements were recorded per anatomical trait. Micrographic analysis was conducted using ImageJ 1.52a, calibrated against microscope images with a 1±0.01 cm scale across magnifications (4x, 10x, 40x, and 100x).

| Statistical analysis

Statistical analyses were performed using Primer v.6.1.15 and its PERMANOVA+ 1.0.5 extension, alongside R v.1.1.423 (R Core Team, 2024). A nested PERMANOVA was applied with leaf/elevation as the factor, using 9,999 permutations and a

TABLE 2 Calibration curve using increasing concentrations of gallic acid, prepared from a stock solution of gallic acid at 3×10 M, 20% (w/v) NA_2CO_3 , and Folin-Ciocalteu reagent at 1N(*) Reaction blank.

Value of the calibration curve	Gallic (mL)	Water (mL)	Folin (mL)	NA_2CO_3 (mL)
0*	0	0	0.25	0.25
1	0.1	1.4	0.25	0.25
2	0.2	1.3	0.25	0.25
3	0.3	1.2	0.25	0.25
4	0.4	1.1	0.25	0.25
5	0.5	1.0	0.25	0.25
6	0.6	0.9	0.25	0.25
7	0.7	0.8	0.25	0.25

significance threshold of $\alpha \leq 0.05$. Principal Coordinates Analysis (PCoA) was performed based on 150 pseudoreplicates per elevation for each of the anatomical traits analyzed in this comparative study.

To determine whether anatomical trait values are clustered by elevation, a Principal Coordinate Analysis (PCoA) was conducted using the full dataset. Before analysis, data were normalized and standardized using Euclidean distance metrics.

| Phytochemical Analysis. Determination of Total Phenolic Content Using the Folin–Ciocalteu Method and the sulfuric vanillin test.

Phytochemical studies were performed at the Laboratory of Natural Products, at the Faculty of Sciences of the University of the Andes. Phenolic compound extractions with methanol were conducted using healthy, fully expanded, exposed canopy leaves randomly sampled from different trees until a composite 500g sample was obtained from 15-20 individuals. For this reason, we were only able to process samples from three out of the four forests selected across the elevation gradient, located at the lower (3,549 m a.s.l), middle (3,836 a.s.l.) and upper (4,223 m a.s.l.) limit of this gradient, which corresponded to this species' distribution range within the Sierra Nevada National Park. The reason that we excluded the forest located at 4,080 m a.s.l. was the limited number of individuals that composed this very reduced forest (only 8 trees). Therefore, the composite sample would have been biased. The other, more powerful reason that we took into consideration when we excluded this forest from the phytochemical studies was that the evergreen leaves produced by high elevation trees (including *P. servicea*) have slow development (Ely, personal communication); therefore, it would have been unethical to harvest a large, composite sample from such a limited number of trees.

Following collection, samples were oven-dried at 50°C for four days; then thoroughly ground, weighed, and subjected to Soxhlet methanolic extraction using 250g of the dry sample per elevation. Methanol, while effective in extracting flavonoid-type polyphenols, is non-specific; therefore, extracts were treated as complex mixtures of methanol-soluble compounds (Julkunen-Tiitto, 1985). Percentage contributions of phenolic compounds were determined relative to the initial dry weight before extraction. Methanolic extracts underwent full-range spectrophotometric scanning (210–710 nm) and the quantification of phenolic contents were assayed through the Folin–Ciocalteu method, that uses gallic acid as reference (Table 2).

The Folin–Ciocalteu reagent contains tungsten and molybdenum ions that form blue chromogens upon reduction by dihydroxy-substituted polyphenols such as gallic acid (Gutiérrez *et al.*, 2008). Phenolic content was quantified through the Folin–Ciocalteu spectrophotometric assay, based on the Beer–Bouguer–Lambert law: $Abs = C \cdot l \cdot \epsilon$, where Abs represents absorbance, C is the concentration of the analyte, l is the optical path length (1 cm), and ϵ is the molar extinction coefficient ($M^{-1} \cdot cm^{-1}$). The relationship displays a linear behavior (Y = mX + b) with a zero intercept, allowing accurate quantification across standard ranges.

Values were expressed as gallic acid equivalents (GAE) through interpolation from a standard calibration curve (Fig. 2), developed using increasing dilutions of gallic acid (Table 2). Gallic acid was initially dissolved in distilled water, followed by the addition of Folin–Ciocalteu reagent. The solution was mixed, incubated for 5 min at ambient temperature, and treated with Na_2CO_3. After 30 min of reaction time, the final volume was adjusted to 4 mL with distilled water. Absorbance was measured at 754 nm (peak wavelength for maximum absorbance) on a Shimadzu UV-1240 spectrophotometer, maintaining readings within the range of 0–3 Abs units. Linear correlation parameters were derived from the calibration curve equation (Fig. 2).

Total phenolic content was expressed in gallic acid equivalents (GAE) per g of dry weight (DW) of plant material, inferred from the gallic acid calibration curve (Fig. 2). This represents the soluble fraction of total polyphenols. Polyphenol content was calculated as the sum of GAE and the dry weight of the precipitated fraction. To distinguish extract components in leaves collected at different elevations, phenolic content was determined using thin-layer chromatography (TLC) on silica gel, with a mobile phase of ethyl acetate: methanol (2:1), and plates developed with sulfuric vanillin reagent using Z234214 Sigma-Aldrich® TLC Plates. The plates were observed under UV lamps at 254 and 366 nm to detect relevant compounds and developed using sulfuric vanillin reagent (100 mL of 1% vanillin in ethanol + 2 mL of 98% sulfuric acid), applied by spraying and briefly heating the plates. When vanillin is protonated in acidic solution, it has a weak electrophilic radical that reacts with the ring of many flavonoid compounds at positions 6 or 8. This intermediate compound dehydrates and forms a product that is visually perceived in the range of light red to deep cherry red, which indicates a positive reaction. In the case of flavonoids, hydroxyl groups at C-5 and C-7 do not react effectively with vanillin when C-4 contains a carbonyl group or C-7 bears O-glycosylations. Therefore, this reaction is applied as a specific test to detect aglycone flavanols; although, interference by dihydrochalcones and anthocyanins may occur (Sarkar & Howarth, 1976).

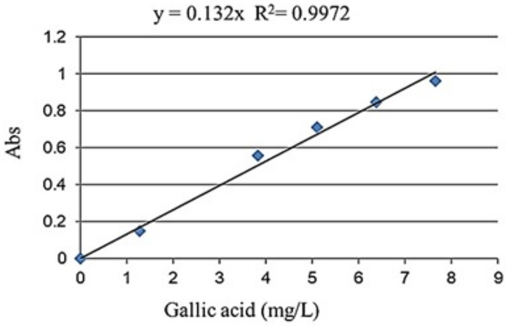

FIGURE 2 Calibration curve of phenolic content based on increasing dilutions of gallic acid. Correlation coefficient derived from the lineal equation is indicated above.

RESULTS

| Altitudinal Variation in Forest Structure and Conserved Leaf Traits in *Polylepis* Forests

Along the altitudinal gradient ranging from 3,549 to 4,223 m a.s.l., water availability decreases with increasing elevation, creating a hydric gradient. Forests located at the lower boundary of this gradient (3,549 m a.s.l.), near Laguna La Fría and Laguna Los Anteojos (3,836 m a.s.l.) occupy muddy soils rich in organic leaf litter. These *P. sericea* forests exhibit a well-defined three-layered vertical stratification, with canopy trees that reach heights of 5–7 meters and display a physiognomy like that of contiguous páramo forests at the same elevation (Fig. 3 A, B).

Conversely, *P. sericea* forest islands situated at elevations above 4,000 m a.s.l. grow on steep, rocky slopes, and display shorter and more open canopies (Fig. 3C). At 4,223 m a.s.l. (the upper limit of gradient) *P. sericea* forms monospecific shrublands that rarely exceed 1.5–2.0 m in height. At a distance, these stands composed of stunted, dwarf-like trees visually resemble high-Andean páramo shrubs (Fig. 3D) and contrast markedly with the conspecific individuals occurring at lower elevations.

Although microenvironmental characteristics distinctly influence overall plant size, leaf size and specific leaf area (SLA) remained unaffected, as the latter did not vary significantly across forest sites at different elevations (Table 3).

FIGURE 3 *Polylepis sericea* forests sampled along the altitude gradient of the Mukumbarí Cable Car System in the National Park Sierra Nevada, in Mérida, Venezuela. (A, B) Forest located adjacent to La Fría Lake (3,549 m a.s.l.), (C, D) Forest located adjacent to Los Anteojos Lake (3,836 m a.s.l), (E) Forest located at 0.5 Km from Loma Redonda (4,087 m a.s.l.) y (F, G) Shrub-like P. sericea forest located in the Alto de La Cruz (4,223 m a.s.l.)

Comparative anatomical analysis confirmed that *P. sericea* leaflets are composed of approximately 80% chlorenchyma (Fig. 4, Table 3), a trait indicative of a heliophilous leaf architecture, adapted to the intense radiation levels of high-Andean páramo environments.

Surprisingly, neither leaf blade thickness nor the proportion of palisade parenchyma vs spongy parenchyma (assessed via transverse sections) showed significant variations across the altitudinal gradient. Likewise, cuticle thickness, as well as the thickness and width of adaxial epidermal cells, hypodermal cells, and palisade parenchyma, remained statistically consistent

TABLE 3 Specific leaf area, leaf blade thickness, and percentage (%) of palisade vs spongy parenchyma in *P. sericea* leaf samples from four forests located along the altitudinal gradient. No significant differences were found between the samples ($\alpha \leq 0.05$)

Anatomical trait	La Fría (3,549)	Los Anteojos (3,836)	Loma Redonda (4,087)	Alto de La Cruz (4,223)
Specific leaf area (cm2/g)	64.15	85.74	87.19	71.68
Leaflet blade thicknes (μm)	278,989	282,817	302,751	295,132
% Palisade parenchyma	60,76	63,15	62,36	63,77
% Spongy parenchyma	18,03	14,58	15,41	16,85

TABLE 4 Mean values (±Standard errors) of the anatomical traits studied in transverse leaflet sections of *P. sericea* samples collected from four forests occurring across the 674 m altitude gradient in the National Park Sierra Nevada de Mérida. Groups with significant differences were assigned different letters. Parameters without letters showed no significant differences ($\alpha \leq 0.05$).

Anatomical trait	La Fría 3,549	Los Anteojos 3,836	Loma Redonda 4,087	Alto de La Cruz 4,223
Cuticle thickness μm	8.07 ±0.66	6,76 ±0,55	7.13 ±0.58	8.91 ±0.73
Adaxial epidermal cell height μm	13.96 ±1.14a	14,53 ±1,19a	16.60 ±1.36	13.93 ±1.41a
Adaxial epidermal cell width μm	15.69 ±1.28	15,19 ±0,59	16.86 ±1.38	17.62 ±1.44
Hypodermal cellcell height μm	23.30 ±1.91	24,70 ± 2,02	24.35 ±2.00	24.35±2.00
Hypodermal cell width μm μm	27.02 ±2.21	29,16 ± 2,39	29.74 ±2.43	27.80 ±2.28
Palisade parenchyma cell height μm	56.26±4.61	56,51 ±4,63	62.58 ±5.13	62.26 ±5.10
Palisade parenchyma cell width μm	10.10±0.83	9,43 ±0,77	11.22 ±0.92	10.38 ±0.85

across populations at varying elevations (Table 4). The only anatomical trait that differed minimally was the thickness of the adaxial epidermis, which was slightly higher in the leaves collected from the trees of the forest situated close to the Loma Redonda cable car station (4,087 m a.s.l.; Table 4).

Principal Coordinate Analysis (PCoA) was conducted using normalized data for all anatomical attributes evaluated, with Pearson correlation coefficients greater than 0.02 incorporated, except for adaxial epidermal cell height, as it was the only variable showing significant differences among elevations. However, according to this analysis, no clear segregation of anatomical groups was evident across the altitudinal gradient (Fig. 5); since as we mentioned previously, the only exception observed was a very slight increase in the height of the adaxial epidermal cells in leaves the forest at 4,087 m a.s.l. (Loma Redonda Station).

The results of this comparative anatomical assay revealed that contrary to our initial assumptions, *P. sericea* exhibits highly conserved leaf traits across a 674 m elevation gradient in the National Park Sierra Nevada.

| Localization of Phenolic Compounds and Phytochemical Studies

Qualitative tests carried out with ferric compound stains confirmed the presence of polyphenolic compounds in all the chlorenchyma cells (Fig. 6 A-H), primarily associated with the chloroplasts (Fig. 6), and at higher concentration in the palisade parenchyma cells, which harbor the greatest number of chloroplasts within the chlorenchyma tissues (Fig. 6 I, J). The intensity of the stains did not increase linearly along the altitudinal gradient; however, preparations corresponding to the samples collected at

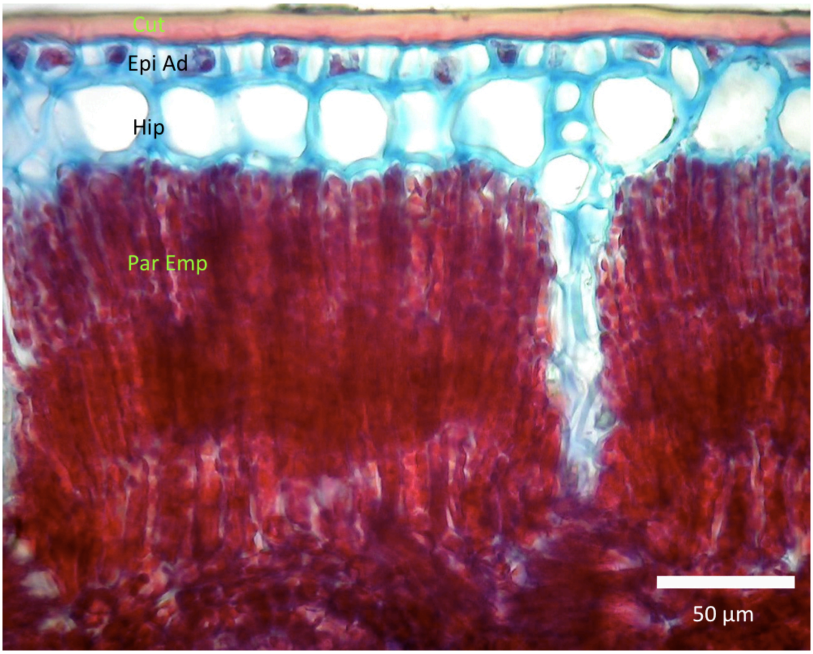

FIGURE 4 Free-hand section of a leaflet of *P. sericea* stained with Alcian Blue/Safranine preparation. Picture taken using 40x magnification. Leaf sample collected from the forest adjacent to Los Anteojos Lake (3,836 m a.s.l.), located in the National Park Sierra Nevada. Cut: Cuticle, Ad epi.: Adaxial epidermis, Hip: Hipodermis; Pal par: Palisade parenchyma.

4,223 m a.s.l. (Fig. 6 G, H, J) exhibited a notably darker coloration compared to the other three forests located at lower elevations (Fig. 6 A-F). These differences became more evident when we compared the preparations of samples collected from the trees situated at the extremes of the 674 m altitudinal gradient (3,549 and 4,223 m a.s.l., respectively, Fig. 6 J, K). The parenchymal tissues of the leaf samples collected in the forest located at the lower limit of the gradient, adjacent to Laguna La Fría (3,549 m a.s.l., Fig. 6 I), presented a matte green coloration that contrasted noticeably with the dark brown to black coloration (resulting from the sequestration of a higher concentration of iron ions) observed in the chlorenchyma cells of leaf samples collected at the upper limit of the gradient (4,223 m a.s.l., Figure 6 J). These differences evidence an increase in the content of condensed polyphenolic compounds in the trees of the forest exposed to higher levels of radiation along this elevation gradient.

The UV spectrophotometric scans of methanolic leaf filtrates obtained from the lower, middle, and upper limits of the 674 m altitudinal gradient displayed a very similar pattern, consisting of a high absorbance peak at 212 nm. It is noteworthy that this single-peak pattern was not the expected typical two-peak pattern, indicative of the presence of flavonoids in the extract. These discrepancies suggest the absence of the double C2-C3 bonds in the flavonoid skeleton that are usually related with catechins and/or flavanones. Moreover, we must also consider that the absorption produced by the ring B (Band I) may superimpose with the absorption spectrum generated by the ring A (Band II) (Mabry *et al.*, 1970).

However, the UV absorption spectrum corresponding to samples from the forest located at 4,223 m a.s.l. revealed an additional absorbance peak at 255 nm, evidence of a greater diversity of aromatic compounds in the filtrate. This compound could not be detected by quantification tests using vanillin–sulfuric acid, since the latter is unable to detect compounds with double bonds at the C3 position, such as flavones (Sarkar & Howarth, 1976). According to Mabry et al. (1979), the Band II absorbance peak of many flavones with oxidized A rings—corresponds to compounds such as 7-hydroxy-4 keto-flavonoids—that

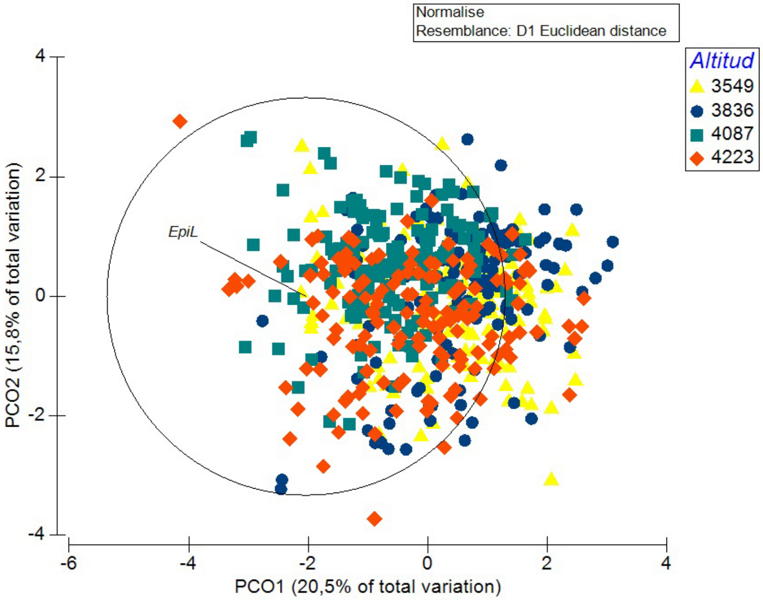

FIGURE 5 Principal Coordinate Analysis (PCoA) based on the anatomical traits measured in the leaf samples collected in three forests of *P. sericea* sampled along the 3,459-4,223 m elevation gradient in the National Park Sierra Nevada de Mérida. From each sample, 30 pseudo-replicates were generated using 9-15 different samples per elevation, resulting in 270-450 measurements per trait, per elevation.

fall in the 250–280 nm range.

Nevertheless, if the flavonols or flavones are methylated or glycosylated at their 3, 5, or 4' hydroxyl groups, hypsochromic shifts (absorbance peaks at shorter wavelengths) can be observed in their UV spectra. In plants, flavonoids with O-glycosidic substitutions are more common than those with C-glycosidic ones (Rijke et al., 2006), which is why it is not unusual to find UV spectra with absorbance peaks below 250 nm also associated with Band II. The UV spectrum of many flavones and flavonols is characterized by the presence of an absorbance peak between 240–280 nm, that is attributed to their A ring, which—when methylated or glycosylated—produces absorbance peaks at shorter wavelengths (Mabry *et al.*, 1970). In the UV spectrum, flavonoids are characterized by absorption peaks in the range of 240–280 nm of the ring A, commonly denoted as of Band II, whereas peaks in the range of 300–380 nm belong to Band I of the ring. Based on the above, we presume that the methanolic filtrates are polyphenolic flavonoid-like compounds, given that they all share a strongly oxidized aromatic ring, with absorbance peaks around 212 nm. Nevertheless, the identity of these polyphenolic compounds can only be verified with more precision through high-performance tests such as liquid chromatography (HPLC), that enables the separation and identification of the components obtained in the extracts using spectroscopic techniques.

Once we had obtained the absorbance pattern of the extracts, we proceeded to determine the concentration of hydroxyl-substituted polyphenols using the Folin–Ciocalteau reagent assay (Table 6), employing a gallic acid calibration curve, with the purpose of determining the concentration of hydroxyl-substituted polyphenolic compounds required to reduce the metal ions present in the Folin–Ciocalteau reagent. This concentration was calculated by interpolating the absorbance values of each extract. It is important to note that it was necessary to dilute all methanolic filtrates obtained (Table 6), after which we proceeded to multiply the absorbance values by the corresponding dilution factor for each sample, and then determine the grams of polyphenols in solution (Table 7).

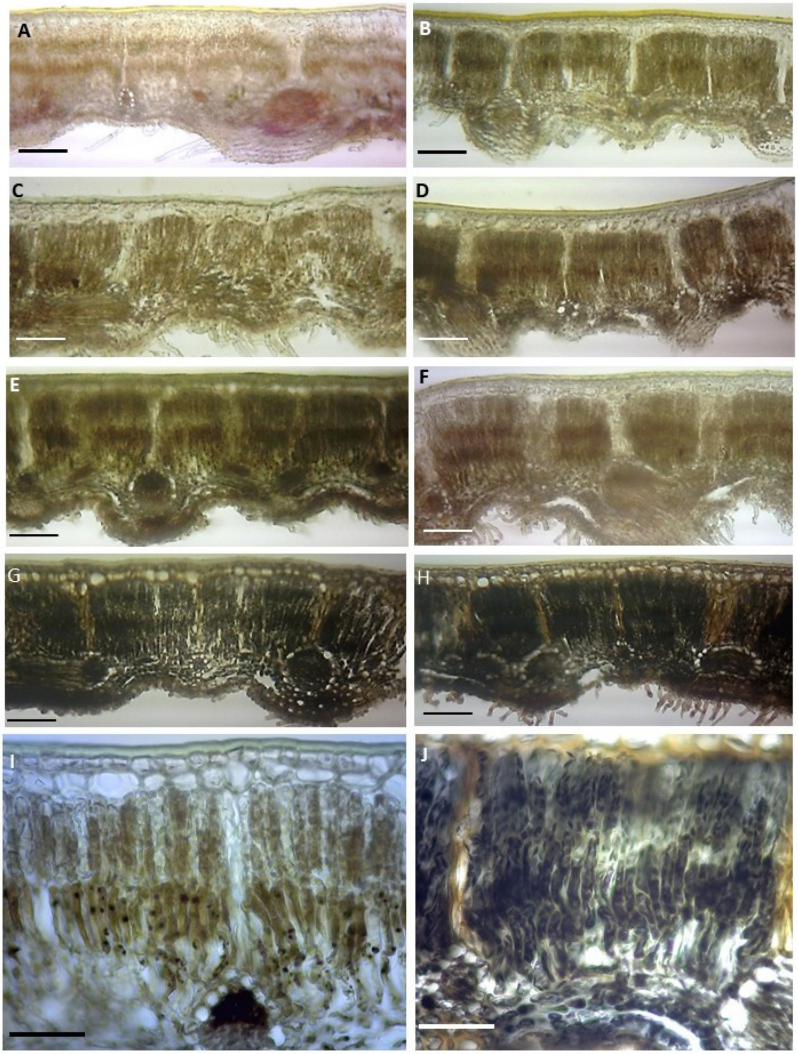

FIGURE 6 Free-hand, transverse sections of *P. sericea* leaflets stained with $FeCl_3$ (left) and $FeCl_4$ (right). Notice in both cases the positive reaction of the ferric reagents in the chlorenchyma cells, particularly in the palisade parenchyma cells. A, B: Samples collected at 3,549 m a.s.l. C, D: Samples collected at 3,836 m a.s.l. E, F: Samples collected at 4,087 m a.s.l. G, H: Samples collected at 4,223 m a.s.l. Pictures taken with 10X magnification. Details of the palisade chlorenchyma cells stained with FeSO4. Pictures taken with 40X magnification. I: Sample collected at 3,549 m a.s.l. J: Sample collected at 4,223 m a.s.l.

TABLE 5 Absorbance calibration curve, estimated with increasing concentrations of gallic acid and the leaf tissue samples collected from the foliage tissues collected in the forests located in the lower, middle and upper limit of the 674 altitudinal gradient. (*) Phenol concentrations were determined based on the final concentration volume of the spectrophotometer measurement cuvette, that had a capacity of 4 mL.

Reference point	[Phenols] (mg/L) in the cuvette*	Abs (754 nm)
0	0	0
1	1.276	0.1514
2	3.828	0.4187
3	5.104	0.577
4	6.38	0.7108
5	7.655	0.8452
6	8.931	0.9601
La Fría (3,459 m.s.s.l.)	3.178	0.4195
Los Anteojos (3,836 m.s.s.l.)	0.97	0.128
Alto de La Cruz (4,223 m.s.s.l.)	1.721	0.2272

TABLE 6 Percentages of polyphenols in *P. sericea* leaves determined through methanolic extractions derived from the foliage tissues collected in the forests located in the lower, middle and upper limit of the 674 altitudinal gradient. The contents of polyphenols (g) equivalent to gallic acid that reacted with Folin-Ciocalteu reagent were calculated based on the dry weight of the extracts obtained through rotary evaporation.

Location/altitude (m a.s.l.)	% Methanolic extract of foliar tissues	Polyphenols (g) equiv. to gallic acid	% Foliar polyphenols
La Fría (3,549)	17.423	1.986	0.17
Los Anteojos (3,836)	13.993	0.096	0.044
Alto de La Cruz (4,223)	18.064	0.172	0.078

Contrary to our hypothesis, the percentage of polyphenols did not increase linearly across the 674 m gradient. According to this last analysis, the highest content of polyphenols obtained from the methanolic extracts corresponded to the samples collected from the *P. sericea* forest located at lower limit of this gradient (3,549 m a.s.l.), followed by the samples from forests located at the upper and middle elevations (4,223 and 3,836 m a.s.l., respectively) (tables 6 and 7).

Qualitative staining with ferric reagents (Figure 6) evidenced the highest accumulation of polyphenolic compounds in the chlorenchyma tissues of the samples collected at the highest elevation 4,223 m asl; yet the highest percentage of total hydroxyl-substituted polyphenols determined via gallic acid (Table 7) corresponded to the sample collected of the lowest elevation (3,549 m a.s.l.). These discrepancies suggest that the leaves collected from forests located at the extremes of this 643 m gradient in both cases store high concentrations polyphenolic compounds, but of different compositions. The extract obtained from the trees located at 3,549 m a.s.l. suggests a tendency to store aromatic-structure compounds devoid of steric hindrances, that enable them to react with the Folin–Ciocalteu reagent—unlike the extract from the forest located at 4,223 m a.s.l. Nevertheless, both analyses proved that the pattern of polyphenolic compound accumulation did not increase linearly across this 643 m altitudinal gradient. Moreover, we must also consider in our results that although methanol is the most effective solvent for extracting polyphenolic compounds, whether tannins or flavonoids, like any organic solvent, it can interact with secondary metabolites present in the solution and facilitate their dissolution during the extraction process, potentially altering the final extraction product. By generating a complex mixture of polyphenolic compounds in each extract, there is no direct way of determining the precise composition of these extracts, and it may be necessary to use separation, purification (in some cases crystallization) methods to achieve an accurate identification of the polyphenolic compounds. Since our objective was to quantify the polyphenolic compounds, we proceeded to explore the differences in polyphenol composition through an estimation using vanillin–sulfuric acid.

Differences in the polyphenol composition were assayed comparing the band pattern of the three extracts on a TLC on silica gel using vanillin–sulfuric acid. According to this test, only the sample collected at lowest elevation (3,459 m a.s.l.) exhibited a light, cherry red coloration, indicative of the presence of phenolic compounds from the flavonol group (flavon-3-ol, see methodology), that confirmed the presence of glycosylated or oxidized flavonols at carbon positions 5 and 7. Now, if we associate these results of the vanillin–sulfuric acid test with the quantification of phenolic compounds using gallic acid as a reference (tables 6 and 7), we can affirm that the highest concentration of foliar polyphenolic compounds reacting to the Folin–Ciocalteu reagent corresponded to the forest located at 3,459 m a.s.l., and not to the one located at the upper limit of the gradient (4,223 m a.s.l.). Based on these results, we must reject the hypothesis that the phenolic content accumulated in the leaves of *P. sericea* increases linearly across its distribution gradient in the National Park Sierra Nevada.

Now taking into account all phytochemical tests conducted—that is, the iron-based stains on anatomical leaf preparations (Figure 6), the UV absorption spectra, the reduction reaction using the Folin–Ciocaltaeu reagent (Table 6), and the dehydration reaction using vanillin–sulfuric acid, which primarily depend on the presence of a phenol group with ortho (1,2) dihydroxy substitutions without steric hindrances—it is reasonable to assume that the majority portion of the polyphenolic compounds present in the *P. sericea* extracts possess a structure similar to hydroxyl-substituted flavonoids, with a composition that varies amongst individuals growing at different elevations across this altitudinal gradient. Nevertheless, to more accurately determine the nature of these polyphenolic compounds, it is necessary to conduct future tests to confirm this assumption, using identification techniques such as tandem mass spectrometry, as suggested by de Rijke *et al.* (2006).

DISCUSSION

| Vegetation Structure of *Polylepis sericea* Forests along the altitude gradient

According to Arnal (1983), *Polylepis* forests are not characterized by an exclusive floristic composition; on the contrary, their composition is strongly influenced by the surrounding open páramo vegetation. Our sampling of *Polylepis sericea* forests across the 3,459–4,223 m a.s.l. gradient confirmed this assertion—particularly in the case of the forest located in the Alto de la Cruz (4,223 m a.s.l.), that lacks vertical stratification and where trees rarely exceed 1.5 meters in height and are surrounded by sclerophyllous shrubs, typical of the high-Andean open páramo.

This dwarf shrub-like forest contrasts strongly with the stratified *P. sericea* forests found at lower elevations along the altitudinal gradient (3,549, 3,836, and 4,087 m a.s.l.) that harbor other woody species such as *Gynoxys moritziana* and *Libanothamnus neerifolia*, as well as a relatively high diversity of forbs and bryophytes (Delgado, 2015). The low stature of the *P. sericea* trees located at 4,223 m as.s.l. and absence of other the woody species typically associated to this species at lower elevations, as well as the scarcity of surrounding vegetation are likely the result of the harsher environmental conditions at this elevation. Above 4,100 m a.s.l., the frequency of nighttime frosts increases and mean daytime temperatures decrease due to the intensified winds, despite the increase in levels of incident radiation, due to the diminished cloud cover. Other environmental constraints that limit plants' development above this elevation are the highly drained, rocky, oligotrophic soils (Azócar & Rada, 2006; Azócar & Monasterio, 1980). All of these adverse environmental conditions, particularly the intense winds have undoubtedly a severe effect on plants' overall development. Strong, continuous winds alter plants' general architecture; erect trunks and branches become prostrate or decumbent, and these modifications are typically accompanied by a greater investment in underground organs versus aerial ones (Gardiner *et al.*, 2017). These conditions also affect carbon assimilation and respiration rates and their effect may be appreciated in the limited development of the plants associated to these environments (Körner, 2007; Ma *et al.*, 2012; Li *et al.*, 2022; Liu *et al.*, 2024).

The harsher environmental conditions of the upper limit of this 674 m gradient undoubtedly have a negative impact on the physiognomy and floristic diversity of the *P. sericea* forests at situated at 4,220 a.s.l. in the Sierra Nevada National Park. This inference is supported by previous comparative studies concerning physiognomic and floristic differences in plant communities occurring across broad latitudinal and altitudinal gradients (Liu *et al.*, 2021); Momberg et al., 2021)(Momberg *et al.*, 2021). Nonetheless, the presence of *P. sericea* patches at 4,223 m a.s.l. demonstrates a notable resilience in this species that enables it to tolerate the hydric (Arnal, 1983; Azócar *et al.*, 2007), thermal (Goldstein *et al.*, 1994; Rada *et al.*, 1985; Azócar *et al.*, 2007; Rada *et al.*, 2011), and light stress (Azócar & Rada, 2006) induced by the environmental conditions of high elevation tropical Andean ecosystems.

| Anatomical studies

This comparative study involving specific leaf area (SLA) and the targeted anatomical leaf traits across the 3,549–4,223 m a.s.l. altitudinal gradient confirmed that *P. sericea* has a xeromorphic leaf structure, typical of plants associated to high elevation ecosystems (Ely *et al.*, 2005; Ely & Torres, 2003; Yang *et al.*, 2022). This study also revealed highly conserved leaf traits; neither SLA, nor the seven anatomical traits we analyzed varied significantly across the 674 m gradient, with exception of the minor increase in the height of adaxial epidermal cells in the leaves collected from the forest located a 4,080 m a.s.l. Increases in the levels of irradiance across altitudinal gradients have proven to modify leaf blade thickness and SLA and in *Nothofagus cunninghamii*, a sclerophyllous tree of Tasmania(Hovenden & Vander Schoor, 2005). Foliar plasticity, in terms of leaf or leaflet blade thickness, height of the epidermal cells and the proportion of palisade vs spongy parenchyma may also vary across altitudinal gradients, depending on plants' growth-form; according to an extensive, comparative study conducted by He *et al.* (2017), that involved 916 species exposed to similar environmental constraints (mainly temperature and precipitations), from both temperate and tropical forest units across a 3,700 km north-south latitudinal gradient in China. Liu *et al.* (2021) described similar results, using these same leaf traits in their comparative study involving 60 woody species across a 2,000 m elevation gradient in Tabai Mountain, China. However, foliar phenotypical and physiological plasticity in plants adapted to extreme environments may differ depending on the species phylogenetic group, according to the comparative study performed by Zhou *et al.* (2019) in *Dryas octopetala* var. *asiatica*, *Rhododendron confertissimum* and *Vaccinum uliginosum*, three sclerophyllous, perennial shrubs of the Chinese tundra growing at 2,028 m a.s.l. Nevertheless, our results suggest that this was not the case of *P. sericea*, given that we did not detect any significative changes in the seven leaf traits analyzed in this species across its altitudinal distribution gradient in the Sierra Nevada National Park, despite the changes in the environmental conditions across this 674 m gradient.

Phenotypic plasticity in leaf traits across altitudinal gradients in response to changing environmental conditions is generally more accentuated in populations of annual herbaceous plants compared with woody ones, as we may infer from the study conducted in *Arabidopsis arenosa* by Bertel *et al.* (2022) in the Austrian Alps. These authors found notable differences between ecotypes cultivated in Alpine foothills and those of Alpine summits. Outstanding differences concerned: an increase in the size of the leaf blade, lower trichome density, a higher proportion of palisade parenchyma vs spongy parenchyma, and chloroplasts containing larger starch granules as well as less grana stacking within the thylakoids. It is likely that herbaceous plants from temperate latitudes—with predominantly annual reproductive cycles—exhibit greater histological plasticity, compared to perennial forbs and woody plants adapted to extreme, high elevation ecosystems, where plants typically exhibit higher leaf durability (1-3 years) and share xeromorphic traits that facilitate their adaptation to these environments.

Our results contradict findings reported by Colmenares-Arteaga *et al.* (2005), whom reported a decrease in SLA, as well as taller adaxial epidermal cells in the individuals collected in the forest located in Loma Redonda (4,087 m a.s.l.). However, when we compared our results with those of Colmenares-Arteaga *et al.* (2005), we found evident discrepancies in the dimensions of the epidermal cell of both leaf surfaces. This raises concerns about the measurement methods used by these authors. Nevertheless, our statistical analyses also differed from those used by Colmenares-Arteaga *et al.* (2005), and these differences could also account for such discrepancies.

The lack of anatomical plasticity has previously been described in woody species from tropical Andean ecosystems such as *Chaetolepis lindeniana*, a sclerophyllous dwarf shrub from the páramos of Venezuela and Colombia that maintains a remarkably conserved anatomical structure across its broad altitudinal distribution range of 1,100 m (3,000–4,100 m a.s.l.) in the Venezuelan Andes, despite changes in leaf size and thickness (Ely & Torres, 2003). The absence of anatomical plasticity is not uncommon in plants subjected to extreme environmental conditions (Yang *et al.*, 2022; Liu *et al.*, 2024). Liu *et al.* (2024) evaluated phenotypic plasticity in woody and herbaceous plants from the Mongolian and Tibetan steppes. Their findings proved that species of the Tibetan steppes exhibited remarkably conserved leaf structures across broad altitudinal gradients; in contrast with those of the Mongolian plains, given the harsher environmental conditions of the Tibetan steppes, particularly at elevations of 4,500 m a.s.l. where plants are exposed to intense and prolonged radiation (including UV), as well as abrupt fluctuations in seasonal and daily temperatures. Leaf traits examined by these authors also included SLA, cuticle thickness, epidermal cell height, palisade parenchyma thickness, and the palisade/spongy parenchyma ratio. These findings suggest that under extreme conditions, morphological differences regarding size and vegetative development are not always associated with anatomical plasticity, as was the case in this study.

| Chelating Agents and altitudinal variation in UV-protective phenolic compounds in *Polylepis sericea*

The accumulation of polyphenolic chelating agents strongly associated with the photosynthetically active cells of the mesophyll in both palisade and spongy parenchyma cells confirm the adaptation of *P. sericea* to high-radiation conditions (Agati *et al.*, 2012). According to our results, the highest concentration of chelating compounds observed in chlorenchyma tissues corresponded to the individuals of *P. sericea* growing at 4,223 m a.s.l., which suggests that the synthesis and accumulation of condensed polyphenols in the foliar tissues of *P. sericea* are influenced by environmental conditions along the altitudinal distribution gradient of this species. An increase in the concentrations of polyphenolic compounds; specifically, flavonoids, flavones and catechins, in response to seasonal drops in the air temperatures have been described in *Polylepis cuadrijuga*, a species of the Colombian Andes (Vélez *et al.*, 1998). Therefore, we may assume that low temperatures can enhance the synthesis of these secondary metabolites in this genus. Nevertheless, reference studies concerning phytochemical responses of high elevation woody species of the Tropical Andes are still scarce.

Although in this particular study we did not assess the relationship between the concentrations of flavonoid-like chelating compounds and the photosynthetic performance in *P. sericea*, it is reasonable to assume that an increase in the concentration of chelating polyphenols should increase this species' tolerance to elevated levels of radiation, thanks to their role as antioxidant molecules in the mitigation of the photoinhibitory and photooxidative damage caused by prolonged exposure to UV radiation (Morales *et al.*, 2009; Fini *et al.*, 2011; Del Valle *et al.*, 2020; Kim *et al.*, 2021; Cesarino *et al.*, 2022). Nevertheless, it is necessary to conduct future studies involving comparative radiation and microclimate measurements at different elevations, as well as detailed soil analysis, that should include soil nutrient and water content, pH, chemical properties and gross rhizosphere composition, in order to fully understand this species' adaptation strategies in high elevation paramos.

According to Agati *et al.* (2012), the antioxidant phenolic compounds stored in the organelles of *Phillyrea latifolia* were either hydroxycinnamates or dihydroxy-substituted flavonoids. Hydroxycinnamates may also function as antioxidants; however, in sun-exposed leaves, these may be replaced by phenylpropanoids, which accumulate in stomatal guard cells, chloroplast envelopes, vacuoles, and the cell nucleus (Agati *et al.*, 2013). Our observations of ferric-stained chlorenchyma tissue preparations of *P. sericea* under light microscopy at 100x magnification confirmed that chelating compounds indeed accumulated within the chloroplasts. While the light microscope does not allow clear visualization of the chloroplast envelope, it is reasonable to infer that phenylpropanoids and other polyphenols are effectively stored there and contribute to protect the photosynthetic machinery against oxidative stress induced by excessive radiation.

High levels of UV-B radiation promote the formation of reactive oxygen species ($^1O_2$ and H_2O_2) produced by excessive excitation of photosystem II in the chloroplasts. ROS can alter thylakoid membrane structure and function and trigger photoinhibition. Fortunately, these conditions also stimulate the synthesis of phenolic compounds such as flavonoids, whose intracellular antioxidant action protects the photosynthetic complex from ROS-induced damage (Apel & Hirt, 2004; Agati *et al.*, 2012; Zlatev *et al.*, 2012). In sun-exposed leaves, flavonoid-type phenolic compounds typically accumulate in the uppermost layers of palisade parenchyma, while hydroxycinnamates tend to concentrate in the lower ones (Agati *et al.*, 2012). In *P. sericea*, chelating agents were found throughout the chlorenchyma, yet they were evidently in higher concentrations in the first two layers of the palisade parenchyma, which lead us to believe that they could be flavonoid-like phenolic compounds, derived from phenylpropanoid precursors (Agati *et al.*, 2013).

UV spectrophotometric scans also suggest that the methanolic extracts obtained from leaf samples collected along the 3,549–4,223 m gradient are not hydroxycinnamates, but rather dihydroxy-substituted phenylpropanoids. This would suggest that in *P. sericea* the phenylpropanoid biosynthetic pathway is positively regulated toward flavonoid production over hydroxycinnamate synthesis, given their superior capacity to absorb UV radiation and mitigate oxidative stress caused by ROS. Previous studies in *Ligustrum vulgare* (Tattini *et al.*, 2004) and *Ginkgo biloba* (Zhao *et al.*, 2024) support this conclusion. Moreover, the presence of flavonoids and triterpenoids in tissues of leaves and branches *P. incana*, a species of this genus that grows in the high elevation Ecuadorian Andes Catalano *et al.* (1995) suggests that the synthesis and storage of role of the dihydroxy-substituted flavonoids isolated in leaf tissues of *P. sericea* could in effect play an important role in mitigating photooxidative stress generated by ROS, due to prolonged exposure to UV-B radiation and freezing temperatures (Spitaler *et al.*, 2008; Zlatev *et al.*, 2012; Mierziak *et al.*, 2014; Cesarino *et al.*, 2022; Salam *et al.*, 2023). An enhanced in the phenylpropanoid biosynthetic pathway induced by elevated photosynthetically active radiation (PAR) may enhance the leaf's potential to eliminate ROS and potentially mitigate the negative effects generated by the prolonged exposure to higher energy wavelengths such as UV-A, B, or C, which affect sensitive cellular targets like the photosynthetic apparatus (Tattini *et al.*, 2004; Agati *et al.*, 2013; Mierziak *et al.*, 2014).

A tentative explanation that may elucidate the differences observed in the content of polyphenolic compounds in the leaf samples of specimens of *P. sericea*, that did not follow a linear trend (as we had originally hypothesized) could be related to the energy cost of synthesizing secondary metabolites, since their synthesis is greatly influenced by local environmental conditions (Mierziak *et al.*, 2014; Thakur *et al.*, 2019). Under conditions of extreme environmental stress (prolonged exposure to UV radiation, drought and freezing temperatures) plants often shift metabolic priorities towards the synthesis of phenolic compounds, tannins and flavonoids, rather than terpenes or lipids (Wang *et al.*, 2025), due to the stronger protective roles these compounds provide against stress factors (Agati & Tattini, 2010; Fini *et al.*, 2011; Salam *et al.*, 2023). Prolonged exposure to high UV levels during the day, combined with below zero temperatures during the night could be constraining the diversity of secondary metabolites synthesized by the individuals growing at 4,223 m a.s.l. on dry, rocky substrates, which exhibited the highest concentration of polyphenols in the chlorenchyma tissues, which are likely condensed flavonoids (Mabry *et al.*, 1970; Zhou *et al.*, 2019). If this is the case, it would in part account for the small stature observed in the trees of *P. sericea* located at this elevation, that contrasted strongly with the heights registered in trees of this species growing at lower elevations along this gradient.

At the lower limit of this gradient (3,549 m a.s.l) precipitations are more abundant, the soils are moist and rich in organic matter, below zero temperatures are rare (Ely *et al.*, 2011) and UV radiation is lower. These more favorable conditions could explain the synthesis of higher concentrations of phenolic compounds of the leaves of P. sericea trees growing at this elevation; as well as the presence of dihydroxy-substituted flavanol glycosides and other unknown phenolic compounds that were not detected in the extracts isolated from the samples collected at higher elevations. We should also consider the possibility that the unspecific phenolic compound isolated from the trees growing at 3,549 m a.s.l could be precursors of phytoalexins, whose synthesis may be induced due to the interactions between P. sericea and herbivorous organisms or pathogens (Mierziak *et al.*, 2014; Thakur *et al.*, 2019; Misra *et al.*, 2023). Another possibility that should be considered is that these compounds could also be acting as precursors of allelopathic compounds, given that at this elevation, the *P. sericea* forests are composed by other woody species such as *Gynoxys moritziana* and *Libanothamnus neerifolia*. Members of the Rosaceae family are known for synthesizing allelochemical compounds (Możdżeń *et al.*, 2021). Unfortunately, little is known about the phytochemistry of these interactions, especially in high elevation woody plants. Site-specific abiotic conditions may affect the nature of polyphenolic compounds synthesized by the same species, as previous phytochemical studies conducted in *Rosa* spp. (Ghazghazi *et al.*, 2012) and *Quercus suber* (Hoceini-Bentaha *et al.*, 2025) confirm, and we believe that this could also the case of *P. sericea*.

Based on our results, we believe that stable leaf traits associated to moderate phytochemical plasticity and the specialized physiological adaptations (low temperature resistance coupled with a highly efficient photosynthetic and respiratory performance) have facilitated the successful adaptation of *P. sericea* to the harsh environmental conditions that characterize high elevation paramo ecosystems of the northern Andes.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

Data available at: https://doi.org/10.53157/ecotropicos.6vej-j65x

AUTHOR CONTRIBUTIONS

- **Conceptualization:** FE
- **Data curation:** DGM
- **Formal analysis:** DGM, FE, CRP
- **Funding acquisition:** FE
- **Investigation:** DGM
- **Methodology:** DGM, FE, CRP
- **Project administration:**
- **Resources:** FE, CRP
- **Software:** DGM
- **Supervision:** FE, CRP
- **Validation:** FE, CRP
- **Visualization:** DGM
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- **Writing – review & editing:**, DGM, FE, CRP

RESUMEN

Atributos foliares conservados y plasticidad fitoquímica en *Polylepis sericea* Wedd. (Rosaceae) en un gradiente altitudinal de Los Andes Venezolanos

Este estudio examinó la plasticidad anatómica foliar, basada en atributos estándar y la plasticidad fitoquímica, en términos de acumulación y composición de compuestos polifenólicos en *Polylepis sericea* a lo largo de un gradiente altitudinal de 674 m (3.549–4.223 m s.n.m.) en los Andes venezolanos. Los análisis morfoanatómicos revelaron una estructura anatómica altamente conservada, dada la ausencia de variaciones significativas en el área foliar específica (SLA), grosor de la cutícula, altura de las células epidérmicas en general y en la proporción de clorénquima en empalizada/esponjoso. El perfil fitoquímico incluyó tinción férrica y espectrofotometría ultravioleta de los extractos foliares metanólicos. El estudio reveló la acumulación de agentes polifenólicos quelantes, principalmente flavonoides dihidroxilados y fenilpropanoides en el clorénquima, particularmente en las capas superiores. El contenido total de fenoles no incrementó linealmente, ya que la mayor concentración correspondió a la muestra recolectada a menor elevación (3.549 m s.n.m.). Sin embargo; las mayores concentraciones de flavonoides oxidados se observaron en el clorénquima de las muestras recolectadas a 4.223 m s.n.m. Estas diferencias, aunadas a la presencia de flavonoles glicosilados u oxidados en las muestras de 3.459 m s.n.m. sugieren que la síntesis y tipo de compuestos polifenólicos en *P. sericea* están influenciados por factores ambientales sitio-específico y que estos metabolitos secundarios desempeñan un papel clave en la mitigación del estrés fotooxidativo generado por condiciones ambientales extremas. La estructura foliar conservada asociada a la plasticidad fitoquímica hallada en este estudio podría ayudarnos a comprender las adaptaciones desarrolladas por plantas leñosas de alta montaña.

Palabras clave: Andes tropicales, atributos morfoanatómicos foliares, estrés UV, flavonoides, fenilpropanoides, polifenoles.

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