

Stomatal morphometry of Andean species and their relationship with spatial variation

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Stomata play a fundamental role in maintaining plant homeostasis by regulating the balance between water loss and CO₂ absorption required for photosynthesis. Analysis of stomatal traits across environmental gradients provides insight into how plant species are uniquely adapted to their environment and their potential response to environmental change. In the Andes, there is limited information on how stomatal traits vary spatially and with climatic conditions. This study aimed to characterize the morphometry of Andean woody species and corroborate the relationship of stomata characteristics with leaf traits in response to environmental variation. We measured the following traits on each species: stomatal size (SS), stomatal width (SW), stomatal density (SD), stomatal relative area (SRA), leaf thickness (LT), leaf area (LA), and specific leaf area (SLA). These traits were then analyzed to determine their relationships with mean annual temperature (MAT), mean annual precipitation (MAP), mean solar radiation (MSR), latitude, and longitude at the sampling sites. Our analyses showed that stomatal traits largely vary among species. Some species (e.g., *Morella pubescens*) exhibit high plasticity, suggesting greater resilience to unfavorable climatic conditions. However, the relationships with MAP, MSR, and MAT varied, suggesting that these species employ different mechanisms to avoid water stress and optimize water use. Moreover, we observed relationships between stomatal traits, particularly SS and SW, with latitude and longitude. Similarly, we identified significant correlations between leaf and stomatal traits. Our results suggest that the functional traits of stomata in the individual evaluated from these species respond to their geographic origin and, therefore, to the climatic conditions of their habitat.

Keywords: Stomatal Density, Stomatal Size, Stomatal Conductance, Plasticity, Functional Traits, Andean Woody Species

Introduction

Climate change is altering plant communities along with the associated processes and functions of ecosystems (Twalla et al. 2022). Investigating plant functional traits, particularly the relationship between trait variation and climatic factors, can yield valuable insights into the potential impacts of these changes on critical ecosystem functions, such as the regulation of carbon and water cycle (Zawude Bakure et al. 2022). Even the functional traits of vegetation in mountain forests are already being affected by the unusual temperature in-

crease (Feeley et al. 2020). Leaf traits are particularly significant in this context (Yu et al. 2022), as stomata are essential for maintaining plant homeostasis through gas exchange and regulating water loss and CO₂ uptake (Liu et al. 2018). This implies that it supports ecological processes vital to the balanced functioning of ecosystems (Twalla et al. 2022).

Previous studies have indicated species-specific variation in stomatal morphometric characteristics, such as stomatal length (SL), width (SW), size (SS), and density (SD) (Bertolino et al. 2019, Durand et al.

2020). These traits often reflect the prevalent environmental conditions of the habitats where plants have evolved (Driesen et al. 2020). Stomatal traits may also evolve at different rates across species, with some showing relatively rapid changes and others exhibiting little to no change over millions of years (Wang et al. 2022). Nevertheless, traits and environmental patterns can be observed at the individual level (Olusoji et al. 2023), despite the considerable variation existing at the species level. This suggests that the strongest relationships are likely to emerge from analyses conducted at the individual level (Auger & Shipley 2013). Consequently, an improved understanding of how stomatal traits evolve in response to environmental variation is essential for predicting plant responses to climate change.

Research on forest plant species has demonstrated that stomatal traits can be highly plastic (He & Liang 2018). For example, some studies indicate that SD is directly proportional to light intensity, including blue wavelengths (Lin et al. 2015), and inversely proportional to CO₂ levels in the environment (Zaida et al. 2014). Similarly, water stress has been shown to influence SL and SS in some species (Mitra et al.

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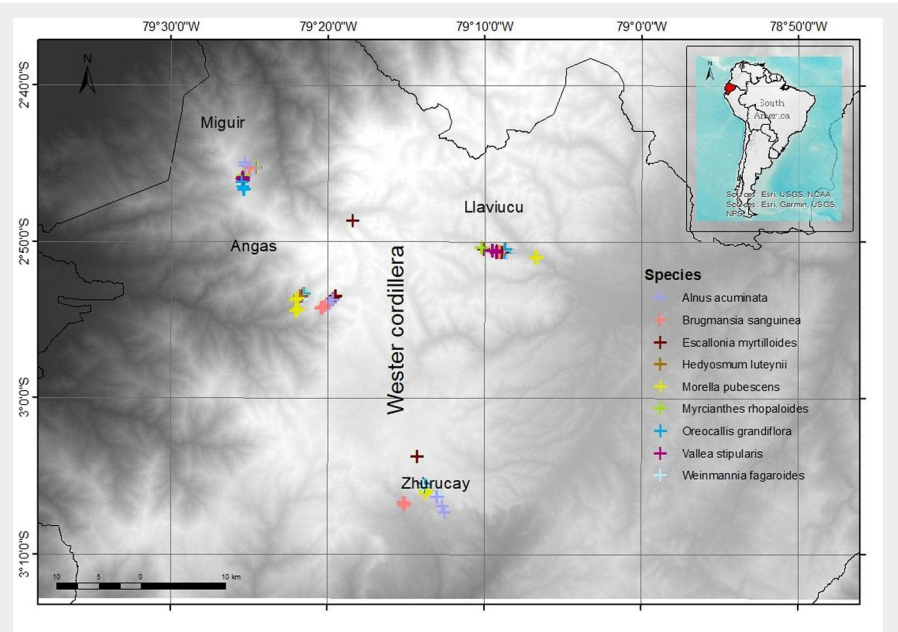


Fig. 1 - Location of sites and individuals across the province of Azuay in Ecuador.

2015), whereas stomatal relative area (SRA) is associated with solar radiation, latitude, and longitude (Matthews & Lawson 2019). Some studies showed that temperature can influence stomatal traits (e.g., SL and SD), whereas others did not (Wang et al. 2019). Plants can also adjust stomatal size and density according to their spatial position within the habitat (Fanourakis et al. 2019), resulting in substantial variation in stomatal traits among forest species (Chen et al. 2022). Overall, these findings make it challenging to conclusively relate patterns of stomatal density and their distribution in the leaf to environmental variables (Pirasteh-Anosheh et al. 2016). Likewise, stomata can also be related to the functional traits of the leaves themselves, such as leaf area and specific leaf area. Indeed, previous research reported that stomatal size and density are correlated with leaf area (Sun et al. 2021). Stomata also show a relationship with leaf thickness, as most stomata are located on the underside of leaves, where gas exchange mainly occurs (Urban et al. 2017). Addition-

ally, there is a relationship of stomata characteristics with other traits such as plant height and petiole size (not studied here), that allow the plant to receive solar radiation, thereby increasing SD, SL, and therefore, SS (Hong et al. 2018). Previous research on stomata has been conducted in *Alnus* (Esperón-Rodríguez & Barradas 2016) and *Hedyosmum* spp. (Kong 2001), but not in native Andean species. However, these studies have been primarily descriptive and have not attempted to relate climatic or spatial variables to functional traits. The Andes host an array of diverse ecosystems and habitats, including forests under different climatic conditions. Understanding how stomata interact with the climate in these habitats will help us better understand plant adaptations and responses to these varying environmental conditions. This research aims to (i) characterize the morphometry of nine native Andean woody species throughout a wide altitudinal gradient, (ii) confirm stomatal and leaf traits correlation in response to environmental variation. We hypothesize that

Tab. 1 - Type of the nine studied species, number of individuals, and number of images analyzed for each species.

Code	Species	Individuals	Images	Type
Aa	<i>Alnus acuminata</i> Kunth	20	100	Tree
Bs	<i>Brugmansia sanguinea</i> (Ruiz & Pav.) D. Don	20	100	Shrub
Em	<i>Escallonia myrtilloides</i> L. f	20	100	Tree
Hl	<i>Hedyosmum luteyngii</i> Todzia.	20	100	Tree
Mp	<i>Morella pubescens</i> Benth.	20	100	Shrub
Mr	<i>Myrcianthes rhopaloides</i> (Kunth) McVaugh	20	100	Tree
Og	<i>Oreocallis grandiflora</i> (Lam.) R.Br.	20	100	Shrub
Vs	<i>Vallea stipularis</i> L.f	20	100	Shrub
Wf	<i>Weinmannia fagaroides</i> Kunth	20	100	Tree

stomatal traits show environmental plasticity, and leaf traits, including stomatal traits, respond to environmental changes. The results of this study will improve our understanding of how these species may respond to a rapidly changing climate.

Materials and methods

Study site

The study was carried out from January to March 2019 in four forest sites: Llaviucu, Zhurucay, Angas, and Miguir, located on the two slopes of the western Andes cordillera in the province of Azuay, southern Ecuador (Fig. 1). The study sites are distributed along an altitudinal gradient ranging from 2800 to 3500 meters a.s.l.

Species collection

Samples of nine native species were collected on two slopes of the western cordillera in Azuay. The nine species selected are ecologically important to the Andean forest and are distributed across the four study sites. Five of these species were trees (a total of 100 individuals), and four were shrubs (a total of 80 individuals – Tab. 1). The individuals were randomly selected, with a minimum distance of 100 m between individuals of the same species. Leaf samples were collected from branches exposed to solar radiation. For each individual, a branch with leaves was cut, placed in a moistened plastic bag (Pérez-Harguindeguy et al. 2013), and taken to the Laboratory of Seeds and Forest Ecology at the University of Cuenca for processing.

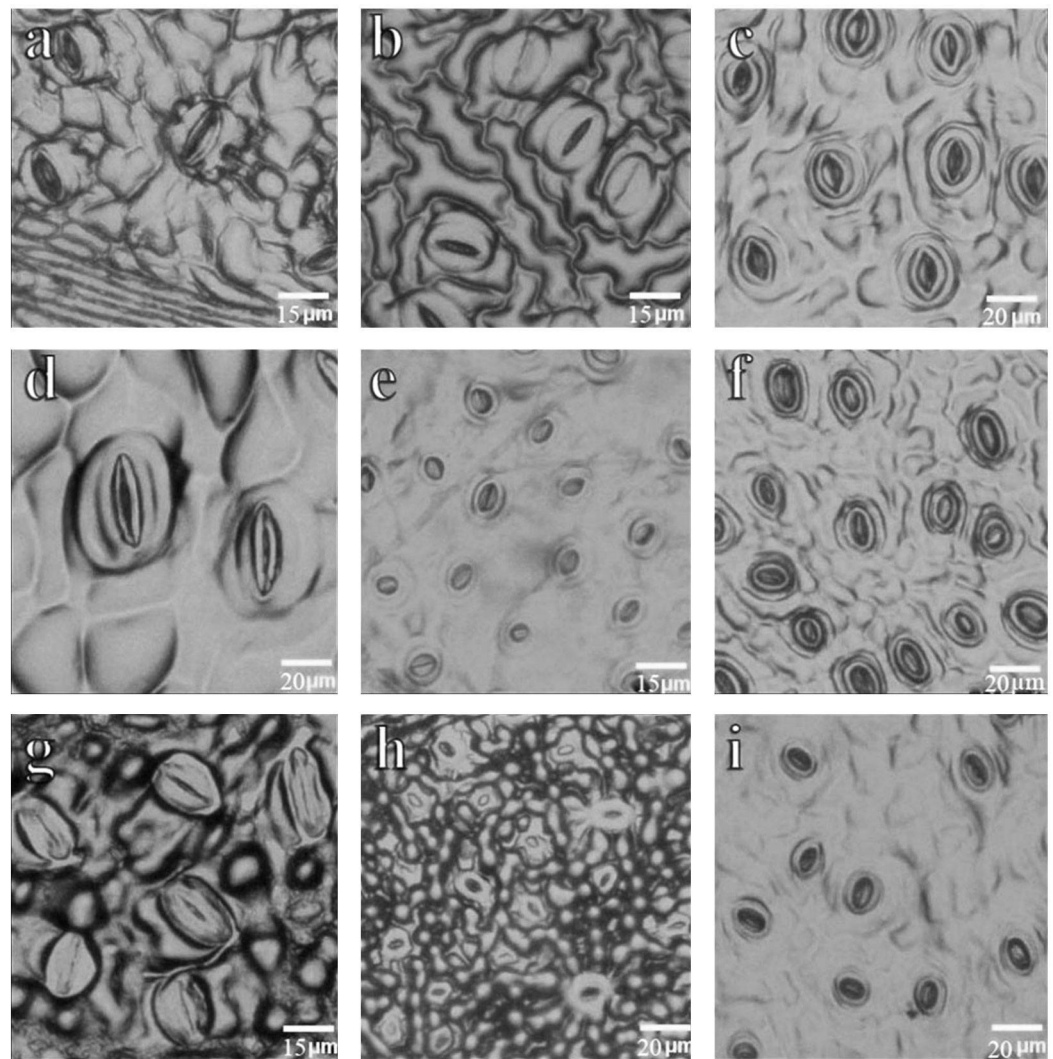
Quantification of stomatal functional traits

Stomatal data were obtained by applying transparent nail polish or cyanoacrylate glue on the underside of each leaf. Once the nail polish was dry, the epidermis layer was removed and placed on a slide marked with sample information (Wall et al. 2022). For each species, 100 images (one per leaf, totaling 900 images – Fig. 2) were processed using a microscope (Olympus™, <https://www.evidentscientific.com>) at 100× magnification, equipped with a camera (Lumenera™ – <https://www.lumenera.com>) and image capture software (Infinity Analyze v. 7). Stomatal length, width, and size were measured following Cai et al. (2024). To measure stomatal density (SD), the number of stomata was counted and extrapolated to 1 mm². For each image, the SL and SW of five randomly selected stomata were measured and averaged using ImageJ (<https://imagej.net/ij/> – Peel et al. 2017).

The stomatal relative area (SRA) was also quantified. The SRA represents the anatomical restriction on the maximum gas exchange capacity of the leaf, which determines the maximum stomatal conductance. The SRA was calculated as follows (Sun et al. 2021 – eqn. 1):

$$SRA = SS \cdot SD \cdot 10^{-4} \tag{1}$$

Fig. 2 - Stomata of the nine species selected. (a): *Alnus acuminata*; (b): *Brugmansia sanguinea*; (c): *Escallonia myrtilloides*; (d): *Hedyosmum luteynii*; (e): *Morella pubescens*; (f): *Myrcianthes rhopaloides*; (g): *Oreocallis grandiflora*; (h): *Vallea stipularis*; (i): *Weinmannia fagaroides*.



Leaf functional traits

Leaf thickness (LT) was measured with a digital caliper (Marenco et al. 2009). Leaf area (LA) was obtained by scanning the leaf, and the area was measured in ImageJ (Wigley et al. 2020). For specific leaf area (SLA), the sample was placed in an oven for 72 hours at 60 °C, weighed, and the dry weight divided by LA (Pérez-Harguindeguy et al. 2013).

Climate and spatial data

Mean annual temperature (MAT), mean annual precipitation (MAP), and mean daily solar radiation (MSR) were extracted from TIF images from WoldClim (<https://www.worldclim.org>). The data covers the years 1970 to 2000, and the images have a pixel size of approximately 1 km² (Cerasoli et al. 2022). Latitude and longitude were obtained *in situ* using a GPS device.

Statistical analysis

To describe the stomatal traits of woody species, we used metrics of central tendency and dispersion. To confirm the effects of climate, spatial variables, and leaf traits individually on SS, SD, SRA, and SW, mixed linear models (LMMs) were fitted,

with species/forest as a nested random effect, and marginal R² was evaluated (Liu et al. 2023). Also, we assessed the interaction of climate variables and leaf traits separately on stomatal functional traits using LMM (Liu et al. 2023). Scatter plots were constructed to represent each stomatal relationship with climate factors. Statistical analyses and climate data extraction were performed in R (R Core Team 2024) using the lme4 library (Wickham et al. 2024).

Results

The species exhibiting the highest mean stomatal size (SS) was *H. luteynii* (474.8 ± 197.9 μm²), which also had the maximum stomatal size (926.2 μm²). In contrast, the species showing the lower SS were *V. stipularis* and *M. pubescens* (76.3 ± 51.7 and 105.2 ± 79.4 μm², respectively). The above three species also recorded the largest and smallest stomatal lengths (SL: 34.4 ± 6.9, 14.3 ± 3.7, and 12.4 ± 4.6 μm, respectively) and widths (SW: 16.9 ± 4.0, 6.3 ± 3.9, and 9.4 ± 4.2 μm, respectively), and a coefficient of variation (CV) ranging from 0.2 to 0.3. Likewise, *H. luteynii* and *O. grandiflora* had the lowest mean stomatal density (SD: 132.7 ± 28.9 and 237.4 ± 37.2 mm², respec-

tively). In addition, *V. stipularis*, *B. sanguinea*, and *O. grandiflora* exhibited the lowest stomatal relative area (SRA: 3.3%, 4% and 4.4%, respectively). Conversely, *M. pubescens* showed a stomatal density (SD) of 652.2 per mm² and a CV of 0.4, making it the species with the highest values for this trait. Finally, *E. myrtilloides* had the largest SRA (7.7 %), while *M. pubescens* showed the largest CV (0.6 – Tab. 2).

Climatic variables

Linear regression analysis revealed that most pairwise relationships between stomatal functional traits and climatic variables were significant, though weak and positive, except for mean annual precipitation (MAP). However, the distribution of climatic data was unequal across the range analyzed, with a limited number of records with precipitation > 900 mm (Fig. 3c, Fig. 3f, Fig. 3i). The temperature trend for all stomatal traits was negative, with a marginal R² < 0.10, with exception of SL (R² = 0.16, p-value < 0.001 – Fig. 3i). As for SRA and SW, it was not statistically significant (Fig. 3h, Fig. 3j). The MSR showed a positive relationship (p-value < 0.05) with SS (Fig. 3a), SD (Fig. 3b), and SW (Fig. 3d),

Tab. 2 - Measures of central dispersion of the four stomatal traits for each species. (STD): standard deviation; (CV): coefficient of variation. For species labels, see Tab. 1.

Species	Stomatal Size SS (μm ²)			Stomatal Density SD (mm ²)			Stomatal relative area - SRA (%)			Stomatal Length SL (μm)			Stomatal Width SW (μm)		
	Mean ±STD	CV	Min -Max	Mean ±STD	CV	Min -Max	Mean ±STD	CV	Min -Max	Mean ±STD	CV	Min -Max	Mean ±STD	CV	Min -Max
Aa	187.3±101	0.5	44.7-366.9	380.5±112.4	0.3	201.5-622	6.1±1.7	0.3	3.7-10.2	22.2±4.5	0.2	13.4-28.8	10.4±4.4	0.4	2.5-16.7
Bs	155.1±87.6	0.6	2.6-273.9	278.3±68.4	0.2	171.4-407.8	4±1.7	0.4	1.7-8.7	22.8±6.9	0.3	10.5-34.4	8.1±4.2	0.5	0.3-14.4
Em	202±69.4	0.3	65.9-297.7	457.3±102.2	0.2	312.3-708	7.7±2.3	0.3	4.4-13.6	22.5±3	0.1	17.5-28.6	11.5±3.9	0.3	4.7-16.5
HL	474.8±197.9	0.4	159.8-926.2	132.7±28.9	0.2	75.5-196.7	6.2±1.8	0.3	3.6-9.7	34.4±6.9	0.2	21.9-47.6	16.9±4	0.2	9.3-24.8
Mp	105.2±79.4	0.8	14.9-256.2	652.2±275.6	0.4	280-1122.3	6.8±4.2	0.6	2.3-16	12.4±4.6	0.4	6.3-20.5	9.4±4.2	0.4	3-17.1
Mr	116.9±80.3	0.7	18.3-268.4	647.6±111.9	0.2	420.6-827.8	6.6±2.5	0.4	3.6-12.6	15.5±5.2	0.3	5.9-22.6	8.6±4.4	0.5	2.9-16.4
Og	207.1±86.7	0.4	49.7-334	237.4±37.2	0.2	167.4-308	4.4±1.7	0.4	2.1-8.4	24±4	0.2	17.2-30.1	10.8±3.8	0.3	2.8-15.3
Vs	76.3±51.7	0.7	3.4-156.6	561.4±170.2	0.3	346.4-975.8	3.3±1.1	0.3	1.7-5.6	14.3±3.7	0.3	5.1-18.1	6.3±3.9	0.6	0.4-11.3
Wf	131.9±84.9	0.6	12-251.5	540±113.5	0.2	331.1-735.3	6±2.1	0.4	2.8-10.5	16.7±6	0.4	5.7-23.5	9.1±4.5	0.5	2.7-14.7

with a cluster of data points around 14,000 KJ m⁻² day⁻¹. Similar results were obtained for the relationship between climatic variables and leaf traits (Fig. S1 in Supplementary material).

Leaf functional traits

All stomatal traits demonstrated dependence on leaf area (LA); specifically, the regression of this trait with SD showed an R² of 0.04 (Fig. 4b), while SW had an R² of 0.13 (Fig. 4e). Also, a significant relationship between SLA and SD, SRA, and SL was found, however, the variance explained (R²) values were >0.04 (Fig. 4f-Fig. 4j). On the contrary, Fig. 4k to Fig. 4o indicates that LT is independent of stomatal traits. Additionally, Fig. 4 shows that most data lie on the left side of the scatter plots of leaf traits versus stomatal traits.

Spatial variation and patterns

We found significant associations of spatial variables with four stomatal traits (Fig. 5). All relationships between stomatal characteristics and latitude were negative, whereas those with longitude were positive. LMMs revealed a dependence of SS (Fig. 5a), SD (Fig. 5b), and SW (Fig. 5e) on latitude; this last model had an R² of 0.15. A similar trend was observed for the longitude, which revealed a significant association with SL. Likewise, the best fit (R² = 0.33) was achieved with SW (Fig. 5j). Furthermore, the unique stomatal trait showing no significant relationship with geographic coordinates of the sampling sites was the stomatal relative area (SRA - Fig. 5c, Fig. 5h).

Climatic variables and leaf traits models

The analysis of climatic variable interactions indicated that stomatal density (SD) responded to all three climatic variables analyzed (p-value < 0.05), whereas SL and SW showed an exclusive dependence on MAT (mean annual temperature) and MAP (mean annual precipitation). In leaf trait models, most stomata variables interact with specific leaf area (SLA), and to a lesser extent with leaf area (LA) and thickness (LT). In addition, in the performed models, the random effect for species/forest was found to be statistically significant. On the contrary, SRA showed no association with climatic variables, whereas SD did not relate to any predictor. However, the variance explained (R²) in each case was relatively low (2% to 18%), while the ICC ranged from 0.1 to 0.8; the latter value suggests

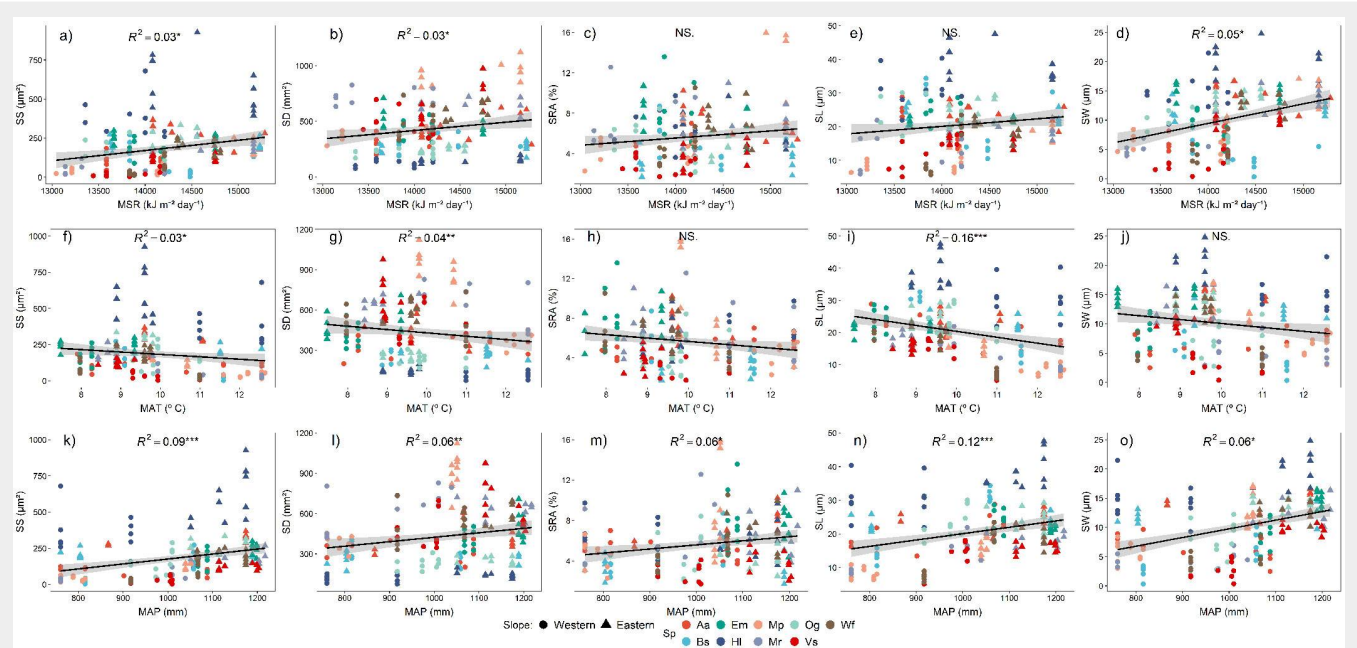


Fig. 3 - Associations of stomatal traits with climatic variables.

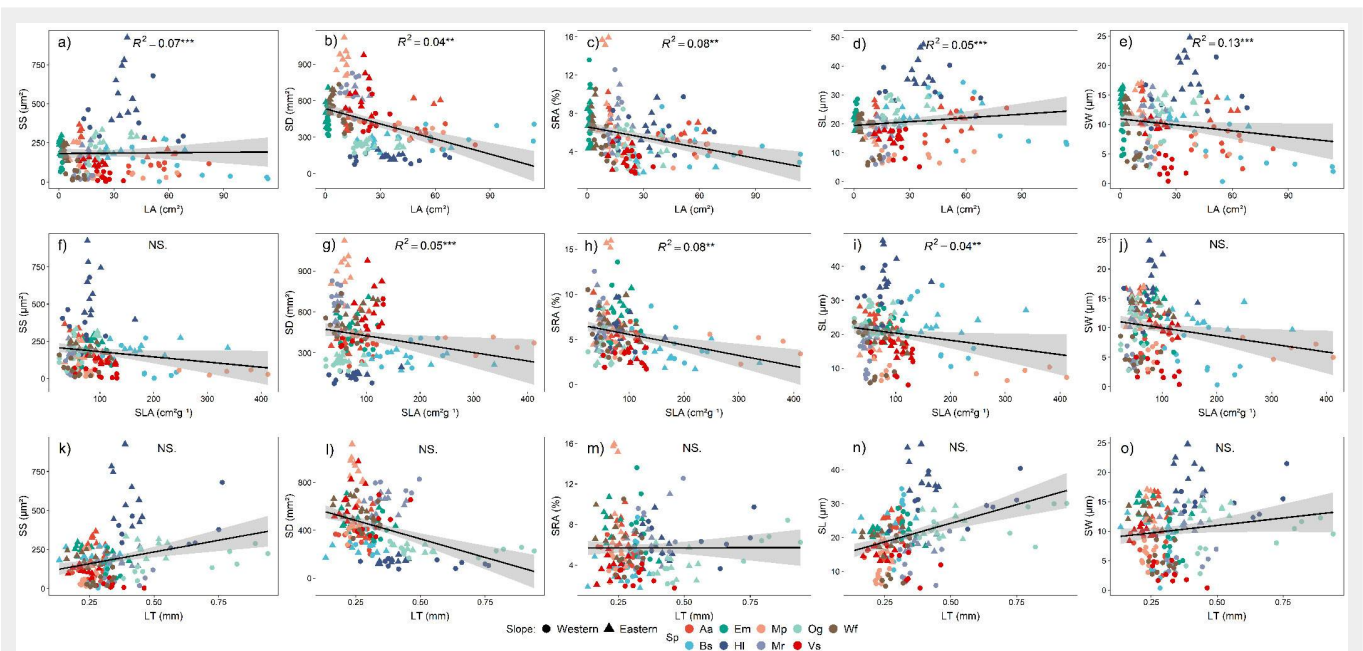


Fig. 4 - Associations of stomatal traits with leaf traits.

that our results are due to the interaction between species/forest (Tab. 3).

Discussion

Examining the coefficients of variation for each stomatal trait revealed relative differences in plasticity among the nine species analyzed in our study (Pélabon et al. 2020). Our results indicate that *M. pubescens* has the greatest plasticity of four out of five stomatal traits, while *V. stipularis* was the most plastic in terms of stomatal width. Species with larger stomata tend to exhibit reduced stomatal density, thereby facilitating more efficient regulation of water and gas exchange (Haworth et al. 2023). This trend was evident in the tree species *H. luteynii*. Species with reduced size of stomata tend to increase their density, as is the

case with *M. pubescens* (Tab. 2). This suggests that this species may be better adapted to the climatic conditions of its habitat and potentially more resilient to climate change impacts.

The stronger relationships observed in this study were between stomatal traits and leaf functional traits (LT) and MAP. In contrast, the relationships with the other climatic and spatial variables appeared to be less robust (Fig. 3, Fig. 4, Fig. 5). Leaf area (LA) was related to all stomatal traits, likely due to the fact that a larger leaf area could host larger stomata. However, this leads to a reduced stomatal density or width, and these patterns were found in our study (Liu et al. 2018, Peel et al. 2017). This indicates that correlation exists between stomata, leaf traits, and mesophyll

architecture for the proper functioning of this process (Daningsih et al. 2022).

In addition, as higher stomata density leads to lower length (SL) or size (SS) of stomata, they will close more quickly, thereby avoiding unnecessary water loss (Dow et al. 2017). Indeed, a smaller SL can facilitate rapid stomatal opening and closing through altering cell turgor (Zhang et al. 2020). Besides, lower LA and SLA allow the plant to mitigate water stress in dry environmental conditions (Wei et al. 2024). Our findings suggest that stomatal length is associated with a decrease in their density on the leaf, likely preventing unnecessary water loss (Liu et al. 2023). In addition, coordination among stomatal traits was verified; for example, SL and SS tend to reduce while SD increases (Fig. S2 in Supple-

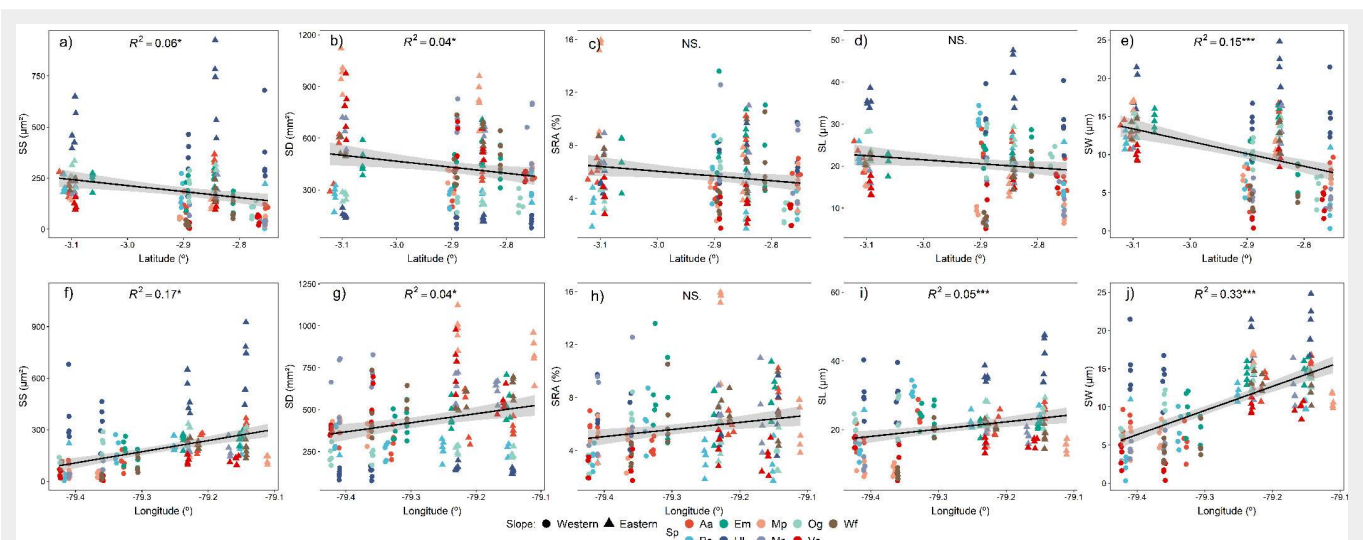


Fig. 5 - Associations of stomatal traits with geographic variables.

Tab. 3 - LMM performed for each stomatal trait with climatic and leaf variables. (df): Degrees of freedom; (STD): standard deviation; (ICC): intraclass correlation coefficient; (MAT): mean annual temperature; (MAP): mean annual precipitation; (SLA): specific leaf area; (LA): leaf area; (LT): leaf thickness; (***): $p < 0.001$; (**): $p < 0.01$; (*): $p < 0.05$; (NS): not significant.

Group	Trait	Predictors	df	F	Var exp (%)	Random Effect	ICC	STD	σ^2
Environmental	SS	MAT $\times$ log(MAP) ^{NS}	1	0.01	11	Species/Forest	0.82	56.95	4169.80
						Residuals	-	66.07	-
	SD	MAT $\times$ log(MAP) $\times$ log(MSR) [*]	1	0.98	12	Species/Forest	0.87	99.75	6230.50
						Residuals	-	78.93	-
	SRA	MAT $\times$ log(MAP) ^{NS}	1	0.20	4	Species/Forest	0.28	1.41	3.48
						Residuals	-	1.86	-
	SL	MAT $\times$ log(MAP) ^{**}	1	4.45	17	Species/Forest	0.10	2.51	11.05
						Residuals	-	3.32	-
	SW	MAT $\times$ log(MAP) [*]	1	4.31	2	Species/Forest	0.66	3.99	3.45
						Residuals	-	1.86	-
Leaf traits	SS	log(SLA) $\times$ LT ^{NS}	1	0.36	2	Species/Forest	0.85	122.10	7403.30
						Residuals	-	86.04	-
	SD	LA $\times$ log(SLA) $\times$ LT ^{**}	1	3.52	12	Species/Forest	0.83	98.91	3807.60
						Residuals	-	61.71	-
	SRA	LA $\times$ log(SLA) $\times$ LT ^{**}	1	5.15	14	Species/Forest	0.47	1.36	3.43
						Residuals	-	1.85	-
	SL	LA $\times$ log(SLA) ^{**}	1	3.37	11	Species/Forest	0.86	5.08	10.17
						Residuals	-	3.19	-
	SW	LA $\times$ LT ^{**}	1	7.38	8	Species/Forest	0.85	4.27	3.37
						Residuals	-	1.84	-

mentary material).

Stomatal density was negatively related to specific leaf area (SLA, Fig. 4e), indicating that as SLA increases, stomatal density tends to decrease. This aligns with previous studies (Peel et al. 2017), possibly due to the balance between the leaf area and its mass (SLA), which is related to the number of guard cells (Galmés et al. 2007). Although the relation between leaf thickness and stomatal variables was found to be statistically insignificant, our study revealed a negative trend with stomatal density. This is because leaf gas exchange is regulated by the architecture of the mesophyll, stomata density, and characteristics of the stomata, which together determine the maximum gas flux (Liu et al. 2018, Sakoda et al. 2019).

According to the LMM results (Tab. 3), climatic variables at the sampling site, such as precipitation (MAP) and temperature (MAT), influence the functional traits of individuals. Earlier studies have demonstrated that these factors play a significant role in determining stomatal morphometry (Du et al. 2021). Our findings suggest that the stomata may respond to different climate conditions. For example, MAP was positively related to stomatal density, size, length, and width, as well as to stomatal relative area, consistent with prior research (Sun et al. 2021). This supports the hypothesis that plants develop mechanisms for optimal water use, such as the efficient opening of stomata to minimize water loss (Bertolino et al. 2019), while also maintaining photosynthetic rates or adjusting stomatal conductance to cool leaves (Lin et al. 2015). But this may also result in unnecessary water loss (McAusland et al. 2016).

In this study, all stomatal variables were negatively, though weakly, related to mean annual temperature. Previous research reported the same trend (Sun et al. 2021). Higher temperatures allow the leaf to modify stomatal length, width, and size (Hill et al. 2014), thereby increasing water-use efficiency and CO₂ absorption (Hughes et al. 2017). Solar radiation influences stomatal transpiration, increasing leaf and mesophyll temperatures, thereby altering plant respiration and photosynthesis (Lin et al. 2015). Additionally, leaf exposure to wavelengths at which photosynthesis occurs may modify stomatal size and density (Wang et al. 2019).

Our findings indicate that the individuals of the collected species have adapted their leaf (Fig. S1 in Supplementary material) and stomatal traits to the local climate (Fig. 3) in order to maintain the proper plant functionality. For example, a reduced stomatal density and an increased leaf area can contribute to keeping the leaves cool and refreshed (Lin et al. 2015, Wang et al. 2019).

Our results also indicate that stomatal size and density, and stomatal relative area are associated with longitude or latitude (Fig. 5), consistent with previous research (De Boer et al. 2016, Du et al. 2021). Other studies have analyzed geographic patterns in stomatal traits across large gradients in longitude and latitude, such as in China, reporting similar results. Likewise, stomatal functional traits, such as stomatal density, increase with increasing latitude and longitude (Wang et al. 2015, Du et al. 2021). In our study, the geographic gradients are relatively short, suggesting that this relationship may be robust even at shorter geographic distances.

The length, width, and size of stomata decrease as the sampling sites are closer to the western Andes cordillera. This trend is linked to the influence of moisture from the Pacific and Amazon regions (Esquivel-Hernández et al. 2019). This moisture influences precipitation levels: the western slopes, influenced by the Amazon, experience higher precipitation, while the eastern slopes, affected by the Pacific, receive less. In our study, two forests are located on the eastern slope of the western Andes Cordillera and two on the western slope, where we observed higher values for stomatal traits.

Conclusions

The stomatal functional traits of nine shrub and tree species showed varying levels of adaptation to their local environments. Species such as *M. pubescens* and *V. stipularis*, which exhibit high plasticity, may better adapt to climate change. In contrast, *E. myrtilloides* may be negatively affected by climate change due to its lower plasticity in stomatal traits. Other species, such as *H. luteynii*, which had the highest stomatal length and size but the lowest

stomatal density, may exhibit variable responses to climate change.

Generally, the variation in stomata characteristics responds to climatic variables, especially precipitation. Leaf functional traits are correlated with stomatal traits, especially LA and SLA. This means that the plant adjusts its functional traits to save and optimize resources. In addition, our findings showed that plants exposed to higher temperatures have reduced stomatal length, width, and size, likely as a mechanism to reduce water loss.

We observed a significant relationship between stomata characteristics and geographic coordinates (latitude and longitude) of the sampling sites. While these relationships have been observed across wide ranges of latitude and longitude, our study indicates that such relationships also hold across relatively short geographic gradients. Overall, these results suggest that native Andean species exhibit varying degrees of stomatal trait plasticity and possibly varying abilities to cope with climate change. Future research on additional Andean woody species is needed to shed light on how these critically important plant communities are likely to respond to current and future climate changes.

List of abbreviations

SL: stomata length; SW: stomata width; SS: stomata size; SD: stomata density; LA: leaf area; SLA: specific leaf area; SRA: stomatal relative area; LT: leaf thickness; MAT: mean annual temperature; MAP: mean annual precipitation; MSR: mean solar radiation; LMM: linear mixed model; Aa: *Alnus acuminata*; Bs: *Brugmansia sanguinea*; Em: *Escallonia myrtilloides*; Hl: *Hedyosmum luteyui*; Mp: *Morella pubescens*; Mr: *Myrcianthes rhopaloides*; Og: *Oreocallis grandiflora*; Vs: *Vallea stipularis*; Wf: *Weinmannia fagaroides*; ICC: Intraclass correlation coefficient; σ^2 : variance.

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Supplementary Material

Fig. S1 - Relationship between leaf traits and climate variables.

Fig. S2 - Relationship between SD with SL, SD, and SS.

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