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Original article

Does the functional leaf anatomy of *Justicia calycina* (Acanthaceae) reflect variation across a canopy gradient in the Southern Brazilian Amazon?

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ABSTRACT

Justicia calycina is a subshrub well distributed in northern South America. Despite its widespread occurrence, little is known about its anatomy and plasticity in different environments. Our study aimed to evaluate how leaf anatomical traits of *J. calycina* adjust in forest areas with variations in canopy openness in the Southern Amazon, Mato Grosso State, Brazil. Forest areas were: Open and Dense Ombrophilous Forest, and Seasonal Deciduous Forest (rocky outcrop). We performed anatomical measurements in leaves sampled on 10 individuals in each forest site, with the help of usual anatomy techniques. Foliar anatomy of *J. calycina* showed similar pattern among the studied forests, with significant difference ($p<0.05$) in four leaf traits among 22 studied: vascular bundle diameter, palisade parenchyma thickness, stomatal density and trichome density in adaxial epidermis. These leaf traits showed high plasticity index, with higher values in Dense Forest and in Deciduous Forest. Even though *J. calycina* showed overall little anatomical variation among forest types, the few attributes that differed are fundamental in the photosynthesis process. The adjustment in leaf anatomical traits under different luminosity conditions demonstrates a degree of phenotypic plasticity in the species, which contributes to its distribution in different forest phytophysiognomies in Brazilian Amazonia.

Keywords: Justicieae, structural attributes, phenotypic plasticity, sun leaves, tropical forest.

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Introduction

Tropical plant communities show variable environmental conditions, especially in microclimatic and edaphic factors. The coexistence and development of plant species is directly related to the potential that they have to adjust phenotypic characteristics in response to environmental variations (Pireda *et al*. 2019). The ability of plants to alter their phenotype in different environments is defined as phenotypic plasticity (Sultan 2000).

Leaf traits are among the most plastic in plants in response to environmental variations (Sultan 2000; Gratani 2014). Leaves are directly involved in photosynthetic processes and ecosystem processes (Garnier *et al*. 2001; Diaz *et al*. 2004). Leaf morphological characteristics can significantly externalize the environmental stresses that plants are suffering or, conversely, show plasticity in response to anatomical adjustments (Garnier *et al*. 2001; Diaz *et al*. 2004; Rosado & Mattos 2007). In this sense, plants that have greater phenotypic plasticity are more apt to coexist in different environments, allowing for a wide geographic distribution (Gratani 2014).

Variations on thickness of tissues (*e.g*., epidermis and mesophyll parenchyma) and density of stomata and trichome are usually found related to luminosity, water availability and soil fertility conditions (Dickison 2000; Gratani 2014). These anatomical variations are associated with regulating light and CO_2 profiles within leaves and maximizing photosynthetic efficiencies (Dickison 2000; Crang *et al*. 2018). Sun leaves need to prevent high sunlight irradiance and/or water stress, and may have larger cells or more layers in palisade parenchyma making them more efficient in capturing and distributing of light through the mesophyll (*e.g*., Rabelo *et al*. 2013; Gratani 2014; Campbell *et al*. 2018). Sun leaves may also have more stomata per unit of leaf area, thicker epidermis and cuticle which favors greater light reflection, preventing leaf overheating (Rossatto *et al*. 2009; Fang & Xiong 2015).

The Acanthaceae family has high diversity in Brazil (471 native species; Flora e Funga do Brasil 2020 2022), but only few studies addressed leaf anatomical responses in different environmental conditions (*e.g*., Larcher & Boeger 2006; Cassola *et al*. 2019). For *Justicia calycina* (= *Justicia acuminatissima*), some studies pointed to great morphological variability (Wasshausen & Wood 2004; Wasshausen 2006; Verdam *et al*. 2012). This species is a perennial subshrub that occurs in several different phytophysiognomies and is well distributed in northern South America, including Brazil and a number of other countries (Tropicos 2022). The species also has potential medicinal properties (*e.g*., Corrêa & Alcântara 2012; Cordeiro *et al*. 2019). Thus, it is important to deepen studies on the leaf morphoanatomy of *J. calycina* to understand its wide geographic distribution. Cassola *et al*. (2019), for example, observed changes in leaf anatomy of three *Justicia* species under different growth conditions. Besides, anatomical studies associated with taxonomy, phylogeny and degree of plasticity are useful to evaluate environmental heterogeneity, contributing to the conservation and management plans of natural vegetation (*e.g*., Metcalfe & Chalk 1979; Gupta & Shukla 2012; Gratani 2014; Sokoloff *et al*. 2021).

In this context, our study aimed to evaluate how leaf anatomical traits of *J. calycina* vary among three forest areas with canopy openness variation in the Southern Brazilian Amazon. Considering that the forests in this region have different types of vegetation and environmental conditions, we expect that anatomical traits will differ among areas (structural adjustments), enabling acclimation and adaptation of *J. calycina*. We also expect that in forest areas with higher luminosity intensity, *J. calycina* will show typical variations of sun plants in leaf anatomical traits, such as thicker tissues and increased stomata and trichome density. These anatomical traits are among those that most vary in relation to environmental conditions, especially in sun-shade comparisons (Dickison 2000; Gratani 2014).

Material and methods

Studied areas

Our study with *Justicia calycina* (Nees) V.A.W.Graham (Fig. 1A) was developed in three sites with different forest types in Southern Amazon, Mato Grosso State, Brazil: Open and Dense Ombrophilous forest, and Seasonal Deciduous Forest (Fig. 1B). The species is abundant in the three areas.

The Dense Ombrophilous Forest (hereafter Dense Forest) was sampled at the Reserva Surucuá, a particular reserve with about 50 hectares in the urban area of Alta Floresta municipality. In the studied forest portion (9°52'42"S, 56°05'55"W, 290 m altitude; Fig. 1C), the degree of anthropogenic disturbance is relatively low, the forest canopy reaches 20-30 m in height and the understory is dense and rich in shrub species (E. Gressler, personal communication).

The Open Ombrophilous Forest (hereafter Open Forest) was sampled in the Parque Ecológico Municipal C/E (9°52'39"S, 56°05'29"W, 280 m altitude), a public reserve located about 1 km from the Reserva Surucuá, comprising an area of 9.19 hectares (Fig. 1C), and average canopy height of 15-20 m (Colpini *et al*. 2009). The forest fragment suffered intense anthropic influence, showing many clearings and exotic plants (Varella *et al*. 2018).

The Seasonal Deciduous Forest (hereafter Deciduous Forest) was sampled in the Reserva Particular do Patrimônio Natural Mirante da Serra reserve (1616.7 ha) located in the municipality of Novo Mundo, about 40 km away from the other two studied forests. We sampled *Justicia calycina* at two points in a rocky outcrop with transitional area of

Deciduous Forest and Dense Forest (9°34'59"S, 55°55'09"W, 290 m altitude, and 9°35'06"S, 55°55'05"W, 325 m altitude) with little/no anthropic disturbance (Fig. 1D). At these points the forest canopy is relatively open (20-25 m high) and most species are deciduous, losing their leaves in the dry season (Sasaki *et al*. 2010).

Rainy seasons in the region of Alta Floresta and Novo Mundo occur between October and April, and dry seasons between May and September (Caioni *et al*. 2014). Open and Dense Forest are under the same meteorological conditions. In these areas, in 2016, total annual rainfall was 1911 mm, mean annual temperature 27.5 °C and mean annual air humidity 75.0 %. For the same year, in the Deciduous Forest, total annual rainfall was 2566 mm, mean annual temperature 27.2 °C and mean annual air humidity 85.6 % (Fig. S1). Those data were collected in two meteorological stations, one installed in the Dense Forest and one installed about 3 km of distance from the Deciduous Forest (V Dubreuil, unpubl. data). There is no meteorological data available for 2017 in the Deciduous Forest, due to local equipment failure.

We measured luminosity conditions in each studied forest using hemispheric photographs taken with a digital professional camera (Nikon DSLR D600), equipped with a fisheye 8 mm lens (Sigma EX DG 1:3.5) and mounted on a tripod at 1.0 m aboveground. Photographs were taken on 27-28th September/2016, from 07:00 to 11:00 a.m., in points close to the sampled individuals in Dense Forest (9 points), Open Forest (3) and Deciduous Forest (4). We analyzed canopy openness (%) in the photographs using the program Gap Light Analyzer version 2.0 (Frazer *et al*. 1999). Hemispheric fisheye photographs are widely used in ecological studies to estimate light conditions in forest understory (*e.g.*, Dahlgren *et al*. 2007). In the case of *J. calycina*, we took photographs when the equipment was available in our institution, which coincided with the beginning of the growing season (Lauton *et al*., unpubl. res.) and may represent the general luminosity conditions under which the leaves collected in April/2017 for anatomy developed.

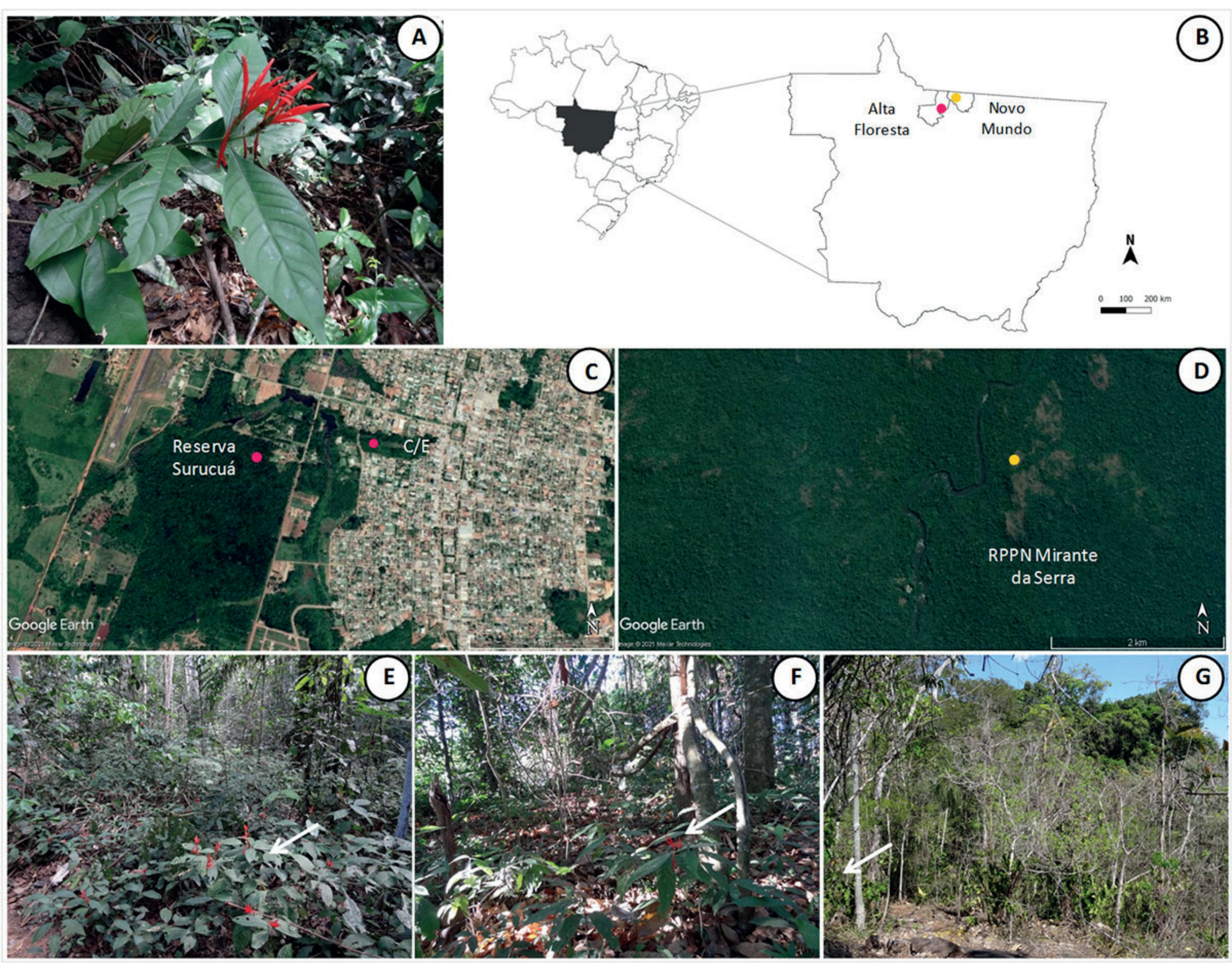

Figure 1. Individual aspect of *Justicia calycina* (Acanthaceae) (**A**) and studied areas in Southern Amazon, Mato Grosso State, Brazil (**B-G**). Reserva Surucuá (Dense Forest) (**C, E**). Parque Ecológico Municipal C/E (Open Forest) (**C, F**). RPPN Mirante da Serra (Deciduous Forest) (**D, G**). Arrows indicate *J. calycina* individuals in each forest site.

Anatomical measurements

In April/2017 (end of the rainy season), we randomly sampled four adult leaves (fully expanded and developed) from the middle plant part (between the first, second and third knots) of 10 adult individuals of *Justicia calycina* in each studied forest, to minimize influence of developmental stage on the analysis. We also collected fertile material (Fig. 1A), which we deposited at the Southern Amazon Herbarium (HERBAM). Vouchers: M.B. Lauton 01 (26587 – 25/IV/2017), M.B. Lauton 02 (26588 – 27/IV/2017) and M.B. Lauton 03 (26589 – 28/IV/2017). We fixed the leaves with FAA_{50} (formaldehyde, glacial acetic acid and 50 % ethanol, with proportion of 5:5:90, respectively) for 48 hours, and later we preserved in 70 % ethanol according to the modified method of Johansen (1940).

From each individual we analyzed two leaves, totaling 60 samples, without visually signals of senescence or damage caused by herbivores or pathogens. We used the method of Roeser (1962) to make freehand cross sections (with razor blades), in the median region of each leaf, obtaining thin sections which were stained with Astra blue and Basic Fuchsin. The median region was selected because it is an area with more uniform tissue distribution in the leaf lamina (Appezzato-da-Glória & Carmello-Guerreiro 2012). For the analysis of the epidermis, we used the technique of printing both leaf surfaces with a drop of universal cyanoacrylate ester instant adhesive on histological slides, as described by Segatto *et al.* (2004).

We photographed each histological slide using a digital image capture device, coupled to a Leica ICC50 photomicroscope, with the support of software LAZ EZ 1.7.0. For the comparison among forest sites, we measured 22 leaf anatomical traits (anatomical variables) using the Anati Quanti software (Aguiar *et al.* 2007; Tab. 1, Fig. S2).

Statistical analysis and plasticity index

We compared luminosity conditions (canopy openness) among the three forest sites using Kruskal-Wallis test in STATISTICA 8.0 software. Forest types differed significantly in luminosity ($H = 11.76$, $p = 0.0028$) and Deciduous Forest showed higher values than the other two forest sites (Fig. 2).

All the statistical analyses with anatomical data were performed using R package version 4.0.3 (R Development Core Team 2020). First, we tested the assumptions for normality and homoscedasticity using Shapiro and Levene tests. We tested the relationships among anatomical variables with Spearman's correlation (Tab. S1) and considered a strong correlation when the pairs of variables showed a value above 0.70 (Kendall 1938). In such cases, we eliminated one of the variables from the subsequent analysis using the potential adaptive explanation of the variable as an elimination criterion. Thus, the variables midrib size (MIDS), spongy parenchyma thickness (SPT) and stomatal index (STI) were excluded (Tab. S1).

We performed a PCA (Principal Components Analysis) with data of the three studied forests using the anatomical variables with the highest factorial load values for significant axes, selecting them for comparison among the forest types. To identify which anatomical variables contribute significantly to distinguish the studied forest sites, we performed the Kruskal-Wallis test. After that, we applied Dunn's multiple comparison test with Bonferroni's correction (PMCMR package; Pohlert 2014) on the variables with positive results in the previous tests.

For the variables which were significantly different among forest types we calculated the plasticity index (PI), which ranges from 0 (no plasticity) to 1 (maximum plasticity), with values higher than 0.6 interpreted as high plasticity (Valladares *et al.* 2000; 2006).

Results

Qualitative analysis

In the three studied forest sites, *Justicia calycina* showed hypostomatic leaves, midrib with a bicollateral vascular bundle in an open arc and 2-3 accessory bundles, one-layered adaxial and abaxial epidermis, dorsiventral mesophyll and both epidermal surfaces with trichome and cystoliths (Figs. 3, 4).

We observed only slight qualitative differences in cross and paradermal sections among the forest sites. The epidermal cells showed sinuous contour, with visually less sinuosity and thicker walls in Deciduous Forest (Fig. 3). Both the adaxial and abaxial epidermal surfaces presented cystoliths and numerous trichomes (or their scars) mainly on the abaxial surface (Fig. 3). In the midrib we observed little visual variation among forest types in relation to the size of vascular bundles (larger in Dense Forest) and a slight difference in the collenchyma position, which in the Open Forest was more dispersed than the other two forests (Fig. 4A, C, E).

In the leaf lamina, anatomical traits were visually similar among forest sites (Fig. 4B, D, F). Palisade parenchyma showed a single layer and spongy parenchyma showed four to five layers. The cuticle was relatively thin (less than 25 % of the adjacent epidermal cell size) and stomata occurred in the same level of other epidermal cells.

Quantitative analysis

The PCA showed an overlap of the three studied forest sites regarding anatomical variables, indicating a common anatomical pattern among the areas, with a greater distribution of sample range in the Deciduous Forest (Fig. 5). The studied forests differed significantly in four anatomical variables among the 22 analyzed ($p < 0.001$; Tab. 1).

In the midrib, the vascular bundle diameter (VBD) showed higher values in Dense Forest. In the leaf lamina (cross-section), the thickness of palisade parenchyma (PPT) was significantly higher in Deciduous Forest. In the paradermal section, the variables which differ significantly among forest types were stomatal density (STD) and trichome density in adaxial epidermis (TRIAD), both with higher values in Open and Deciduous Forest (Tab. 1).

Discussion

Our results showed that leaf anatomical traits of *Justicia calycina* were overall similar among the three forest sites – Dense, Open and Deciduous Forest – studied in the Southern Brazilian Amazon. We found only few significant differences among forest sites, which may be related to variations in luminosity conditions. The leaf traits which differed among forests showed high plasticity, representing a certain degree of acclimation/adaptation of *J. calycina* according to Valladares *et al.* (2006).

The thicker palisade parenchyma and higher stomatal and trichome density (PPT, STD and TRIAD, respectively) we verified in the leaves of Deciduous Forest may be interpreted as typical changes of sun plants (*i.e.*, plants under higher luminosity conditions). This forest is located in a rocky outcrop and showed significant greater canopy openness due to the sparse distribution of woody plants (Silva *et al.* 2020).

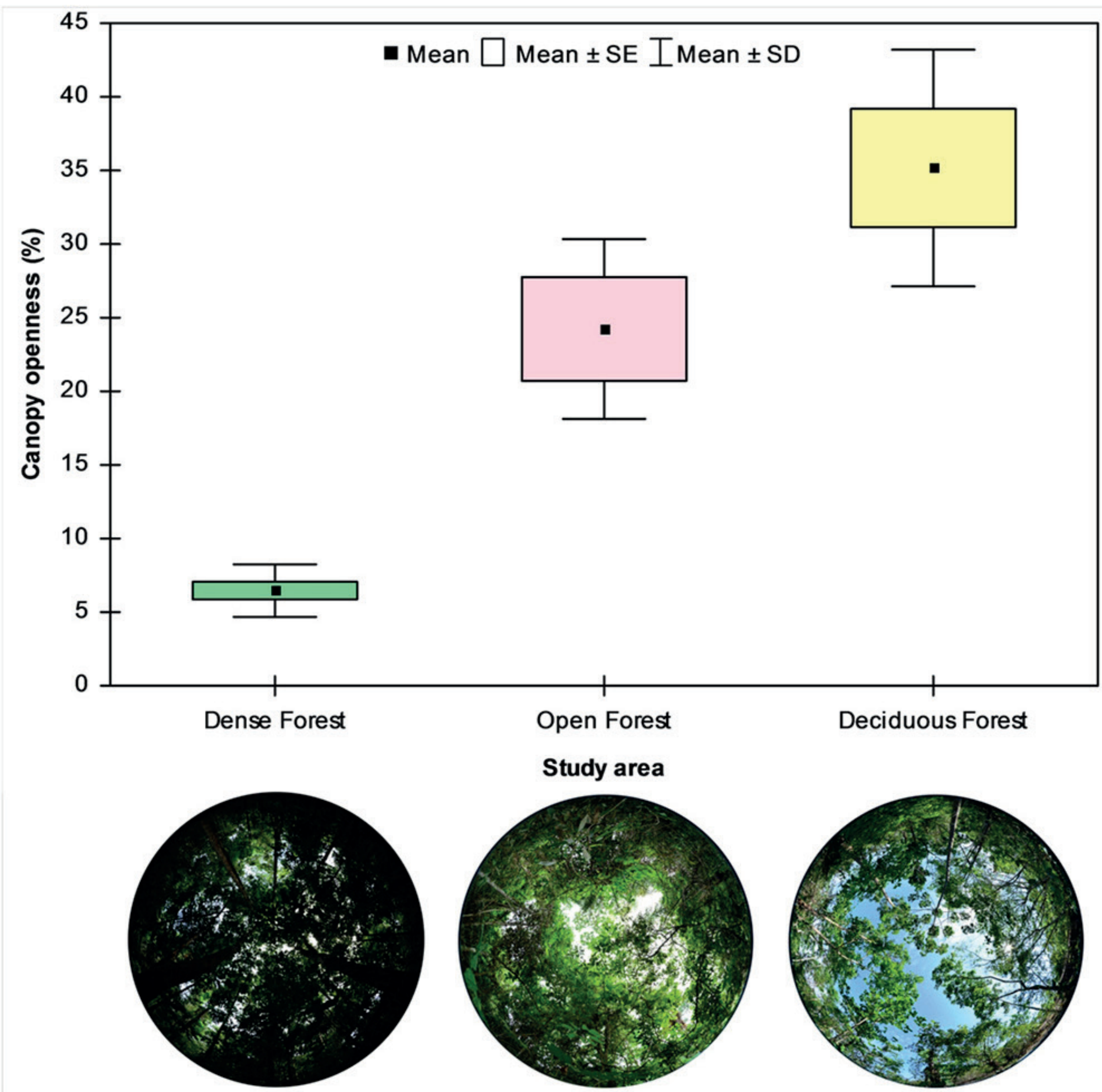

Figure 2. Comparison of canopy openness (%) among the three forests where we sampled leaves of *Justicia calycina* and respective hemispheric photographs examples. Kruskal-Wallis test revealed significant differences among forest sites (H = 11.76, p = 0.0028).

Along with this, at the time of leaf sampling for anatomical measurements (rainy-dry transition season), woody plants of the Deciduous Forest start to shedding leaves (Sasaki *et al.* 2010; E. Gressler, personal communication), which contributes to making this forest even more illuminated in comparison to the other two studied forests (both evergreen). Sun leaves usually contain an increased number of palisade layers and greater palisade cell elongation (Lange *et al.* 1981; Dickison 2000; Gratani 2014). The elongation of palisade cells in sun leaves has been related to a reduction in mesophyll resistance to CO_2 and optimization of light absorption allowing light to channel deeper into lower leaf cell layers, such as the spongy cells where the light is scattered (Vogelmann & Martin 1993; Théroux-Rancourt & Gilbert 2017).

Regarding stomata, the higher density (STD) we observed in the Open and Deciduous Forests may also be explained by higher luminosity in these forests. Stomata of a given species can largely vary in quantity, distribution, size and shape in response to environmental factors (Willmer

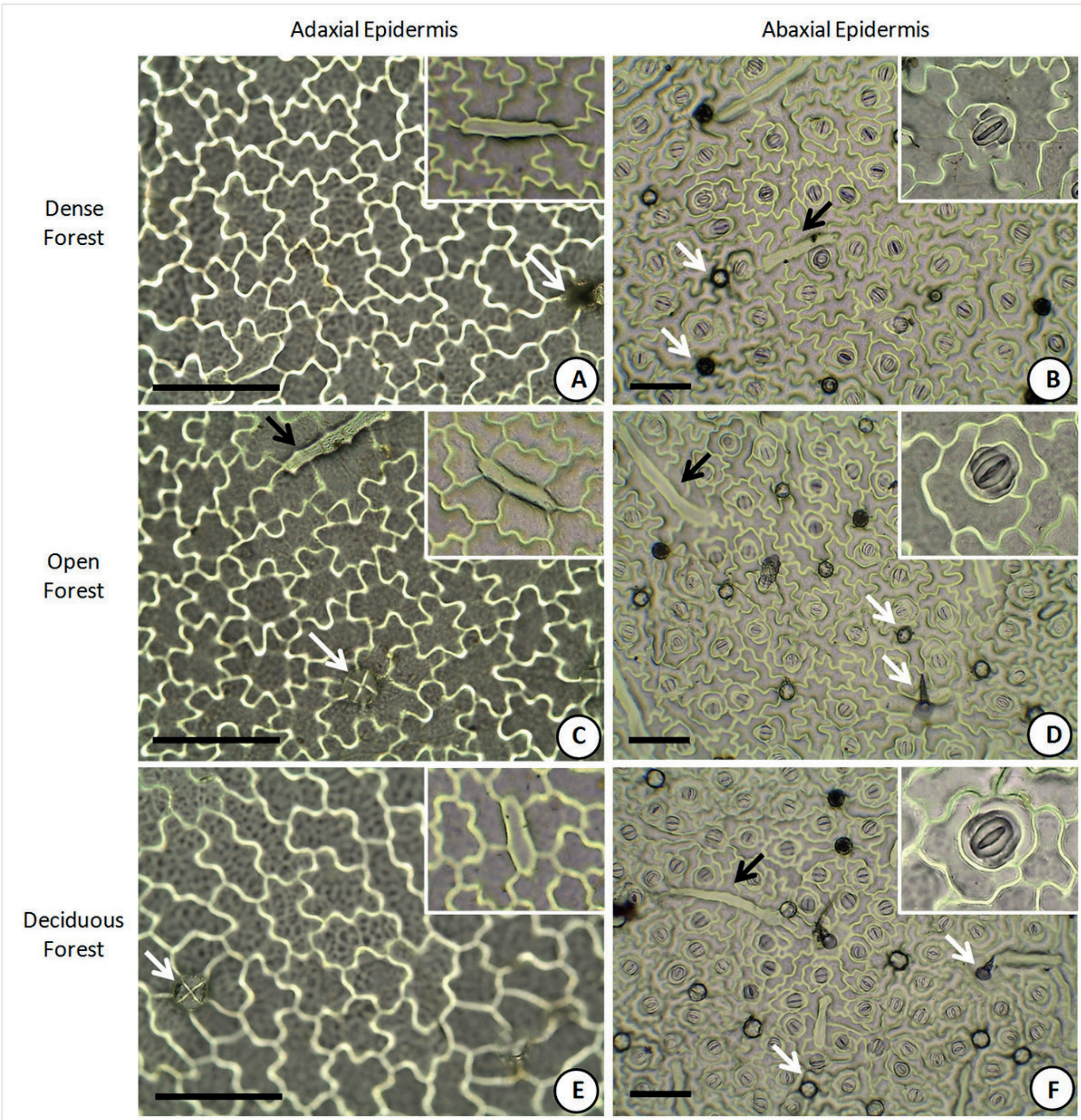

Figure 3. Paradermal sections of the leaf lamina of *Justicia calycina* in three forest types in Southern Brazilian Amazon: Dense Forest (A-B), Open Forest (C-D) and Deciduous Forest (E-F). Adaxial epidermis with cystoliths in detail (A, C, E). Abaxial epidermis with stomata positioning in detail (B, D, F). White arrows indicate trichome or scars and black arrows indicate cystoliths. Bars: 100 µm.

& Fricker 1996; Larcher 2003; Camargo & Marenco 2011). Sun leaves usually present high stomatal frequency, being necessary to maximize CO_2 absorption rates, to water saving and to prevent excessive leaf heating under high irradiance (Givnish 1988; Bertolino *et al.* 2019; Lambers & Oliveira 2019). The lower stomatal density in Dense Forest can be an indicator of larger leaves in this forest, because stomata are more spread out in these leaves (Franks & Farquhar 2007).

The higher trichome density in adaxial epidermis (TRIAD) of *J. calycina* leaves from the Decidual Forest may be related to the protection of intense luminosity and desiccation, given the high light intensities of this forest. Highly pubescent surfaces can reflect sunlight and decrease transpiration rates, especially in the adaxial epidermis which is exposed directly to sun and therefore present higher temperature and transpiration than abaxial epidermis (Fahn

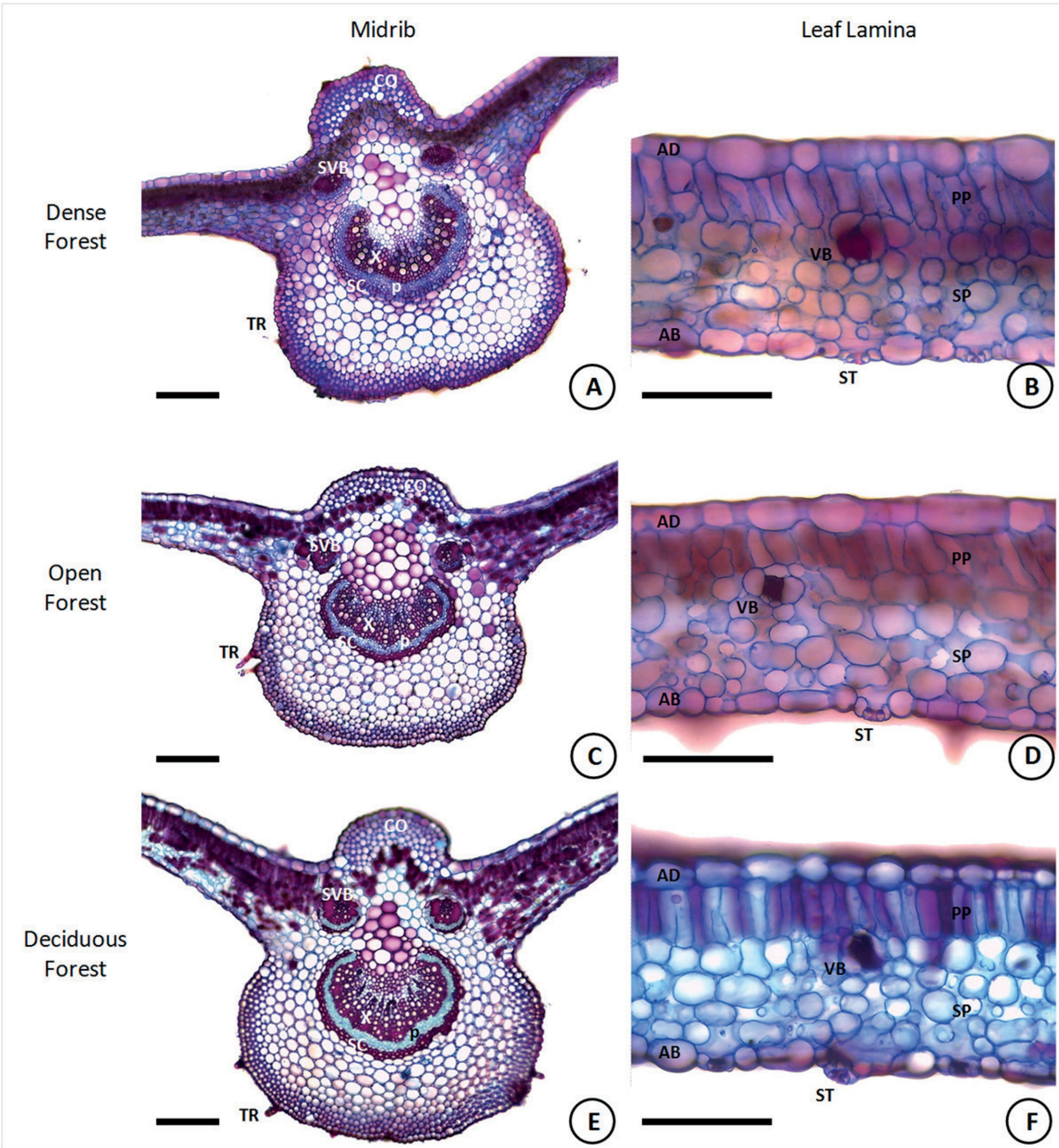

Figure 4. Cross-sections of the leaf midrib (**A, C, E**) and lamina (**B, D, F**) of *Justicia calycina* in three forest types in Southern Brazilian Amazon: Dense Forest (**A-B**), Open Forest (**C-D**) and Deciduous Forest (**E-F**). Abbreviations: P = phloem; X = xylem; SC = sclerenchyma; CO = collenchyma; SVB = secondary vascular bundle; TR = trichome; AD = abaxial epidermis; AB = abaxial epidermis; VB = vascular bundle; PP = palisade parenchyma; SP = spongy parenchyma; ST = stomata. Bars: 200 µm (A, C, E); 100 µm (B, D, F).

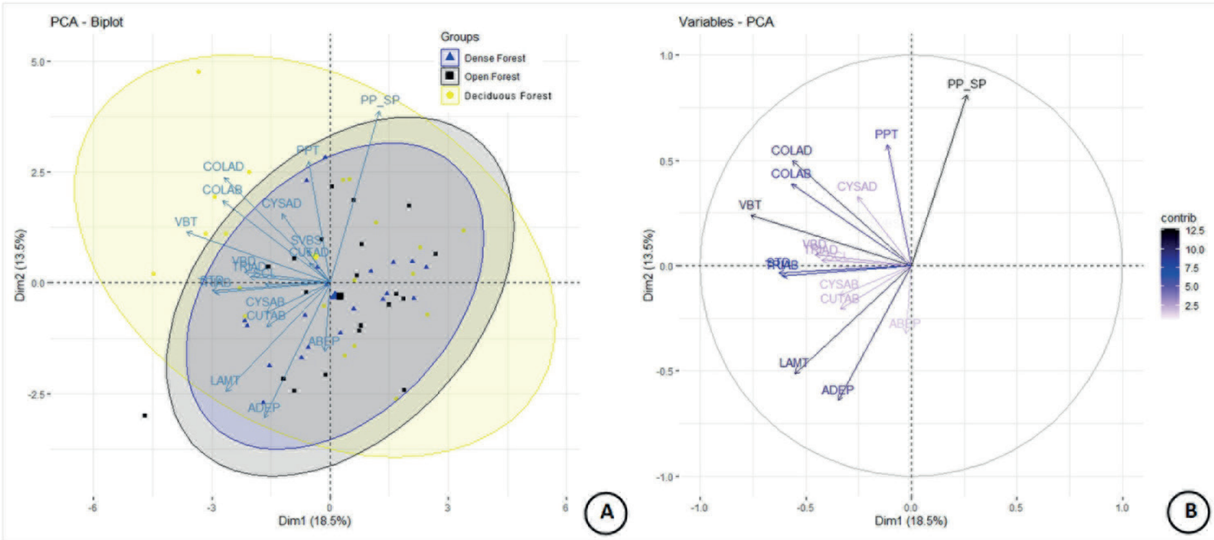

Figure 5. Principal Component Analysis (PCA) with 18 leaf anatomical variables of *Justicia calycina* in three forest sites in Southern Brazilian Amazon (**A**) and contribution of each variable to the distribution of samples (**B**). Forest sites: Dense Forest, Open Forest and Deciduous Forest.

Table 1. Means and standard deviations of 22 leaf anatomical traits measured in *Justicia calycina* in three forest sites, Southern Brazilian Amazon. VBS, SPT and STI were excluded from the analysis due to high correlation values with other leaf traits. *Significant differences among forest sites detected in Dunn's multiple comparison test (p < 0.001); different letters indicate statistical difference on the line. Ns: non-significant result. PI = plasticity index.

Variables	Abbreviations	Dense Forest	Open Forest	Deciduous Forest	PI
Midrib size (μm)	MIDS excluded	1252.43 ± 166.19	1140.83 ± 167.59	1178.10 ± 254.44	
Vascular bundle diameter (μm)	VBD *	616.33 ± 147.96a	485.02 ± 105.99b	515.19 ± 142.22ab	0.63
Vascular bundle thickness (μm)	VBT ns	205.32 ± 32.30	200.32 ± 20.43	204.72 ± 41.51	
Vascular bundle total size (μm)	VBTS ns	821.65 ± 157.21	685.34 ± 116.18	719.91 ± 173.20	
Sclerenchyma thickness (μm)	SCL ns	29.48 ± 4.17	29.52 ± 5.57	29.19 ± 6.27	
Collenchyma thickness adaxial epidermis (μm)	COLAD ns	86.04 ± 11.92	76.49 ± 10.54	84.71 ± 16.36	
Collenchyma thickness abaxial epidermis (μm)	COLAB ns	61.17 ± 13.30	58.36 ± 12.78	60.66 ± 13.18	
Cuticle thickness adaxial epidermis (μm)	CUTAD ns	4.64 ± 1.16	5.73 ± 1.74	5.44 ± 1.64	
Cuticle thickness abaxial epidermis (μm)	CUTAB ns	3.29 ± 1.34	3.11 ± 1.20	3.91 ± 1.09	
Adaxial epidermis thickness (μm)	ADEP ns	26.08 ± 6.43	24.28 ± 6.68	23.48 ± 4.77	
Abaxial epidermis thickness (μm)	ABEP ns	19.40 ± 5.53	20.30 ± 4.01	17.42 ± 5.67	
Palisade parenchyma thickness (μm)	PPT *	36.96 ± 6.40b	37.05 ± 6.19ab	42.30 ± 6.99a	0.52
Spongy parenchyma thickness (μm)	SPT excluded	84.34 ± 14.36	82.26 ± 18.39	80.91 ± 22.18	
Palisade to spongy parenchyma ratio ($\mu m \cdot \mu m^{-1}$)	PP_SP ns	0.45 ± 0.11	0.47 ± 0.13	0.55 ± 0.15	
Secondary vascular bundle size (μm)	SVBS ns	61.80 ± 12.28	64.34 ± 21.42	64.14 ± 9.86	
Lamina thickness (μm)	LAMT ns	167.88 ± 19.49	164.50 ± 19.64	169.32 ± 27.88	
Stomatal index abaxial epidermis (%)	STI excluded	18.63 ± 2.80	22.95 ± 2.70	23.56 ± 4.69	
Stomatal density abaxial epidermis (stomata per mm^2)	STD *	135.24 ± 44.05b	175.48 ± 49.51a	171.82 ± 51.14ab	0.65
Trichome density adaxial epidermis (trichome per mm^2)	TRIAD *	9.42 ± 4.14c	10.80 ± 6.87ab	14.59 ± 4.10a	0.78
Trichome density abaxial epidermis (trichome per mm^2)	TRIAB ns	26.88 ± 7.57	32.28 ± 8.93	31.24 ± 9.44	
Oblong cystoliths density adaxial epidermis (cystoliths per mm^2)	CYSAD ns	2.53 ± 2.22	3.45 ± 2.17	4.36 ± 2.57	
Oblong cystoliths density abaxial epidermis (cystoliths per mm^2)	CYSAB ns	8.38 ± 3.36	8.27 ± 3.02	9.30 ± 3.37	

& Cutler 1992; Roy *et al*. 1999; Dickison 2000; Larcher 2003; Ichie *et al*. 2016).

The epidermal cells of the adaxial surface of *J. calycina* showed thicker walls and slightly less sinuous outline in Deciduous Forest compared to the other forest sites. In environments with high luminosity intensity, such as the Deciduous Forest, epidermal cells usually have thicker walls and less or no sinuosity (Evert 2013). According to Roth (1984), thicker walls increase the protective character of the epidermis.

The greater diameter of the central vascular bundle (VBD) in Dense Forest could indicate that in this forest, due to the significant lower luminosity, plants of *J. calycina* have larger leaves and therefore need to invest more in supporting tissues and major veins (*e.g.*, Chazdon 1985; Niinemets & Sack 2006). Unfortunately, we did not measure the size and area of the collected leaves to confirm this hypothesis.

The few anatomical differences we detected among forests in *J. calycina* may be explained by the interaction of luminosity and other environmental variables such as temperature and humidity. Plants in rocky outcrops, as the studied Deciduous Forest, usually deal with stressful environmental conditions such as high insolation and temperature, desiccant winds, low humidity and poor and shallow soil (Prance 1996; Porembski & Barthlott 2000). According to Porembski (2007), rocky outcrops offer several microhabitats, such as crevices, depressions and soil islands, causing great microenvironmental variation. In the studied Deciduous Forest, the higher annual rainfall may contribute to maintaining a certain degree of humidity in the forest. Another explanation for the few anatomical differences among the studied forests is the intense herbivory observed in *J. calycina* leaves (MB Lauton *et al*., unpubl. res.), which may limit plant phenotypic plasticity (Valladares *et al*. 2007).

In the same region and similar forest types of our study, other studies have also found anatomical variations in understory species related to environmental conditions, though with overall low plasticity index (Ariano & Silva 2016; Dardengo *et al*. 2017; Rocha *et al*. 2019; Müller *et al*. 2020). On the other hand, in savannas and cerrado phytophysiognomies in the Cerrado-Amazon transition of Mato Grosso State, some studies revealed phenotypic plasticity with high occurrence of xeromorphic anatomical traits in most species (Simioni *et al*. 2017; Pessoa *et al*. 2019). It is well known that leaf anatomical traits vary among species, according to their ability to acclimation and intensity of light received (*e.g.*, Rozendaal *et al.* 2006; Markesteijn *et al*. 2007; Liu *et al*. 2019). The plasticity in anatomical responses that we found in *J. calycina* individuals under different luminosity conditions probably contributes to its wide distribution in different forest phytophysiognomies of the Amazon region.

To the best of our knowledge, this is the first study of the functional leaf anatomy of *J. calycina* comparing different forest phytophysiognomies in Brazil. Contrary to our expectations, forest sites with different canopy openness (or light intensity) showed only few significant differences in leaf anatomy of *J. calycina*. The differences we found were related to stomatal/trichome density and thickness of palisade parenchyma/vascular tissues, which are fundamental leaf traits in photosynthetic processes and in plant water transport. Our results can help to deepen physiological studies, directing to integrated studies of plant anatomy and physiology, seeking to elucidate the photosynthetic mechanism of this species and its strategies to deal with different environmental factors.

Supplementary material

The following online material is available for this article:

Table S1 - Lauton MB, Gressler E, Oliveira JA, Simioni PF, Ribeiro-Júnior, Yamashita OM, Silva IV. Does the functional leaf anatomy of *Justicia calycina* (Acanthaceae) reflect variation across a canopy gradient in the Southern Brazilian Amazon? Matrix of Spearman correlations for the 22 leaf anatomical variables of *Justicia calycina* evaluated in three forest sites, Southern Brazilian Amazon. High correlation (≥ 0.70).
Figure S1 - Meteorological data sampled in 2016 in the forest sites where we studied leaf anatomy of *Justicia calycina*, in the Southern Brazilian Amazon. Minimum temperature (Tmin), mean temperature (Tmean) and maximum temperature (Tmax) (A-B). Rainfall and air relative humidity (C-D). The rainy season (October to April) is represented by the boxes in the respective months.
Figure S2 - Leaf anatomical traits measured in *Justicia calycina* in Southern Brazilian Amazon.

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Authors contribution

Maísa B. Lauton: Played equal role. Performed field work and leaf anatomical measurements. Wrote and edited the manuscript; Eliana Gressler: Played equal role. Conceived and designed research. Performed field work and statistical analysis. Wrote and edited the manuscript; Jaqueline A.Oliveira: Performed field work and leaf anatomical measurements; Priscila F. Simioni: Wrote and edited the manuscript; Norberto G. Ribeiro-Júnior: Performed statistical analysis; Oscar M. Yamashita: Wrote and edited the manuscript; Ivone V. Silva: Conceived and designed research. Wrote and edited the manuscript; All authors contributed on drafts and approved the final version for publication.

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