Plant anatomy: history and future directions

Foliar plasticity of *Erythroxylum tortuosum* (Erythroxylaceae) in the *Cerrado*: a representative neotropical genus with medicinal importance

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Abstract

Erythroxylum is common in the Brazilian *Cerrado*. Species from this genus have ecological, pharmacological and popular medical importance. Based on this information, the aims of this study was to perform a descriptive and quantitative morphoanatomical study of *Erythroxylum tortuosum* leaves present in two environments: *cerrado sensu stricto* and *campo rupestre*. To carry out these studies, 24 fully developed leaves were collected and fixed from individuals across both environments. For the quantitative anatomy study, the following measurements were made: leaf area, length, width and mass; stomatal density; polar and equatorial diameter of the stomata; thickness of the leaf blade, mesophyll, epidermis and cuticles on the adaxial and abaxial surfaces; and thickness of the spongy and palisade parenchyma; specific leaf area, leaf mass area and foliar tissue density. This species shows a bald and uniseriate epidermis when viewed in cross section. Stomata are paracytic and occur only on abaxial surfaces. The mesophyll is dorsiventral type, with a dense vascular system, and the vascular bundle is collateral type. The general pattern of the veins is pinnate camptodrome and brochidodrome, with dense veins and pseudosecondary ribs. Significant differences were found for almost all analysed variables, suggesting that different environmental conditions may influence plant development.
Key words: ecological anatomy, medicinal plants, *mercúrio-do-campo*, plant anatomy, plant morphology.

Resumo

Erythroxylum é um gênero bastante comum nos Cerrados Brasileiros. Espécies deste gênero possuem grande importância ecológica, farmacológica e na medicina popular. Baseado nestas informações, o objetivo deste trabalho foi realizar um estudo morfoanatômico descritivo e quantitativo das folhas de *Erythroxylum tortuosum* presentes em duas fitofisionomias: cerrado *stricto sensu* e campo rupestre. Para a realização dos estudos, 24 folhas completamente expandidas foram coletadas e fixadas de indivíduos presentes nos dois ambientes. Para o estudo da anatomia quantitativa, as seguintes medidas foram obtidas: área foliar, largura, comprimento e massa foliar; densidade estomática, diâmetro polar e equatorial do estômato; espessura da lâmina foliar, mesofilo, epiderme e cutícula das faces adaxial e abaxial; e espessura do parênquima paliçádico e esponjoso; área foliar específica, área de massa foliar e densidade dos tecidos foliares. Esta espécie apresenta epiderme glabra e unisseriada, quando vista em secção transversal. Os estômatos são paracíticos e ocorrem apenas na face abaxial. O mesofilo é dorsiventral, com um sistema vascular denso e feixe vascular é do tipo colateral. O padrão geral da venação é pinada, camptódroma e broquidódroma, com veias densas e nervuras pseudosecundárias. Diferenças significativas foram encontradas em quase todas as variáveis analisadas, sugerindo que condições ambientais diferentes podem influenciar o desenvolvimento da planta.
Palavras-chave: anatomia ecológica, plantas medicinais, mercúrio-do-campo, anatomia vegetal, morfologia vegetal.

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Introduction

The Brazilian *Cerrado* is an ecosystem with one of the largest biodiversity in the world (Ribeiro & Walter 2008) and it is considered a hotspot of diversity. However, this domain has been suffering from significant anthropic changes over the years, and now only 19,8% of its original flora remains (Strassburg *et al*. 2017). The *cerrado* can be classified in many ways due to their different vegetation physiognomies, such as *cerrado sensu stricto*, *campo rupestre*, among others (Ribeiro & Walter 2008).

The *cerrado sensu stricto* occupies approximately 70% of the *cerrado* domain (Assunção & Felfili 2004), and its main traits are short trees growing on an inclined and windy slope that show irregular shapes and twists, usually presenting the evidence of fire. The leaves of most species are rigid and leathery (Ribeiro & Walter 2008). According to Ribeiro & Walter (2008), many factors can interfere with tree density, such as edaphic conditions, pH, aluminium saturation, fertility, water conditions, soil depth, fire frequency and anthropic actions.

Campos rupestres occur at altitudes above approximately 900 metres and are associated with outcrops of quartzite, sandstone and iron ore (Vasconcelos 2011). On this vegetation physiognomy, there is a predominance of herbaceous and shrub species and undeveloped small trees. Their floristic composition varies over short distances, and the species density depends on the substrate. The species in these regions have some xeromorphic traits, such as small, thick leathery leaves (Ribeiro & Walter 2008).

In regard to the floristic diversity in this area, *Erythroxylum* P. Browne (Erythroxylaceae) is very common; it is the only genus of this family in the neotropical region, with approximately 240 species (Araújo *et al*. 2015). In Brazil, 135 species were identified, of which 80 are endemic [Flora do Brasil 2020 (continuously updated)]. Their species have high ecologic importance, pharmacological applications and popular medical applications (Loiola *et al*. 2001; Albuquerque *et al*. 2014; Elias *et al*. 2016; Restrepo *et al*. 2019) mainly due to the presence of specific metabolites, such as tropic alkaloids, tannins, terpenes and phenylpropanoids (Evans 1981; Nascimento *et al*. 2012; Silva-Jr *et al*. 2021). According to Zuanazzi *et al*. (2001), increasing interest in this genus occurred in the 19th century due to the discovery of the pharmacological activities of *Erythroxylum coca* Lam. leaves, which were used by the indigenous tribes from the Andes region in South America. This species also produces cocaine, one of the largest illicit markets on the planet (Restrepo *et al*. 2019). However, among the few papers about the foliar anatomy of *Erythroxylum* (Rury 1981; Bieras & Sajo 2004a, b; Mantuano *et al*. 2006; Simioni *et al*. 2017; Silva & Santos 2023), there are only a few records of the relationship between the foliar structure and the environmental traits of the *Cerrado* or *Campo Rupestre* or the association with species plasticity.

Phenotypic plasticity is an alteration of the genotype due to environmental influences (Bradshaw 2006). As such, leaves present structural variation because of some factors, such as water and light availability (Menezes *et al*. 2013), that may cause modifications to foliar area, parenchyma thickness, stomatal density, epidermal cell size and leaf biomass (Cutter 2002). Anatomical studies are fundamental to the evaluation of these traits, especially when they are associated with ecological, physiological and comparative aspects (Metcalfe & Chalke 1979).

In this context, the morphoanatomical traits of *Erythroxylum tortuosum* Mart. (Erythroxylaceae) species occurring in different *Cerrado* vegetation physiognomies can demonstrate species plasticity responses. Moreover, quantitative anatomical evaluations may contribute to further work related to ecology, pharmacology and popular medicine, as well as improve the knowledge and conservation of the *Cerrado* flora. Consequently, this work aimed to analyse the phenotypic plasticity in *Erythroxylum tortuosum* occurring in *cerrado sensu stricto* and *campo rupestre* domains, considering that leaf structure traits are influenced by environmental variation.

Materials and Methods

Plant material

The plant material was collected in areas of altered *cerrado sensu stricto* in the recovery stage and *campo rupestre* above Serra do Campestre in the city of Lavras with coordinates 21°20'00.42"S and 44°58'09.66"W, 1,041 m altitude, and 21°20'45"S and 44°58'38.40"W, 1,159 m altitude, respectively. The climate according to the Koeppen classification is Cwb, a mesothermal climate with a dry winter, mild summer and rainy season in the summer. During 2017–2018, the Lavras weather station registered an annual average temperature

of 22,5 °C, average relative humidity of 67%, average insolation of 6,75 h, average maximum temperature of 27,8 °C and minimum temperature of 16,2 °C, and average daily rainfall of 2.66 mm (INMET 2021).

Voucher species were deposited at the ESAL Herbarium of Universidade Federal de Lavras under numbers 30343 and 30344, one from each study area. Species identification was based on Loiola's work (2001) and specimens deposited in the ESAL herbarium. To carry out the analysis, fully expanded leaves located at the third node of 12 individuals were collected from each area, for a total of 24 observations for each vegetation physiognomy.

Leaf anatomy
The anatomical sections were carried out manually with the help of a steel blade. To study the epidermis, paradermal sections were made and subsequently stained with 1% safranin and mounted on semipermanent slides and coverslips with 50% glycerine (Johansen 1940). To study the leaf blade and petiole, the cross sections were clarified in a 50% sodium hypochlorite solution for approximately 1 minute, washed in distilled water twice for 10 minutes, stained with safrablau solution (0,1% astra blue and 1% safranin in a 7:3 proportion), and mounted on semipermanent slides and coverslips with 50% glycerine (Bukatsch 1972; Kropp 1972).

Photomicrographs were taken with a Nikon light microscope, model Eclipse E100, and a stereomicroscope, both coupled to an Infinity capture camera. The diagrams were made using an Olympus CBB microscope coupled with a drawing tube (clear chamber). Measurements were performed using ImageJ image analysis software.

Stomata counts and measurements were made in three regions: the apex, middle and base of the leaf blade. Three slides of each region were analysed. From the quantitative analyses of the leaf epidermis, stomatal density (number of stomata per mm²) was obtained in each of the regions, as well as the average density of the leaf, the polar diameter average of each region of the leaf (μm), and the equatorial diameter (μm) of the leaf. The total stomatal pore area index (SPI, a dimensionless index of stomatal pore area per leaf blade area) was calculated as stomatal density × cell length guard[2] (Sack *et al*. 2003). The measurement of the stomatal polar diameter was considered equivalent to the guard cell length. The stomatal index was calculated by the equation $EI = NE/(NE + NC) \times 100$, and it represents the value of the stomatal index. NE represents the number of stomata per unit of leaf area, and NC represents the number of epidermic cells in the same area (Salisbury 1927).

For the analysis of the internal foliar tissues, a slide with cross sections was obtained for each individual, and adaxial and abaxial surface cuticle and epidermis thickness (μm), thickness of the palisade (μm) and spongy (μm) parenchyma, thickness of the mesophyll (μm), and leaf blade thickness (μm) were observed.

Leaf venation
Leaves were fixed in $FAA_{70\%}$ (formaldehyde, 5 mL; acetic acid, 5 mL; and 70% ethyl alcohol, 90 mL) for 72 hours (Johansen 1940) and later preserved in 70% ethyl alcohol (Jensen 1962).

For the study of the leaf veins, fragments of leaves that included the primary vein to the leaf edge in the middle region of the leaf were used. The fragments were diaphanized in 5% aqueous sodium hydroxide solution and renewed daily until complete leaf clarification. Afterwards, the material was placed in a 50% (v.v) aqueous solution of sodium hypochlorite until the clarification was complete, and the solution was neutralized with 5% aqueous acetic acid solution (Handro 1964).

The clarified material was washed in distilled water and then washed in 50% ethyl alcohol and stained with hydroalcoholic solution of 1% safranin or acidified 1% aniline blue solution (Handro 1964). After staining, the leaves were mounted between glass slides in glycerinated gelatine (Roman 1971). Leaf fragments that were needed to observe finer details of the veining pattern were mounted between specially sized blades made of glass and slides made of VETEC's Canada Balsam after dehydration and diaphanization in the alcohol-xylol series.

The photomicrographs of the slides were made under a Nikon Eclipse E100 light microscope and stereomicroscope, both coupled to an Infinity capture camera. The diagrams were made using an Olympus CBB microscope coupled with a drawing tube (clear chamber).

Mass and foliar dimensions
To obtain the fresh and dry masses of the leaves, a Shimadzu precision scale (model AY220) was used. To obtain the dry mass, 24 leaves of each vegetation physiognomy were submitted to a drying press. Daily weighing was carried out until a constant dry mass was obtained. Using this method, it was possible to obtain dry and fresh masses in

grams. The fresh mass was obtained at the first weighing on the same day as collection.

To carry out measurements of leaf area, length and width, scans of the leaves were performed using an Epson scanner (Epson Perfection V330 Photo). The images were analysed using ImageJ software and calibrated with a scanned ruler. Three measurements were taken for each leaf: one for length (cm), one for width (cm) and one for area (cm^2).

The water content of the leaves was calculated using the following equation:

H_2O content (%) = [1 - (dry leaf mass/fresh leaf mass) * 100]

The leaf succulence was obtained using the following equation:

S (g H_2O cm^2) = (fresh leaf mass - dry leaf mass)/fresh leaf area

H_2O content and S can be combined to determine the leaf moisture index (Bussotti *et al.* 2002).

Specific leaf area, leaf mass area and foliar tissue density

The specific leaf area (SLA, cm^2 g^{-1}), which describes the amount of foliar area for light capture per unit of biomass invested, was measured as the ratio between the area of fresh leaves and dry mass of leaves (Radford 1967). Moreover, the leaf mass area (LMA, g cm^{-2}), which represents the cost of light interception at the leaf level (Gutschick & Wiegel 1988), was obtained as the ratio between dry mass and leaf area.

For the calculation of leaf tissue density (mg cm^{-3}), the following equation was used:

LTD = leaf dry mass/(leaf area × leaf thickness)

LMA and LTD are considered sclerophilic indices (Bussotti *et al.* 2002).

Statistical analysis

The data were submitted to the Shapiro-Wilk test of normality and Levene test for data homogeneity and then to the T test for parametric data and Mann-Whitney test for nonparametric data with 0,05% significance. R software was used to perform the analyses.

Results

Leaf anatomic description

In paradermal sections, the epidermis is bald, with irregularly polygonal shaped cells, and is larger on the adaxial surface (Fig. 1). In cross section, the epidermis is uniseriate but sometimes presents two layers. Cells with irregular shapes and sizes, tending to a polyhedral shape, with anticlinal walls without sinuosity were observed. In cross section, stomata are located at the same level as the other epidermal cells (Fig. 2). The mesophyll is dorsiventral type, and the palisade parenchyma presents three cell layers with a cylindrical format that is elongated, narrow, and adjacent cells, with few intercellular spaces. The spongy parenchyma presents 3–6 cell layers that vary in shape from circular to irregular and are close to the epidermis of the abaxial surface (Fig. 2).
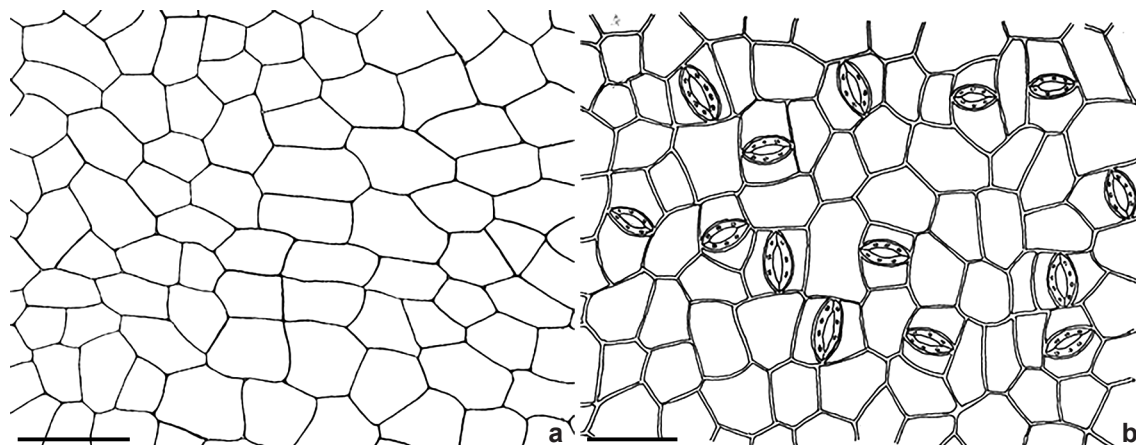

Figure 1 – a-b. Representation of *Erythroxylum tortuosum* adaxial (a) and abaxial epidermis (b) in frontal view. Scale bars = 100 μm.
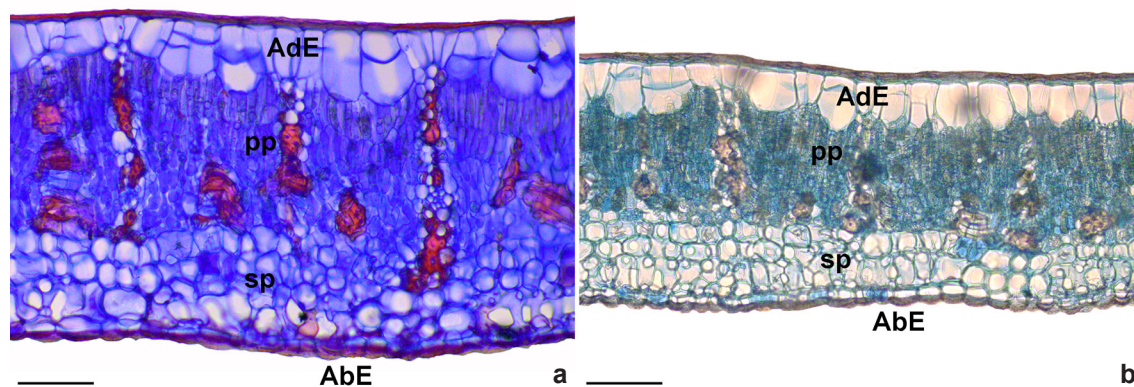
Figure 2 – a-b. Cross sections of *Erythroxylum tortuosum* mesophyll collected at *cerrado sensu stricto* (a) and *campo rupestre* (b). AbE = abaxial epidermis; AdE = adaxial epidermis; pp = palisade parenchyma; sp = spongy parenchyma. Scale bars = 100 μm.

The adaxial and abaxial surfaces of the epidermis that cover the midrib are bald, devoid of stomata and the epidermis cells have a rounded shape. The adaxial surface presents larger cells than the abaxial surface. Below the epidermis, there are some collenchyma layers. The fundamental parenchyma is composed of cells of varying sizes with rounded contours and small intercellular spaces. The vascular system is very dense, open and arch-shaped, forming a "horseshoe", with flat lateral edges, the presence of only one vascular bundle of the collateral type, and fibre caps on the external face of the phloem (Fig. 3).

The epidermis that lines the leaf edge is devoid of stomata, with epidermal cells of a rectangular to rounded shape, which decrease toward the leaf edge. Below the epidermis, the palisade and spongy parenchyma meet at the leaf edge, with rounded cells tending to a polyhedral shape, with few intercellular spaces. It is possible to notice the presence of fibres spread throughout the mesophyll (Fig. 2).

The general pattern of the veins was pinnate camptodrome and brochidodrome, with ovate-type blades and slightly asymmetrical bases. The primary vein is sharp, median, and tapering towards the apex, with a straight course and no branches (Fig. 4). The secondary veins are alternating and ascending, with an acute angle of divergence, moderate thickness and a slightly curved course at the base, with frequent simple or compound intersecting ribs. The tertiary veins are visible in the green leaf. The quaternary ribs have a relatively random course, and the vein close to

the edge is in an arc form (Fig. 4d). The ribs are simple and branched (Fig. 4f), and the halos are well developed, tending to a polyhedral shape (Fig. 4a).
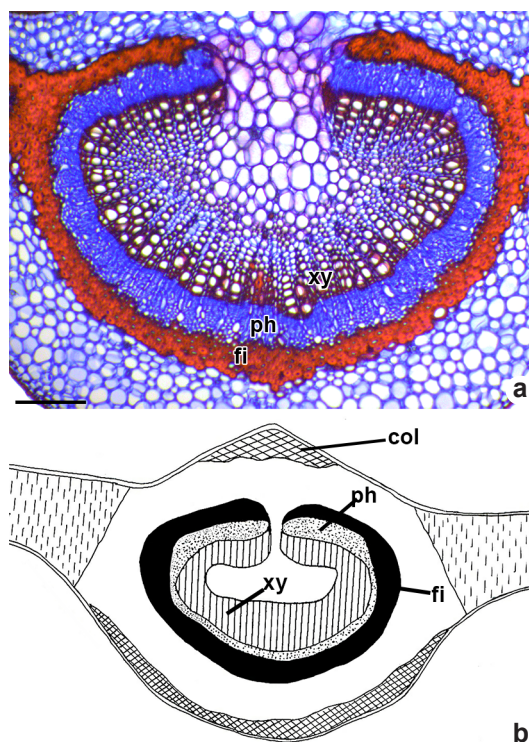

Figure 3 – a. Cross section of *Erythroxylum tortuosum* midrib (scale bar = 150 μm). b. Representation of *Erythroxylum tortuosum* midrib (scale bar = 100 μm). col = collenchyma; fl = floem; fi = fiber; xil = xylem.

Leaf quantitative morphoanatomy
The leaves from *cerrado sensu stricto* plants presented a larger leaf area, length and width than the leaves of *campo rupestre* plants (Tab. 1). The fresh and dry mass are both higher in *cerrado*.

The specific leaf area (SLA) (Tab. 1) did not vary significantly between environments. Among the sclerophilic indices evaluated through the leaf mass area (LMA) and leaf tissue density (LTD), only the LTD presented a significant difference (Tab. 1).
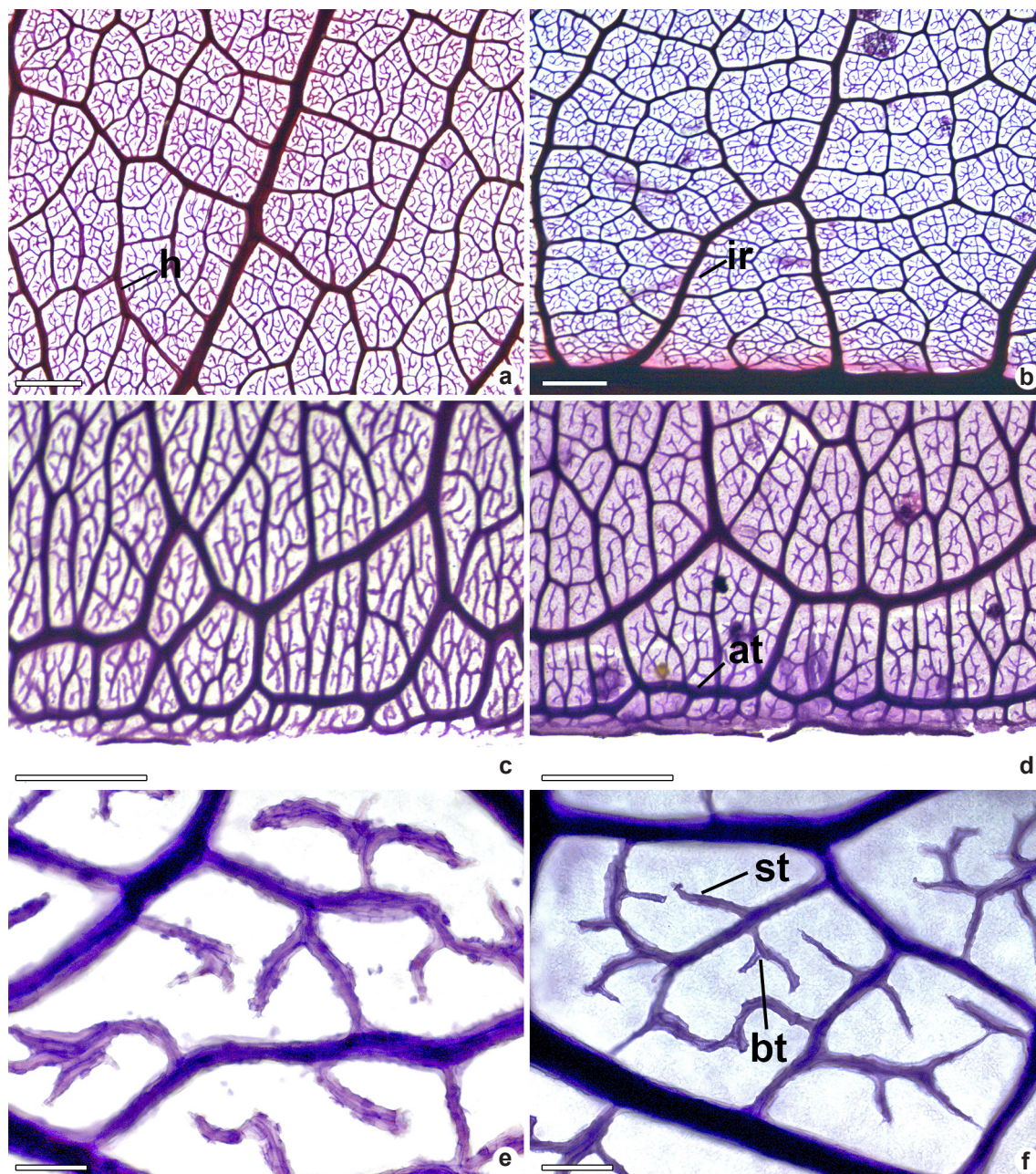

Figure 4 – a-f. Details of secondary and tertiary ribs in *cerrado sensu stricto* (a) and *campo rupestre* (b) diaphanized leaves, leaf edge in *cerrado sensu stricto* (c) and *campo rupestre* (d); details of halos and ribs in *cerrado sensu stricto* (e) and *campo rupestre* (f) leaves. at = arc termination; btbt = branched termination; h = halo; ir = intersecondary rib; st = simple termination. (Scale bars: a-d = 1000 μm; e-f = 100 μm).

Table 1 – Morphological, moisture-related and sclerophilic traits of *Erythroxylum tortuosum* leaves in the *cerrado sensu stricto* and *campo rupestre* environments.

	Cerrado sensu stricto	*Campo rupestre*
Leaf area (cm²)	74,15 (±15,44) a	50,29 (±13,41) b
Leaf length (cm)	22,34 (±2,57) a	19,87 (± 1,70) b
Leaf width (cm)	5,24 (±0,50) a	4,77 (±0,50) b
Fresh mass (g)	3,06 (±0,83) a	2,39 (±0,61) b
Dry mass (g)	2,02 (±0,74) a	1,45 (±0,40) b
Specific leaf area (SLA, cm² g^{-1})	39,89 (±2,33) a	37,71 (±2,65) a
Leaf mass area (LMA, g cm^{-2})	0,028 (±0,001) a	0,030 (±0,002) a
Leaf tissue density (LTD, mg cm^{-3})	0,61 (±0,03) b	0,91 (±0,07) a
H$_2$O content (%)	33,29 (±3,35) a	41,27 (±3,22) a
Succulence (g H$_2$O cm²)	0,013 (± 0,002) b	0,022 (± 0,003) a

The averages followed by different letters in the same row differ from each other by the t test for $p < 0.05$ or the Mann-Whitney test. Source: the authors (2019).

For the leaf moisture index, measured by the water content and succulence index (S), only S presented higher and significant values in *campo rupestre* (Tab. 1). The stomata from *campo rupestre* present higher stomatal density and smaller polar and equatorial diamenters when compared to *cerrado sensu stricto*. Futhermore, the stomata index is higher in *campo rupestre* and the total pore área index is higher in *cerrado sensu stricto* (Tab. 2).

After analysing the thickness of the epidermis, it was observed that there were differences only on the adaxial surface, which presented higher values in *campo rupestre* leaves. In the cuticle, both the adaxial and abaxial faces had higher averages in the

Table 2 – Stomatal traits of *Erythroxylum tortuosum* present in *cerrado sensu stricto* and *campo rupestre* environments.

Traits	*Cerrado*	*Campo rupestre*
Stomatal density (mm²)	248 (±65) b	303 (±67) a
Stomatal density (APEX) (mm²)	261 (±63) b	323 (±67) a
Stomatal density (MIDDLE)	244 (±54) a	299 (±76) a
Stomatal density (BASE)	238 (±76) a	287 (±52) a
Polar diameter (μm)	29,983 (±3,433) a	27,988 (±3,216) b
Polar diameter (APEX) (μm)	30,751 (±3,773) a	27,897 (±2,785) b
Polar diameter (MIDDLE) (μm)	29,569 (±3,443) a	27,602 (±3,413) a
Polar diameter (BASE) (μm)	29,631 (±3,059) a	28,567 (±3,480) a
Equatorial diameter (μm)	19,516 (±3,048) a	17,599 (±1,937) b
Equatorial diameter (APEX) (μm)	19,403 (±3,128) a	16,946 (±2,786) b
Equatorial diameter (MIDDLE) (μm)	18,745 (±2,894) a	17,835 (±3,413) a
Equatorial diameter (BASE) (μm)	20,401 (±3,011) a	18,018 (±3,481) b
Stomatal index (%)	15,55 (±0,37) b	17,56 (±0,36) a
Total stomatal pore area index	0,22 (±0,01) a	0,24 (±0,01) a

The averages followed by different letters in the same rows differ from each other by the t test for $p < 0.05$ or the Mann-Whitney test. Source: the authors (2019).

cerrado sensu stricto leaves (Tab. 3). In addition to the epidermis on the adaxial face, modifications in palisade and spongy parenchyma thickness, hence the mesophyll and leaf blade, were observed. The thickness of the leaf blade, mesophyll, palisade parenchyma and spongy parenchyma are higher in *cerrado sensu stricto* than *campo rupestre* (Tab. 3).

Discussion

In this study, a PP/SP ratio of approximately 66% was observed for both *cerrado sensu* and *campo rupestre* leaves, in addition to the presence of bundle sheath extension and the absence of crystals. These differences possibly reflect structural responses to the distinct environments where these plants were collected for both studies. Beiguelman (1962) and Bieras & Sajo (2004b), studying the species *Erythroxylum suberosum* St Hill. and other *Erythroxylum* species, respectively, found anatomic patterns. The authors also highlighted the open arch of the petiole vascular bundle, acuminate leaf margin and epidermis with two cell layers. Bieras & Sajo (2004a) found a PP/SP ratio of 50%, the presence of crystals and bundle sheath extension.

Some traits, such as higher leaf area and mesophyll thickness are related to specific conditions of each physiognomy we studied. *Campo rupestre* is characterized by underwood vegetation and open environments and is subject to higher incident radiation, with poorer and lower soil water retention capacity (Messias *et al.* 2012; Alves & Silva 2014; Fernandes 2016). The *cerrado sensu stricto* may contain abundant arboreal vegetation (Nettesheim *et al.* 2010), which promotes shading on plant leaves present in this environment, making it more favourable for tree growth than *campo rupestre* (Alves *et al.* 2014). According to Kubinová (1991), leaves that develop in environments with higher radiation present a smaller area than leaves that develop in shaded areas. Gavilanes *et al.* (2016) found similar results when studying *Palicourea rigida* Kunth (Rubiaceae) leaves. They concluded that higher averages observed for leaf traits in plants from *cerrado sensu stricto* may be attributed to less inhospitable environmental characteristics that led to the formation of larger and more functional leaves compared to those from *campo rupestre*.

Brodersen *et al.* (2008) explained that a larger leaf area exposed to solar radiation is related to an increase in the light receiving surface. Thus, these alterations may increase incident light capture and increase plant photosynthetic efficiency (Gobbi *et al.* 2011). Moreover, the decrease in leaf size reduces the air layer in contact with the leaf and allows more heat loss to the environment by convection, requiring less transpiration to cool the leaf down (Pooter 1999). This is an important strategy in environments with higher irradiance and lower water availability in the soil, such as *campo rupestre* (Silveira *et al.* 2016). The carbon gain per leaf mass unit was similar across individuals, although the leaf thickness was higher in the *cerrado sensu stricto* plants (Tab. 3). Due to the fact that the leaves are plastic organs, they may vary in mass and area, as they develop in environments with different light intensities and according to the availability of local resources, such as water and nutrients (Gonçalves *et al.* 2005; Boeger *et al.* 2009); these differences can be found between the *cerrado sensu stricto* and *campo rupestre*. About the succulence results, they are probably related to the smaller leaf area and higher fresh mass and dry mass observed in the leaves from this environment

Between the sclerophilic indices only the LTD presented a significant difference, because they are plastic organs, leaves vary in mass and area, as they difference; it was higher in *campo rupestre*. This result shows that *campo rupestre* leaves may present more compact cells and tissues more compact and/or thicker cell walls and cuticles, with more abundant mechanical and vascular tissues (Witkowski & Lamont 1991), which explains the higher sclerophilic indices, despite the smaller mass, compared to leaves of the *cerrado sensu stricto*. According to Gutschick (1999), sclerophilic indices increase under more stressful conditions with intense radiation, poor nutrients and less water availability (Gutschick 1999; Gonçalves-Alvim *et al.* 2006), as is present in *campo rupestre*. Therefore, a higher LTD favours resistance to drought due to a higher resistance to physical damage promoted by desiccation (Mediavilla *et al.* 2001).

We showed a negative relation between stomatal density and size (Tab. 2). A higher stomatal density is related to plants exposed to adverse environments, similar to *campo rupestre*, and may be an adaptation mechanism of these plants to conditions of low water availability (Souza *et al.* 2019). Additionally, it is associated with more efficiency to gas exchange during periods of higher humidity, the increase of conductance and CO_2, essential to the photosynthetic process (Pearce *et al.* 2006; Siqueira *et al.* 2023). Generally, the

Table 3 – Thickness of the epidermis and cuticle of the adaxial and abaxial sides of the leaves of *Erythroxylum tortuosum* in *cerrado sensu stricto* and *campo rupestre* environments.

	Cerrado sensu stricto	*Campo rupestre*
Adaxial surface epidermis (μm)	75,589 (±13,199) a	56,707 (±13,854) b
Adaxial surface cuticle (μm)	6,214 (±1,526) b	7,988 (±2,592) a
Abaxial surface epidermis (μm)	14,948 (±3,050) a	14,270 (±2,147) a
Abaxial surface cuticle (μm)	6,549 (±1,438) b	7,895 (±2,190) a
Leaf blade (μm)	445,022 (± 40,144) a	332,990 (±41,957) b
Mesophyll (μm)	335,731 (±40,585) a	239,500 (±39,881) b
Palisade parenchyma (μm)	126,553 (±19,548) a	93,727 (±18,219) b
Spongy parenchyma (μm)	192,213 (±37,674) a	144,130 (±29,937) b

The averages followed by different letters in the same row differ from each other by the t test for $p < 0.05$ or the Mann-Whitney test.

increase of the stomatal density is accompanied by the decrease of the stomatal size (Harrison *et al.* 2020). The decrease of the stomatal size causes an alteration on the pore depth due to the smaller area of the guard cells, creating a shorter distance to CO_2 absorption inside the leaf (Franks & Farquar 2007; Franks & Beerling 2009). The presence of small area pores increases the speed of stomatal opening when there is water available and closing rapidly in water deficit, resulting in an improvement of water use efficiency (Drake *et al.* 2013).

Regarding to the thickness of the epidermis, according to Esau (1974) and Dickison (2000), environmental factors may influence the thickness and composition of the cuticle, both of which play an important role in reducing water loss, waterproofing and reflecting sunlight; these characteristics probably vary between the studied environments. The decrease in leaf blade thickness in *campo rupestre* leaves may favour CO_2 diffusion within the leaves by decreasing the pathways through which this gas travels to chloroplasts (Cruz *et al.* 2014). Stomata are the main entrance for CO_2 on leaves during photosynthesis, and after photosynthesis, CO_2 needs to diffuse through the mesophyll until it finds chloroplasts. Each part of this pathway offers resistance to gas diffusion, such as the boundary layer resistance, stomatal resistance, intracellular space resistance and liquid phase resistance (Taiz & Zeiger 2009).

Generally, plants exposed to higher radiation have thicker leaf blades; however, in this case, *Cerrado sensu stricto* plants are theoretically exposed to less radiation compared to *campo rupestre* plants, which presented these characteristics. Nevertheless, it is important to emphasize that both environments can present a high incidence of solar radiation. Furthermore, the higher leaf tissue thickness in *cerrado sensu stricto* is consistent with the dry mass results observed here.

Erythroxylum tortuosum presents plasticity in its foliar structure when it occurs in different environments of the *cerrado* and likely adjusts to differences in environmental conditions, such as irradiance and water availability. In *campo rupestre* where there are higher solar incidente irradiation, poorer soil and lower water retention capacity, and *cerrado stricto sensu* presents arboreal vegetation, promoting shading on the leaves, altering their anatomy and morphology.

Acknowledgements

Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

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