

## Plant anatomy: history and future directions

# Elucidating leaf anatomical traits in *Vereda* plants: different survival strategies

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### Abstract

*Vereda* (palm swamp) is a savanna vegetation type widespread in the *Cerrado*. This environment is characterized by high irradiance levels and waterlogged soils for part of the year, resulting in hypoxic conditions, organic matter accumulation, and nutrient limitation. Due to these characteristics, we expect leaf scleromorphism to be the predominant pattern in the community, thus ensuring the survival of *Vereda* plants in these harsh environments. We examined the leaves of 14 species in Vereda Grande in central Brazil to identify their anatomy. We also analyzed the light availability and edaphic aspects of the soils in which these plants grow. We found high irradiance and soils which were rich in organic matter but low in nutrients; these factors limit plant growth given the elevated aluminum values and low pH (mean of 3.6). Most species had thick leaves with a thick cuticle, a well-developed mesophyll, calcium crystals, phenolic compounds, and sclerenchyma. These results corroborate the expected pattern of scleromorphism predicted by the environmental conditions to which *Vereda* plants are subjected. *Chelonathus alatus* did not show a leaf scleromorphic pattern and avoided stress in the dry period by losing its shoots.

**Key words:** *Cerrado*, leaf emergence, scleromorphism, soil chemical analysis, stress avoidance strategy.

### Resumo

A Vereda é um tipo de vegetação savânica que ocorre ao longo do domínio do Cerrado. Este ambiente é caracterizado pela incidência de altas irradiâncias e solos encharcados durante parte do ano, apresentando condições hipóxicas, com acúmulo de matéria orgânica e limitação de nutrientes. Devido a essas características, esperamos que o escleromorfismo foliar seja o padrão predominante na comunidade, permitindo a sobrevivência das plantas de Vereda nesse ambiente. Avaliamos folhas de 14 espécies em Vereda Grande no Brasil central, para identificar seus padrões foliares anatômicos. Analisamos também a disponibilidade de luz e aspectos edáficos dos solos em que as plantas crescem. Encontramos altas irradiâncias e solos ricos em matéria orgânica, mas pobres em nutrientes, fatores que limitam o crescimento das plantas dado os elevados valores de alumínio e o baixo pH (média de 3,6). A maioria das espécies estudadas apresentou folhas espessas com cutícula espessa, mesófilo bem desenvolvido, e presença de cristais de cálcio, compostos fenólicos e de esclerênquima. Esses resultados corroboram o padrão esperado de escleromorfismo previsto pelas condições ambientais a que as plantas de Vereda estão submetidas. *Chelonathus alatus* não mostrou tais padrões escleromorfos foliares e evitou o estresse no período seco perdendo as partes aéreas.

**Palavras-chave:** Cerrado, emergência foliar, escleromorfismo, análise química do solo, estratégia de evitar o estresse.

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## Introduction

The *Vereda* is a common phytophysiognomy along the entire distribution of Neotropical savannas in Brazil (Ribeiro & Walter 2008). As in any typical savanna, the *Vereda* is formed by two strata: one arboreal, where the palm *Mauritia flexuosa* L.f. (Arecaceae) is abundant, occurring together with scattered eudicotyledons trees, and one continuous shrub-herbaceous layer formed by diverse genera of Poaceae, Cyperaceae, Melastomataceae, and Eriocaulaceae (Araújo *et al.* 2002; Silva *et al.* 2017).

The dominance of *M. flexuosa*, associated with other trees, is incapable of providing sufficient canopy cover, which varies between 5 and 10% in such environments, implying constant and elevated irradiance associated with elevated wind velocities (Munhoz & Ribeiro 2008; Guimarães *et al.* 2017).

In addition to this biotic structure, this phytophysiognomy occurs in association with watersheds or close to gallery forests, mainly in flat areas that accompany drainage lines (Araújo *et al.* 2002; Ribeiro & Walter 2008; Guimarães *et al.* 2017). This spatial structure causes the vegetation to be frequently waterlogged, which alters the chemical and biological characteristics of their soils (Araújo *et al.* 2002). The elevated water content in their soils creates an anaerobic environment, in which organic matter originating from vegetation takes a long time to decompose (Sahrawat 2003), thus creating a very acid edaphic environment where nutrients are limited to plants (Bijos *et al.* 2023).

Sclerophylly is a term used to characterize leaves presenting a set of anatomical and morphological traits, which provide hardness and toughness properties and affect adaptation and resource use processes in certain environments (Read *et al.* 2006). This leaf structure pattern may appear in diverse vegetation types exposed to the most variable light and edaphic conditions in temperate and tropical ecosystems, such as savannas, grasslands, Mediterranean vegetation, and even forests (Seddon 1974; Turner 1994; Rossatto *et al.* 2015; Vitória *et al.* 2019). However, there is a consensus that leaf sclerophylly mainly appears in systems subjected to water deficits, including when flooded (Parolin *et al.* 2006; Read *et al.* 2006), high light irradiance, and nutrient-poor soils (Loveless 1962; Medina *et al.* 1990; Read *et al.* 2006), which are environmental factors that appear in *Veredas* (Fagundes & Ferreira 2016; Nunes *et al.* 2022).

The scleromorphic leaf structure implies that plants would normally build leaves with traits leading to a more conservative use of resources (*e.g.*, thicker leaves with thick cuticle, well-developed mesophyll, and abundant presence of tector trichomes) and capable of a longer lifespan (Rossatto & Kolb 2009; Niinemets 2001; Rossatto *et al.* 2015). This set of traits is common in plants subjected to low availability of nutrients and high light irradiance in their environments (Read *et al.* 2006; Duarte & Dillenburg 2000; Miatto *et al.* 2016). The use of leaf anatomical traits to infer these adaptations has increased drastically in the international literature, and several natural ecosystems have been analyzed concerning these patterns, especially considering a large number of species that may represent the community (Leuschner 2002; Arrivabene *et al.* 2014; Kołodziejek & Michlewska 2015; Rossatto *et al.* 2015; He *et al.* 2018; Paula *et al.* 2019; Ni *et al.* 2022), rather than analyzing just a small number of species. Even with such studies, anatomical leaf strategies at the community level are still poorly understood, especially in high-diversity systems such as phytophysiognomies associated with the *Cerrado* (Fank-de-Carvalho *et al.* 2015).

Considering that *Veredas*: (1) are under the pressure of agricultural advances on natural areas, which affects the drainage of water and risks the persistence of such environments (Rosolen *et al.* 2015); (2) are one of the most frequent phytophysiognomies along the distribution of *Cerrado*; and (3) present few studies which have analyzed leaf anatomical traits in depth in a set of species (Somavilla & Graciano-Ribeiro 2011; Fank-de Carvalho *et al.* 2015; Fagundes & Ferreira 2016), we analyzed leaf anatomical traits in species from different families and growth forms co-existing in a *Vereda* aiming to characterize their leaf anatomical strategies. Our objective was to describe leaf anatomical aspects of trees, shrubs, and herbs in a set of 14 species at a *Vereda* site in Central Brazil. We hypothesized that *Vereda* plants will show a set of scleromorphic anatomical traits in their leaves given their presence in an environment where nutrients are limited and plants are exposed to elevated irradiance.

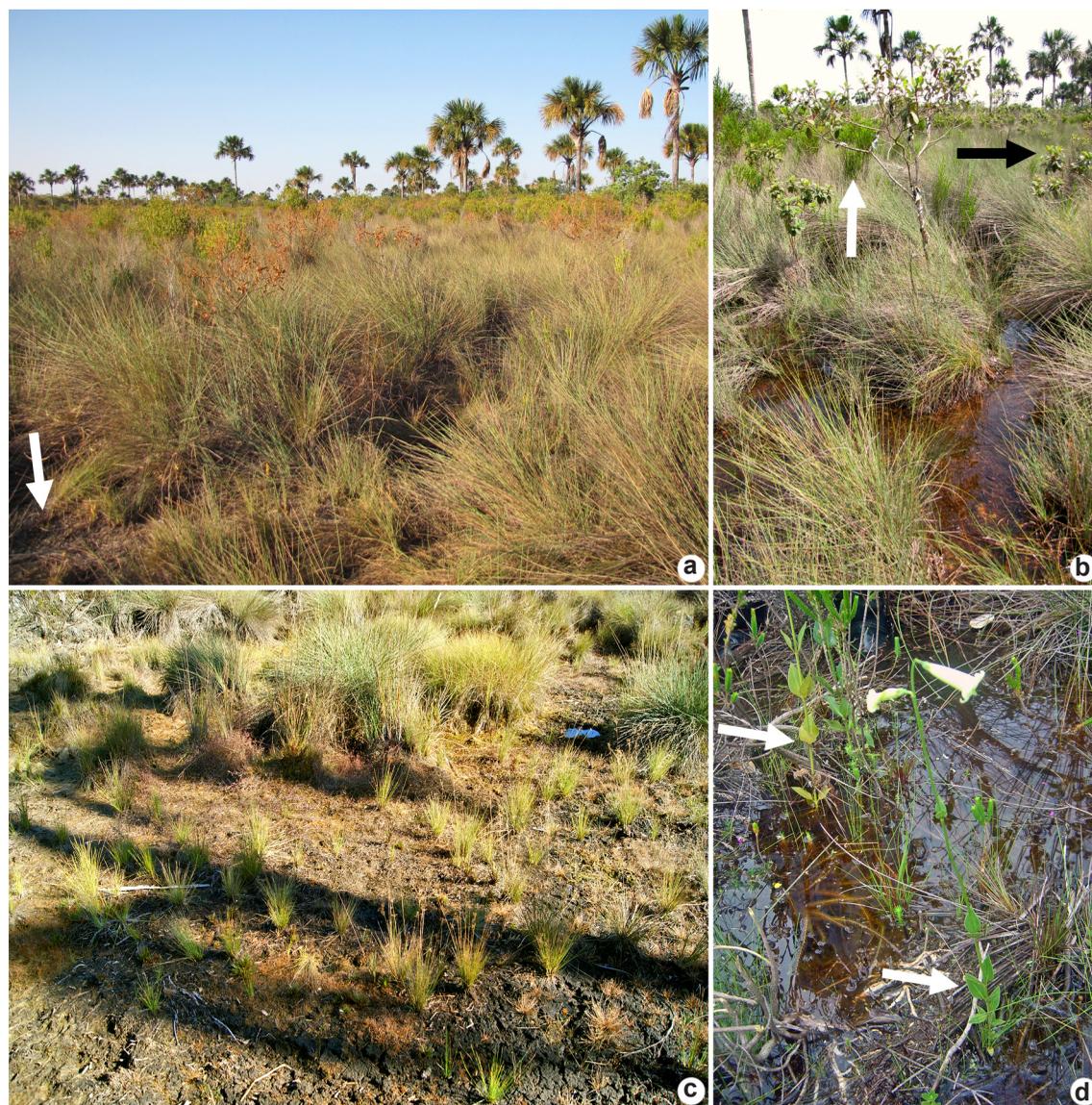
## Material and Methods

### Study area

The study was conducted in the *Vereda Grande*, at the Estação Ecológica das Águas Emendadas (ESECAE), Brasília, Federal District, Brazil (15°34'31.31''S, 47°36'27.82''W). The

climate is tropical Aw according to the Köppen climate classification, with temperatures below 18 °C in the coldest months and an average above 22 °C in the hottest months (Maia & Baptista 2008). The rainy season occurs between September/October and April/May, with 90% of the total rainfall falling during this interval, while the dry

season is marked by intense drought, with 0 mm of precipitation for more than 90 days (Maia & Baptista 2008). Most vegetation in the ESECAE comprises typical savanna (*cerrado sensu stricto*), followed by grasslands and *veredas* (Felfili *et al.* 2008). The diversity of environments in the sampled site is shown in Figure 1.



**Figure 1** – a-d. Photos of the Vereda Grande in different seasons, highlighting the variation in the water table height in the sampling area – a. general view of the *Vereda* with emphasis on the water table below the ground surface (white arrow) at the end of the dry season (September 2010); b. detail of water layer above the ground in the rainy season (picture taken on March 6, 2012) with *Macairea radula* in the frontal plane (black arrow) and *Lavoisiera imbricata* in the background (white arrow); both species are waterlogged; c. detail of the dry season (picture taken in mid-June/July 2012) with water table below ground surface; d. detail of flooded *Chelonanthus alatus* individuals (arrows) during the rainy season (partial submergence).

We measured the photosynthetic active radiation (PAR, in  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of photons) reaching the plants from 10:00 am – 12:00 pm on a typical sunny day without clouds in November 2011 (10 measurements per hour, in four different points at the Vereda Grande) in order to characterize the light environment of the study site. These measurements were performed using a PAR sensor attached to a portable gas exchange system (LcPro, ADC BioScientific Ltd., Hoddesdon, United Kingdom).

Next, we collected four soil samples from different points of the *Vereda* at two different depths to characterize the edaphic environment: the top 0–20 cm and 20–40 cm soil depths. After sampling, soils from the same depth were placed in different plastic bags, sealed, and taken to the laboratory. The pH was determined under  $\text{CaCl}_2$  (Raij *et al.* 1987). Soil phosphorus was determined by spectrophotometry after anion exchange resin extraction (Raij *et al.* 1987). Soil potassium, calcium, magnesium, and aluminum were determined by flame spectrophotometry (Allen *et al.* 1974). Organic matter was determined after the addition of potassium dichromate-sulphuric acid to the soil samples (Sims & Harby 1971). Chemical analyzes were performed at the Soil Analysis Laboratory in the Soils and Fertilizers Department of the FCAV/UNESP Campus de Jaboticabal.

### Species selection and leaf anatomy

In this study, we sampled eudicot species typical of the *Vereda* area (14 species in total), mainly based on the survey performed by Costa (2007) and our field observations. The non-reproductive stage of the species did not allow material to be collected for deposit in a herbarium. The species identification was based on comparison with exsiccates deposited in herbaria in Brasília from materials collected by the authors themselves in previous years, as well as through exsiccates from materials published in the work of Costa (2007); the vouchers relating to the exsiccates used were cited in parentheses. The selected tree species (five species) were: *Cecropia pachystachya* Trécul (Urticaceae) (UB 15680), *Clusia criuva* Cambess. (Clusiaceae) (CEN 75339), *Gomidesia pubescens* DC. (Myrtaceae) (CEN 7196), *Ilex affinis* Gardner (Aquifoliaceae) (HEPH 13864), and *Tapirira guianensis* Aubl. (Anacardiaceae) (UB 187885). The shrub species (seven species) were: *Gaylussacia brasiliensis*

(Spreng.) Meisn. (Ericaceae) (UB 142998), *Lavoisiera imbricata* (Thunb.) DC. (UB 171583), *Lavoisiera grandiflora* A.St.-Hil. ex Naudin (UB 23542), *Macairea radula* (Bonpl.) DC. (UB 143003), *Miconia chamissois* Naudin (UB 142995), *Rhynchanthera grandiflora* (Aubl.) DC. (UB 30706) and *Trembleya parviflora* (D. Don) Cogn. (UB 30700) - all Melastomataceae; and the herbs (two species) were *Desmoscelis villosa* (Aubl.) Naudin (Melastomataceae) (HEPH 2518) and *Chelonanthus alatus* (Aubl.) Pulle (Gentianaceae) (UB 113650). *Lavoisiera* and *Trembleya* are now included in *Microlicia* as *M. cataphracta* (Mart. & Schrank ex DC.) Versiane & R.Romero and *M. parviflora* (D.Don) Versiane & R.Romero, respectively (Versiane *et al.* 2021). However, the authors will use the epithets as published in <http://floradobrasil.jbrj.gov.br/>, access on 03.05.2024. These species occur side by side and are conditioned to waterlogging during the rainy season, which corresponds to the full saturation of the soil pores with a thin layer of water above the soil surface, or to flooding (partial submergence) (Striker 2012); however, their leaves are not submerged or in contact with water.

We performed cross-sections of the middle third of the leaf blade, including the midrib to characterize the leaf anatomy. We collected four to five leaves (one per individual) for each species, which were fully expanded (third or fourth node) with no signs of senescence and completely exposed to the sun during the rainy season of 2012. Younger leaves of *M. chamissois* were also collected for trichome analyses. The middle thirds of the leaves were fixed in FAA70 for 48 h, dehydrated in an ethanol series, and embedded in paraffin (Johansen 1940). The materials were cut using a Leica RM2245 rotary microtome with a thickness of 8  $\mu\text{m}$ . Double staining of sections was performed using Astra blue and basic fuchsin, and Entellan<sup>®</sup> was used as a mounting medium. The mean cuticle and mesophyll thickness was obtained from 20 measurements per analyzed individual. The leaves were kept in a solution of 1:1 (v:v) glacial acetic acid and hydrogen peroxide at 60°C for 12 h to characterize the stomata and trichomes in frontal view, or the time required for complete dissociation of the epidermis (modified from Franklin 1945). After this process, the epidermis faces were washed with distilled water, placed on slides, and stained with 1% safranin. Histochemical tests were performed in paraffin-embedded material to characterize the presence

of lipidic substances (Sudan IV, Jensen 1962) and phenolic compounds (ferric ferricyanide method, Lison 1960) by color change. The slides were analyzed and photographed using a Carl Zeiss Axio Lab A1 light microscope attached to a AxioCam ERC 5s digital camera and AxioVision image capture software.

## Results

### Environmental characteristics

We found elevated irradiance reaching the plants between 10:00–12:00 h ( $1,520 \pm 455 \mu\text{mol m}^{-2} \text{s}^{-1}$  of photons). The Vereda soils are very acidic at both studied depths (mean pH = 3.6), with high aluminum content ( $\text{Al}^{3+}$  and H + Al). The organic matter content was high, whereas the nutrient content was low, especially for Ca and Mg, at both depths (Tab. 1).

### Leaf anatomy

A uniseriate epidermis was a common trait of the species (Figs. 2-4); the exception found was *Ilex affinis*, which displayed a multiseriate epidermis (Fig. 2f). Four species showed parenchymal subepidermal layers (Figs. 2a-b,e; 3d), with more than one layer of cells in *Clusia criuva* (Fig. 2b) and *Macairea radula* (Fig. 3d) and a single layer of cells in *Cecropia pachystachya* (Fig. 2a) and *Gomidesia pubescens* (Fig. 2e).

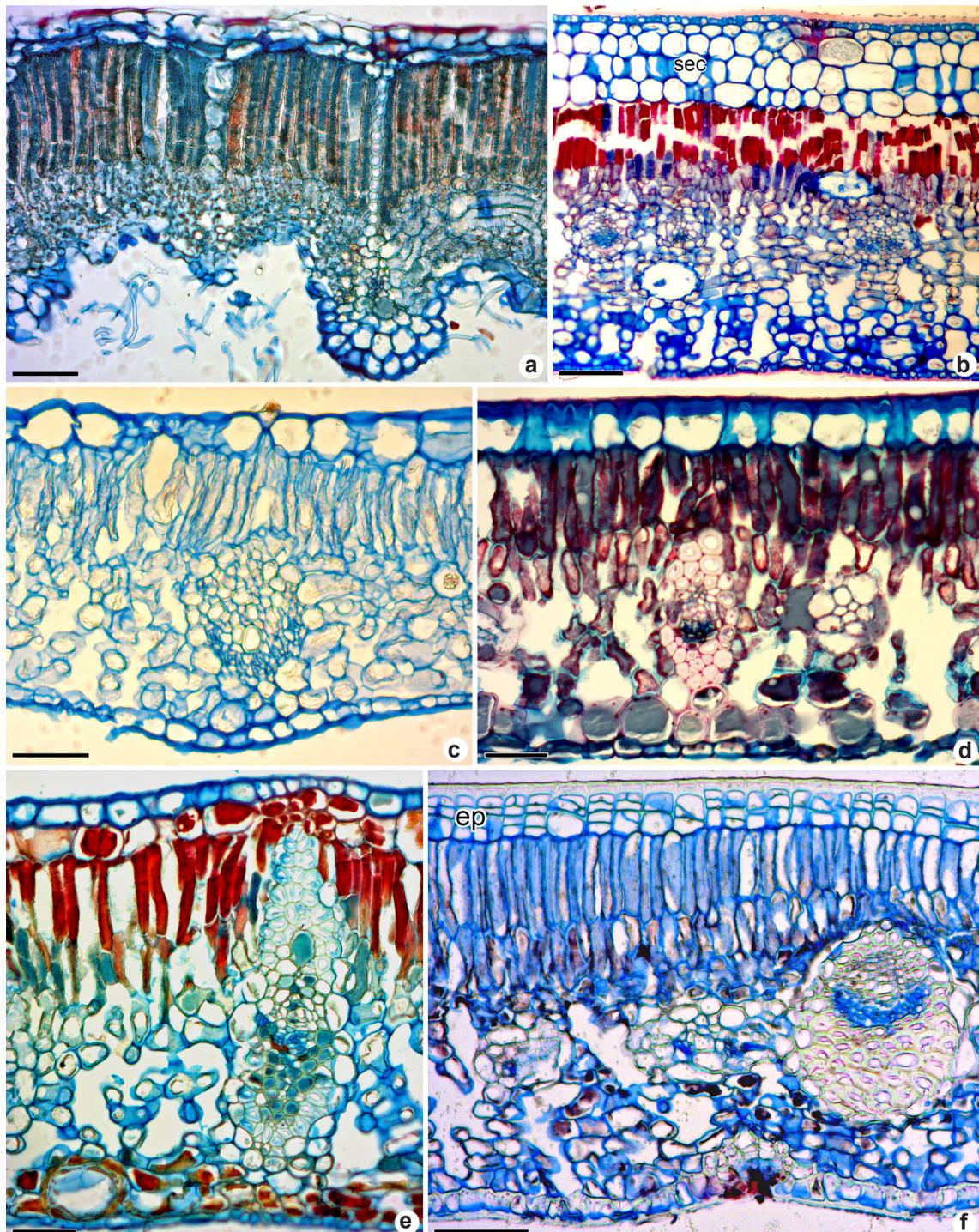
The cuticle was thickened for most species (Tab. 2), except for *Desmoscelis villosa* and *Chelonanthus alatus*, whose cuticles were thinner (around  $1 \mu\text{m}$  thick). The thick cuticle appeared on both surfaces of the leaf, however it was more

common on the adaxial surface (Tab. 2), with values ranging from 4 to  $20 \mu\text{m}$  thick. The cuticular layer in *Gaylussacia brasiliensis* (Fig. 4c) and *I. affinis* presented a lamellar aspect. The epidermis cells on the adaxial surface of *G. brasiliensis* (Fig. 4c) and *G. pubescens* (Fig. 4d), and on both sides of *Lavoisiera imbricata* (Fig. 4e) showed introjections in the periclinal outer cell wall and projections of the protoplasm. The anticlinal cell walls in the frontal view were very sinuous and showed bright dots, indicating differences in the cell wall thickening in the protoplasm introjection and projection regions. The cell wall thickening displays cuticular flanges in the adaxial surface epidermis of *C. pachystachya*, *G. brasiliensis*, *I. affinis* (Fig. 4f) and the abaxial face of *L. imbricata* (Fig. 3b), as well as on both surfaces of *Clusia criuva*. Another trait observed in *I. affinis* was thickening of the inner tangential and anticlinal walls in the abaxial surface epidermis, particularly in the walls of subsidiary cells facing the substomatal cavity (Fig. 4g). These thickenings showed a negative reaction to Sudan IV, indicating the absence of cutinization in the cell wall thickening, which differs from the adaxial surface where the reaction was only negative to Sudan IV in the inner tangential wall of the epidermis cells layer adjacent to the mesophyll (Fig. 4f). No thickening was observed in the inner tangential cell wall of the midvein epidermis.

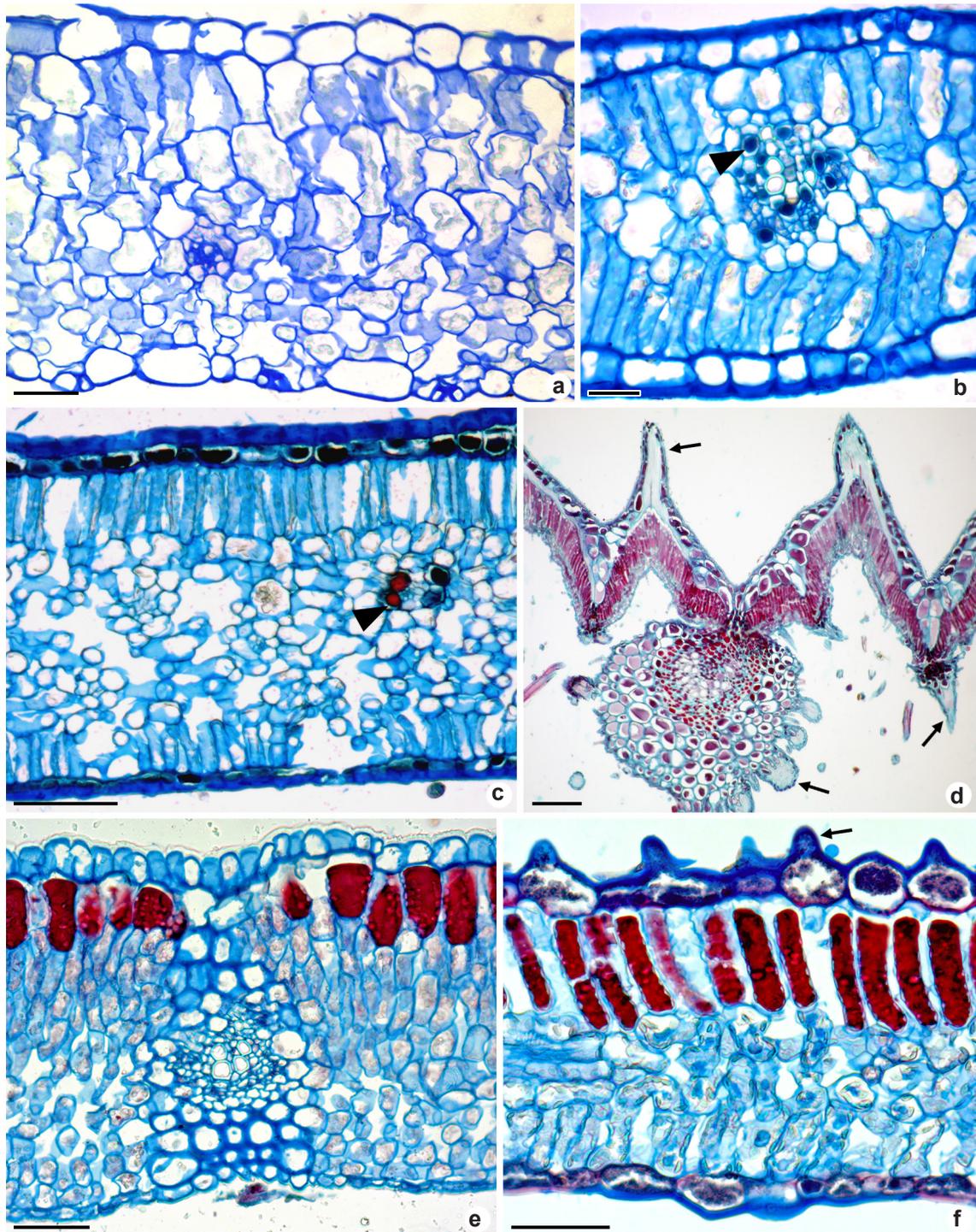
Non-glandular and glandular trichomes occurred in most species (Tab. 2) with variation in shape (Fig. 4h-r), and usually appearing on the abaxial surface. In addition to the glandular trichomes (Fig. 4q-r), there is a papillose adaxial

**Table 1** – Soil chemical properties of the Vereda Grande in the Estação Ecológica das Águas Emendadas, Brasília, DF, Brazil. Mean of four samples followed by the standard deviation of the mean.

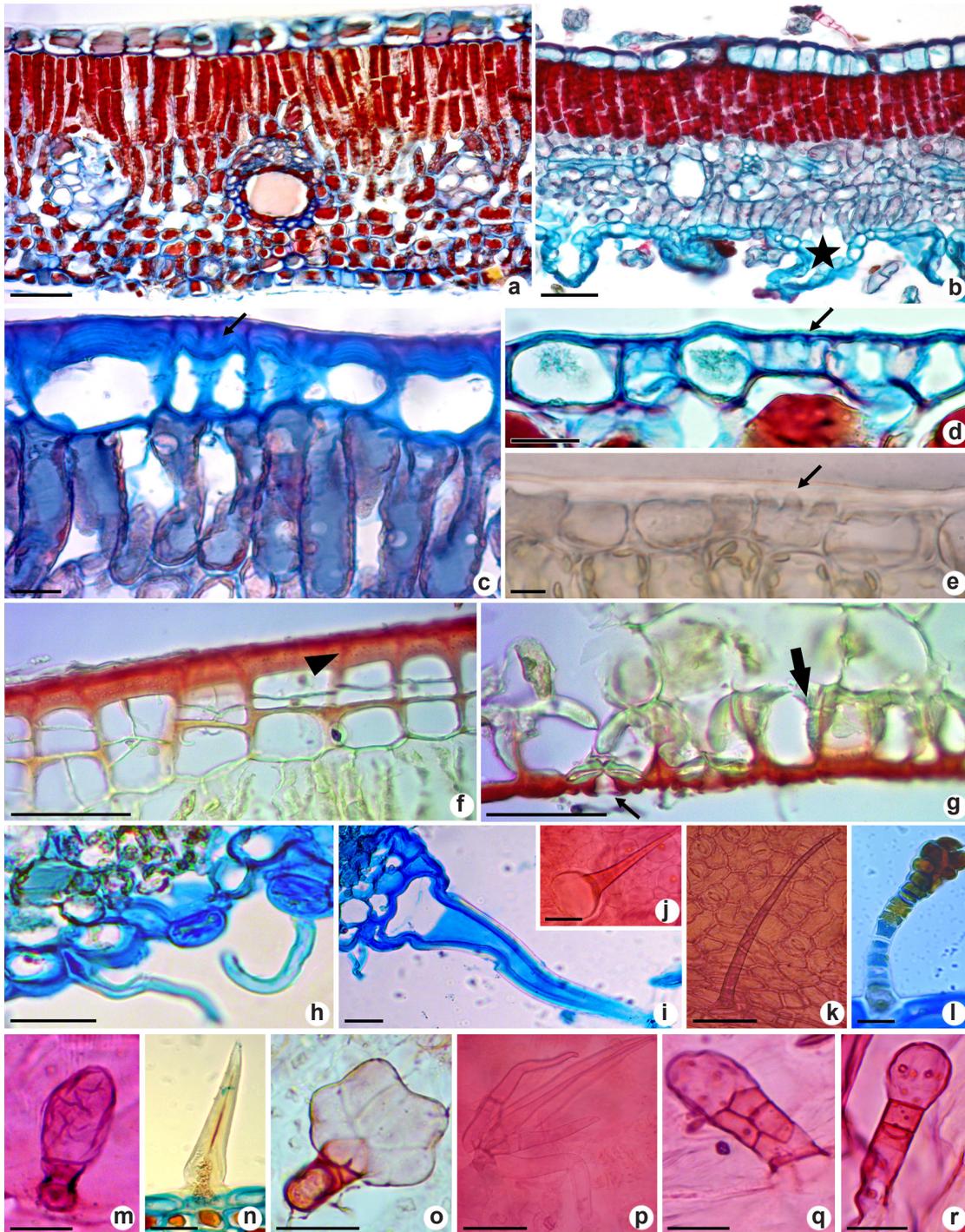
Soil property	Depth	
	0–20 cm	20–40 cm
pH	$3.6 \pm 0.4$	$3.6 \pm 0.4$
Organic matter ( $\text{g} \cdot \text{dm}^{-3}$ )	$169.66 \pm 43.24$	$167.33 \pm 15.27$
P ( $\text{mg} \cdot \text{dm}^{-3}$ )	$22 \pm 2.64$	$13.33 \pm 0.57$
Al ( $\text{mmol} \cdot \text{dm}^{-3}$ )	$36 \pm 9.53$	$27.66 \pm 10.24$
H+Al ( $\text{mmol} \cdot \text{dm}^{-3}$ )	$226.33 \pm 76.95$	$281.33 \pm 75.22$
K ( $\text{mmol} \cdot \text{dm}^{-3}$ )	$2.56 \pm 0.90$	$2.30 \pm 1.2$
Ca ( $\text{mmol} \cdot \text{dm}^{-3}$ )	$2.15 \pm 1.44$	$1.66 \pm 0.57$
Mg ( $\text{mmol} \cdot \text{dm}^{-3}$ )	$2.66 \pm 0.57$	$4.20 \pm 1.44$



**Figure 2** – a-f. Leaf cross-sections of – a. *Cecropia pachystachya*; b. *Clusia criuva* (sec = subepidermal cells in the adaxial face); c. *Desmoscelis villosa*; d. *Gaylussacia brasiliensis*; e. *Gomidesia pubescens*; f. *Ilex affinis* (ep = multiseriate epidermis in the adaxial face). Scale bars: a, c-e = 50  $\mu$ m; b, f = 100  $\mu$ m.



**Figure 3** – a-f. Leaf cross-sections of – a. *Chelonanthus alatus*; b. *Lavoisiera imbricata* – idioblast with phenolic compounds (arrow head); c. *Lavoisiera grandiflora* – phenolic compounds found on vascular bundle sheath (arrowhead); d. *Macairea radula* – emergences on both sides of the leaf (arrows); e. *Miconia chamissois*; f. *Rhynchanthera grandiflora* – papils in the adaxial face of the epidermis. Scale bars: a-c, e-f = 50  $\mu$ m; d = 100  $\mu$ m.



**Figure 4** – a-g. Leaf cross-sections of – a. *Tapirira guianensis*; b. *Trembleya parviflora* (star = showed evaginations in abaxial face epidermis; c-e. introjections in periclinal outer cell wall (arrows) – c. *Gaylussacia brasiliensis*; d. *Gomidesia pubescens*; e. *Lavoisiera imbricata*; f-g. *Ilex affinis* – f. epidermis adaxial face (arrowhead = cuticular flange in the anticlinal wall); g. epidermis abaxial face (thickenings in the cell walls - thick arrow, and stomata outer ledge - thin arrow). h-j. tector trichome in *Cecropia pachystachya*. k-l. *G. brasiliensis* – k. tector trichome; l. glandular trichome. m. glandular trichome in *Desmoscelis villosa*. n. tector trichome in *G. pubescens*. o-p. young leaves of *Miconia chamissois* – o. glandular trichome; p. strelate trichome. q-r. glandular trichome in *Rhynchanthera grandiflora*. Scale bars: a-b, f-g, j-k, p = 50  $\mu\text{m}$ ; c-e, h-i, l-o, q-r = 20  $\mu\text{m}$ .

**Table 2** – Leaf anatomical traits of *Vereda* species. Legend: AB = epidermis abaxial face; AD = epidermis adaxial face; bse = bundle sheath extension; co = midrib cortex; em = emergences; ep = epidermis; g = glandular; hy = hypodermis; lm = leaf margin; md = midrib medulla; mes = mesophyll; ph = midrib phloem; Prism = prismatic crystal; sel = subepidermal layers; t = tector; vb = vascular bundle; vs = vascular bundle sheath; xy = midrib xylem.

Species	Thick cuticle	Trichome	Spongy parenchyma	Druse	Prism	Phenolic compounds	Sclerenchyma
<i>Cecropia pachystachya</i>	AD	t	compact	co/mes/bse	-	sel/mes/md/co/vb	vb/bse
<i>Clusia criuva</i>	AD/AB	-	loose	co/mes/hy	-	mes	vb
<i>Desmoscelis villosa</i>	-	g	loose	em/mes	-	-	em
<i>Gaylussacia brasiliensis</i>	AD/AB	t,g	loose	mes	co	vb/mes/ep/co	vb/bse
<i>Myrcia pubescens</i>	AD	t	loose	-	-	xy/mes/sel/co/vb/vs	bse/ph
<i>Ilex affinis</i>	AD/AB	-	loose	mes	-	-	vb
<i>Chelonanthus alatus</i>	-	-	loose	co	-	-	-
<i>Lavoisiera imbricata</i>	AD/AB	g	compact	co/mes	-	vb	vs/co/em
<i>Lavoisiera grandiflora</i>	AD/AB	g	loose	co/mes	-	ep/vb	em
<i>Macairea radula</i>	AD	t,g	loose	co/mes/md	-	mes/co/ph/vb/bse/ep/xy/md	sel/em
<i>Miconia chamissois</i>	AD	t,g	loose	vb/co/md/mes	-	md/mes/bse	-
<i>Rhynchanthera grandiflora</i>	AD/AB	g	loose	co/mes	-	mes/ep	em
<i>Tapirira guianensis</i>	AD/AB	-	compact	co/ph/mes	-	co/ep/mes/ph/md/en	vb
<i>Trembleya parviflora</i>	AD	g	compact	mes	-	mes/ph/md/co	vb/md/co

surface in *Rhynchanthera grandiflora* (Fig. 3f). *Miconia chamissois* trichome scars were observed on the abaxial surface of fully expanded leaves. However, the young leaves showed glandular (Fig. 4o) and non-glandular (Fig. 4p) trichomes on the abaxial surface and glandular trichomes on the adaxial surface, which were lost during leaf development.

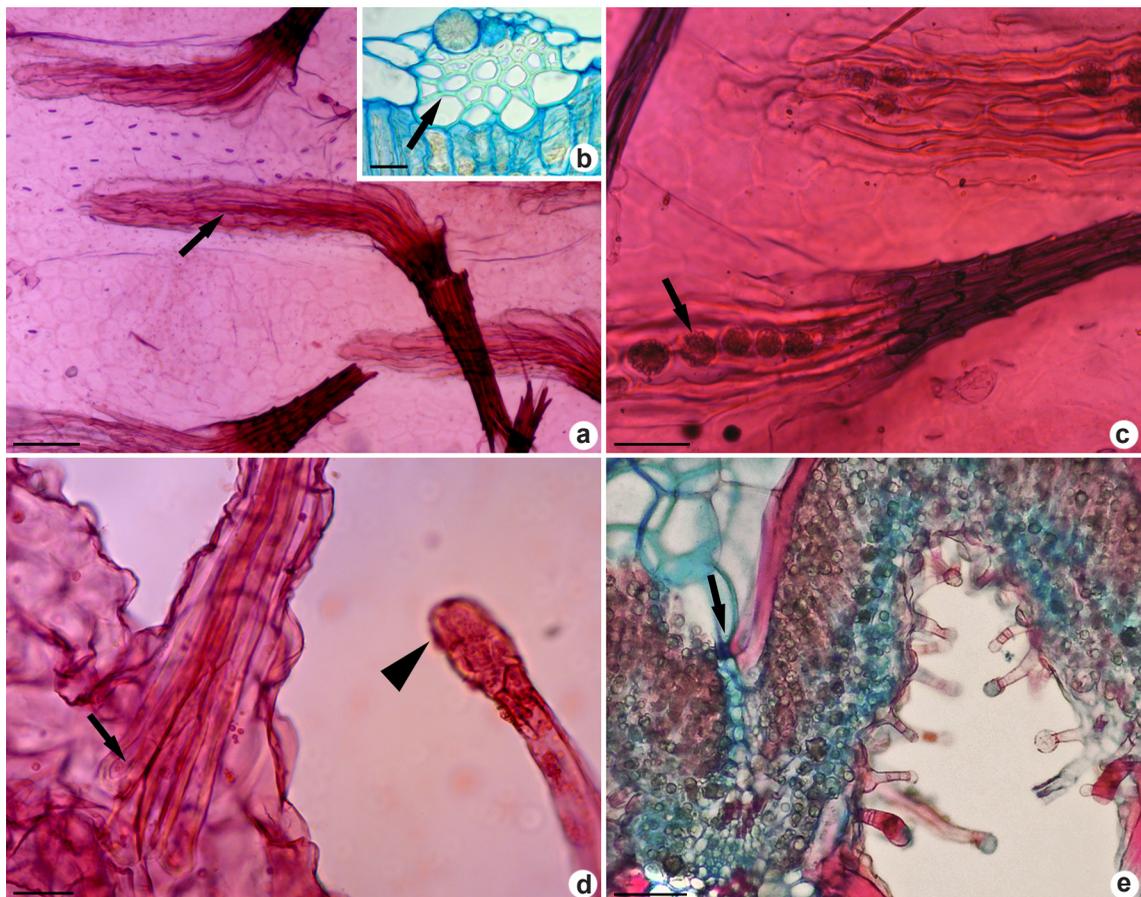
Hypostomatic leaves were a common trait among species, except for *D. villosa*, *C. alatus*, and *L. imbricata*, which showed stomata on both sides of the epidermis. Stomata in invaginations of the abaxial surface were found in *Macairea radula*, and the abaxial surface in *Trembleya parviflora* showed evaginations where stomata were found (Fig. 4b). Outer stomatal ledges (Fig. 4g) were found in most species, except for *G. brasiliensis*, *G. pubescens*, and *C. alatus*.

Dorsiventral mesophyll predominated in the leaves of the study species, except for *L. imbricata* (Fig. 3b), *L. grandiflora* (Fig. 3c), *R. grandiflora* (Fig. 3f), and *T. parviflora* (Fig. 4b), which showed an isobilateral pattern. Some *L. imbricata* leaves presented a dorsiventral mesophyll with the palisade parenchyma facing the abaxial surface. Mesophyll proved to be well developed in all species with average thickness values varying between 115.61  $\mu\text{m}$  and 277.20  $\mu\text{m}$  and reaching greater values in *I. affinis* (348.22  $\mu\text{m}$ ), *G. pubescens* (357.13  $\mu\text{m}$ ) and *C. criuva* (515.66  $\mu\text{m}$ ). The spongy parenchyma had a loose arrangement in most of them, although *C. pachystachya* (Fig. 2a), *L. imbricata* (Fig. 3b), *Tapirira guianensis* (Fig. 4a), and *T. parviflora* (Fig. 4b) showed this tissue compacted (Tab. 2).

Regarding ergastic substances, calcium oxalate crystals occurred in different tissues of

13 species, except in *G. pubescens*. The main shape of the crystal found was the druse, and it was more common in the mesophyll and cortex of the midrib. However, druses were located in different tissues in some species: vascular bundle sheath in *C. pachystachya*, subepidermal layers in *C. criuva*, epidermis and sclereids in the emergency of *D. villosa*, midrib pith in *M. radula* and *M. chamissois*, bundle sheath of *M. chamissois*, and midrib phloem in *T. guianensis* (Tab. 2). Prismatic crystals were observed in the midrib cortex of *G. brasiliensis*. Phenolic compounds were in different leaf tissues in most species (Tab. 2). These compounds were generally deposited in the palisade parenchyma cells in the mesophyll, closer to the adaxial face epidermis (Figs. 2a-b,d-e; 3e-f), and vascular tissue in *L. imbricata* (Fig. 3b).

Sclerified cells (Tab. 2) were typically associated with the vascular bundles (Fig. 2d-f), but they were also observed in the vascular bundle sheath extensions of *C. pachystachya*, *M. radula*, and *G. pubescens* and the emergences of *D. villosa* (Fig. 5a-c), *L. imbricata*, *R. grandiflora* (Fig. 5d), and *M. radula* (Figs. 3d; 5e). Emergences in *L. imbricata* only occurred at the blade edge and on the abaxial side of the midrib. Sclerified cells of emergence in *M. radula* showed contact with vascular bundle sheath cells (Fig. 5e). However, this characteristic was not verified in the emergence cells of the other species. Vascular bundle sheath extension was observed in some species (*C. pachystachya*, *G. brasiliensis*, *M. pubescens*, *M. radula* and *M. chamissois*) and was fully or partially sclerified (Fig. 2a,e) or parenchymatous (Fig. 3e) without intercellular



**Figure 5** – a-e. Emergences – a-c. *Desmoscelis villosa*. Sclerified cells (arrows) – a. paradermic view; b. cross-section; c. druse in basal cells of emergence (arrow); d. *Rhynchanthera grandiflora* – basal (arrow) and apical (arrowhead) cells of glandular emergence; e. *Macairea radula* – emphasis on the contact of the sclerified cells of the emergence with the cells of the vascular bundle sheath extension (arrow). Scale bars: a = 200  $\mu$ m; c, e = 50  $\mu$ m; b, d = 20  $\mu$ m.

spaces. The extension in *G. brasiliensis* was only verified in the abaxial face of the larger caliber vascular bundle.

## Discussion

We confirmed that most of the study species showed a variable combination of scleromorphic structures in their leaves, except for *C. alatus*. We also confirmed that *Vereda* had high light irradiance given its low canopy cover, indicating that plants are exposed to intense solar radiation. The results from soil analysis indicated that the edaphic environment is very acidic with elevated aluminum content. The values found for aluminum content in *Vereda* soils are four times higher than those typically found for typical savannas growing in old weathered oxisols (Souza *et al.* 2015), which points to the acidity of soils as a factor which may limit the absorption of nutrients (Bijos *et al.* 2023) by *Vereda* plants compared with typical savanna vegetation. This limitation can lead plants to adopt a more conservative strategy of resource use in their leaves, which in terms of anatomical traits are reflected in a scleromorphic pattern (Medina *et al.* 1990; Salleo & Nardini 2000): thick leaves with thick cuticle and well-developed mesophyll, associated with trichomes (Loveless 1962; Medina *et al.* 1990; Read *et al.* 2006).

A thick cuticle can be important to reflect part of the elevated amount of incident light energy (Chazdon & Kaufmann 1993), decreasing excess solar radiation on adjacent tissues (Fahn & Cutler 1992). This can prevent the leaves from reaching high temperatures (Hlwatika & Bhat 2002), which could interfere with photosynthesis and cause significant damage to the photosynthetic apparatus (Silva & Rossatto 2022). Furthermore, phenolic acids present in the cuticle play a critical role in plant UV photoprotection and efficient energy dissipation (González Moreno *et al.* 2022). *Desmoscelis villosa* did not show a thick cuticle, which can be linked to its herbaceous habit, and this species has a great number of emergences in its small leaves, which give a woolly consistency to the leaf and can protect the tissues against high temperatures. On the other hand, the absence of a thick cuticle and trichomes in *Chelonanthus alatus* may be related to the fact that this is an herbaceous species which only develops its shoots in the rainy season (Fig. 1d), and is therefore less susceptible to high light intensities associated with high evaporative demands for longer periods.

Along with any physiological adjustments

to deal with higher leaf-air vapor pressure-deficit (Franco *et al.* 2005), the presence of thick inner walls in the epidermis of *Ilex affinis* can help reduce water loss from the mesophyll and substomatal cavity to the environment. This protection was observed in *Cirsium horridulum* (Asteraceae) by the deposition of an internal cuticle in epidermal cells (Pesacrete & Hasenstein 1999). In contrast, we did not find cutin deposition in the inner epidermal cell walls of *I. affinis*, and therefore there was no formation of an internal cuticle. Non-cutinization could be indicative of the high sensitivity of these cells to humidity variation.

Tree species showed hypostomatic leaves, a common trait of this growth form, which was also found in surveys of different physiognomies in the *Cerrado* (Bieras & Sajo 2009; Rossatto *et al.* 2009), the Amazonic Rainforest (Medina *et al.* 1990; Camargo & Marengo 2011), and the Atlantic Rainforest (Boeger *et al.* 2004). Amphystomatic leaves were only found in the shrub species *Lavoisiera imbricata* and in the herbaceous species *D. villosa* and *C. alatus*. The outer cuticular thickenings that form the ledges in the stomata are the first regions to come into contact when there is a loss of turgor in the guard cells, closing the stomata opening. This is followed by the contact between the inner walls of the guard cells (Mauseth 1988). According to the same author, the presence of these ridges decreases water vapor loss, even with the opening of the stomata, because they create a tube that protects the stomatal pore. This trait can be advantageous for leaves exposed to open environments with high irradiance and temperatures, low air humidity, and high incidence of winds, as found in *Vereda* (Maia & Baptista 2008; Munhoz & Ribeiro 2008; Guimarães *et al.* 2017).

Emergences are structures which are commonly found in some genera of Melastomataceae, such as *Lavoisiera*, *Microlicia*, and *Rhynchanthera* (Sousa 1997; Milanez & Machado 2011; Silva *et al.* 2018; Carmo *et al.* 2020), and *Leandra* and *Tibouchina* (Reis *et al.* 2005). Currently *Lavoisiera* is included in genus *Microlicia* (Versiane *et al.* 2021). According to Michelangeli *et al.* (2019) the epithet of *Leandra* is currently accepted in genera *Miconia* as *M. auricoma* (Spring, *ex Mart.*) R. Goldenb. and *M. lacunosa* (Cogn.) R. Goldenb. The epithet of *Tibouchina* listed in this article are now recognized as *Chaetogastra gracilis* (Bonpl.) DC. and *Pleroma stenocarpum* (Schrank & Mart. *ex DC.*) Triana. An ultrastructural study of the emergences of *Microlepis oleaefolia* (*Pleroma*

*oleifolium* (DC.) R. Romero & Versiane in Guimarães 2024) showed communication between the external environment and vascular tissues through cell wall microchannels in the sclerified cells (Milanez & Machado 2008).

We point out in this work the contact of the sclereids of emergences with the bundle sheath extension in *Macairea radula*, indicating that there may be communication of this structure with the vascular tissues as a way to achieve water absorption from the external environment (Boanares *et al.* 2018). This can be advantageous for the species during the dry season when relative air humidity is low. In addition, these emergences are projected onto the surface, constituting a protective layer similar to the trichome cover which helps to maintain the border layer, reducing damage due to high solar incidence (Milanez & Machado 2008). This last function can also be shared by emergences observed in *Desmoscelis villosa*, *M. radula*, and *Rhynchanthera grandiflora*. Emergences in *Lavoisiera imbricata* are only observed on the edges and the main vein, but it is worth highlighting here that the leaves of this species do not have a petiole and are attached to the stem at a closed angle, meaning that they do not expose their leaf blades to high light.

The well-developed mesophyll with high investment in photosynthetic tissue observed in the study species may be related to high light intensities (Larcher 2003) in the *Vereda*, which may aid in optimizing the photosynthesis process (Gratani *et al.* 2006; Rossatto & Kolb 2012). In addition, photosynthesis improvement can occur due to the singular optical properties of the mesophyll cells, as they can regulate internal light distribution (Vogelmann 1993; Vogelmann *et al.* 1996). Thus, the more columnar palisade cells facilitate light penetration into the mesophyll, distributing light more evenly throughout the leaf (Vogelmann & Martin 1993). On the other hand, the more spherical spongy cells and the large fraction of air space within the leaf generate large amounts of scattered light, increasing light absorption by chloroplasts within the mesophyll (Lucia *et al.* 1996).

Leaves with bundle sheath extensions are characterized as heterobaric, and those that do not present this structure are called homobaric (Terashima 1992). The tissues present in the bundle sheath extensions (parenchyma or sclerenchyma) are usually devoid of chloroplasts, compacted, and form a barrier to the horizontal flow of gases within the mesophyll (Terashima 1992; Liakoura

*et al.* 2009). Despite the occurrence of transparent photosynthetically inactive regions in heterobaric leaves, their photosynthetic capacity remained similar and even greater than in homobaric leaves (Liakoura *et al.* 2009). The authors highlight that the cost in terms of C and N investment and photosynthetically active leaf area reduction for the construction of bundle sheath extensions is compensated by their optical function, improved light environment inside mesophyll, and water retrieval. Karabourniotis *et al.* (2000) demonstrated that bundle sheath extensions promote quantitative enrichment of the light microenvironment within the internal layers of the mesophyll, especially the light that is maximally used for photosynthesis. On the other hand, the lack of interruptions in the mesophyll by bundle sheath extensions in homobaric leaves allows higher lateral CO<sub>2</sub> conductance than heterobaric leaves and may even reach values higher than vertical conductance (Pieruschka *et al.* 2005). The internal flux of CO<sub>2</sub> from shaded areas to illuminated areas contributes to the overall increase in photosynthesis in the leaf (Pieruschka *et al.* 2006), especially when there is patchy closure of stomata due to environmental stresses (Terashima 1992). These contributions varied with the degree of lateral CO<sub>2</sub> transport determined by the frequency, size, and length of the interconnected air channels inside the leaves (Pieruschka *et al.* 2008), all of which are intrinsically linked to the thickness and compaction of the mesophyll and the ratio of palisade to spongy mesophyll (it is possible to observe the variation of these characteristics in the different species in Figures 1a-f, 2a-f and 3a-b). In addition to composing the barrier and interacting with the brightness distribution inside the mesophyll and the external environment, the sclerenchymatic cells in the vascular bundle sheath extensions also function as mechanical support to the leaves (Evert 2006).

The presence of calcium crystals in leaves can be related to many functions, like detoxifying aluminum and heavy metals, and light gathering and scattering to optimize photosynthesis (for a review see He *et al.* 2014). Furthermore, calcium oxalate crystals located within mesophyll can function as dynamic internal carbon reservoirs, whereby crystal decomposition releases CO<sub>2</sub> that is subsequently used for photosynthesis in plants exposed to limited conditions of CO<sub>2</sub>, such as total or partial stomata closure during drought stress (Herrera *et al.* 2008; Tooulakou *et al.* 2016). The phenolic compounds may represent a

protective mechanism against UVB rays (Bieza & Lois 2001) and high solar radiation that *Vereda* species are exposed to. Moreover, phenolic compounds have a strong radical scavenging capacity, effectively neutralizing the reactive oxygen species (ROS) (Šamec *et al.* 2021). These radicals are formed both during waterlogging and post-waterlogging, and may cause damage to cells (see Voesenek & Bailey-Serres 2015). Thus, the presence of phenolic compounds with antioxidant activity may have a protective role against ROS in waterlogged environments (Lima *et al.* 2023), such as *Veredas*.

The combination of structural (scleromorphic traits in this study) and physiological characteristics allows some plants to tolerate or avoid environmental stress, for instance when there is a sudden decrease of water in the soil and the possibility of losing water to the environment due to low air relative humidity. Avoiding drought stress in plant cells generally means high investments in structure and metabolism with water control by stomata, causing high energy demands and nutritional adjustments (Bandurska 2022). Although this may be the case for most study species of the *Vereda*, *Chelonanthus alatus* does not share these aspects, as this species does not maintain its shoots during severe drought periods. *C. alatus* is a pioneer geophyte species with a diverse lifespan, which can vary between biannual and perennial (Dejean *et al.* 2011). The renewal of the *C. alatus* population in Vereda Grande was not the object of this study; however, considering the geophytism which enables shoot regrowth, it is possible to associate this characteristic, and not the leaf anatomy, with the strategy of avoiding the driest season.

*Veredas* have very acidic soils, which limits the amount of nutrients available to the plants, leading them to produce leaves with traits linked to the conservative use of resources. The species analyzed have different anatomical strategies for living in both the dry and rainy seasons of Vereda Grande, and most species have anatomical traits linked to leaf scleromorphism, except *Chelonanthus alatus*, which showed a different avoidance strategy in the dry season. New studies focusing on the physiology, germination, establishment of seedlings, and phenology of the species, are necessary to understand the different plant strategies to survive in *Vereda* environments, which are very unstable due to human occupation around this important wetland ecosystem.

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## Data availability statement

In accordance with Open Science communication practices, the authors inform that the data supporting the findings of this study are available within the article. The raw data that support this study will be shared upon reasonable request to the corresponding author.

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