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Morphoanatomy of the vegetative organs of *Wunderlichia azulensis* Maguire & G. M. Barroso (Asteraceae) from a rocky outcrop in northern Rio de Janeiro, Brazil

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Resumo

Morfoanatomia dos órgãos vegetativos de *Wunderlichia azulensis* Maguire & G. M. Barroso (Asteraceae) de um afloramento rochoso no norte do Rio de Janeiro, Brasil. As espécies de *Wunderlichia* (Asteraceae) são endêmicas das regiões central e leste do Brasil, geralmente encontradas em afloramentos rochosos e sujeitas a altas irradiação solar e temperatura. Tais condições sugerem ocorrência de múltiplas adaptações xeromórficas em *Wunderlichia azulensis*. Este estudo visou caracterizar a morfoanatomia dos órgãos vegetativos de *W. azulensis*. Raízes, caules e folhas foram coletados de um afloramento rochoso no Maciço do Itaoca, Campos dos Goytacazes, RJ, Brasil. O material vegetal foi fixado, desidratado e emblocado em resina plástica para seções transversais. Testes histoquímicos foram realizados para identificação das classes químicas nas folhas e tricomas de *W. azulensis*. A morfologia dos tricomas foi avaliada em microscópio eletrônico de varredura. As raízes apresentaram uma estrutura morfoanatômica distinta que pode ser caracterizada como xilopódio. As folhas apresentaram um conjunto de atributos anatômicos adaptativos, como células epidérmicas com paredes anticlinais retas ou levemente sinuosas, alta densidade de tricomas e camada subepidérmica. Os testes histoquímicos revelaram substâncias fenólicas e óleos essenciais no mesofilo. Este estudo contribuiu para o conhecimento sobre as adaptações anatômicas das plantas rupícolas e de ambientes xeromórficos, e sugere futuros estudos para elucidação da estrutura das raízes e caules de *W. azulensis*.

Palavras-chave: Adaptações xeromórficas; *Inselberg*; Micromorfologia; Xilopódio

Abstract

Wunderlichia (Asteraceae) species are endemic to central and eastern Brazil, usually found on rocky outcrops, and subjected to high sun irradiation and temperatures. Such conditions suggest multiple xeromorphic adaptations on the internal structure of *Wunderlichia azulensis*. This study aimed to characterize the morphoanatomy of vegetative parts of *W. azulensis*. Roots, stems, and leaves were collected from the Itaoca massif, a rocky outcrop in Campos dos Goytacazes, Brazil. The plant material was fixed, dehydrated, and embedded in plastic resin to make cross sections. Histochemical tests were conducted to identify the compound classes in *W. azulensis*

leaf tissues and trichomes. The trichome morphology was analyzed using a scanning electron microscope. The roots exhibited a distinctive morphoanatomical structure and can be characterized as a xylopodium. The leaves had a set of anatomical adaptation traits, including epidermal cells with straight or slightly sinuous anticlinal walls, highly dense trichomes, and a subepidermal layer. Histochemical tests revealed phenolic compounds and essential oils in the mesophyll. This study contributed to the knowledge of anatomical adaptations of a rupicolous plant and its xeromorphic environment and suggests further studies are needed to better understand the root and stem structures of *W. azulensis*.

Key words: Inselberg; Micromorphology; Xeromorphic adaptations; Xylopodium

Introduction

The northeastern region of Rio de Janeiro State, Brazil, has a particular physiognomy with many historical signs of anthropic occupation and economical exploitation. In this region, there are distinct Atlantic Forest phytophysiognomies, including ombrophilous and seasonal forests, and mosaics with other forest formations, such as restingas, mangroves, dunes, and islands of rupicolous vegetation on inselbergs (FRANKE et al., 2005).

The rocky outcrop called the Itaoca massif (Maciço do Itaoca) is an inselberg in the Ibitioca district in the municipality of Campos dos Goytacazes, in northeastern Rio de Janeiro. Mauad (2010) recorded 65 families of vascular plants in this site, with Asteraceae among the most representative families. Moreover, species restricted to inselbergs occur on this massif, including those of *Wunderlichia* Riedel ex Benth.

Wunderlichia is endemic to Brazil and comprises five species, which are deciduous and adapted to xeromorphic and rupestrian environments. *Wunderlichia* species are restricted to rocky outcrops in the Distrito Federal and the states of Mato Grosso, Goiás, Tocantins, Minas Gerais, São Paulo, Rio de Janeiro, Espírito Santo, and Bahia (SOUZA-BUTURI, 2022). Initially, their leaves are densely pubescent but later become glabrescent. At the beginning of the flowering period, the plants become visibly leafless, which helps disperse the achenes (BARROSO; MAGUIRE, 1973).

Wunderlichia has traditionally been placed within the tribe Mutisieae s.l. (BREMER, 1994), which is characterized by its variable indument of uni- or multiseriate trichomes composed of uniform cells or modified basal and terminal cells (METCALFE; CHALK, 1950). The tector trichomes in this tribe are usually simple, stellate, malpiguiaceous, or scaly. Depressed glandular trichomes are also common on the abaxial surface (PANERO; FUNK, 2007). However, recent phylogenetic studies placed *Wunderlichia* in the tribe Wunderlichieae, which is characterized by spatulate, glandular trichomes (PANERO; FUNK, 2008; ORTIZ et al., 2009; MARTÍNEZ-QUEZADA et al., 2022).

The function of tector trichomes is attributed to physical protection by forming a barrier against insects and pathogens. These trichomes may also reduce water loss and increase tolerance to abiotic stresses, such as extreme temperatures and ultraviolet (UV) irradiation (YANG; YE, 2013). The glandular trichomes are involved in structural and chemical defense by secreting a variety of secondary metabolites, such as tannins, resinous compounds, and essential oils. The particular traits of the producing cells vary according to the compounds produced (SCHUURINK; TISSIER, 2019).

Studying the anatomy and chemical nature of exudates has contributed to understanding the role secretory structures play in plants (SCHUURINK; TISSIER, 2019). Simultaneously, studies of the morphology and distribution of secretory structures have been used to help delimit some Asteraceae genera (CASTRO et al., 1997; ADEDEJI; JEWOOLA, 2008). From a biotechnological point of view, it is important to characterize the structure and the function of glandular structures, which are usually rich in bioactive molecules.

Having such a restricted distribution is reflected in the low number of studies of *Wunderlichia* species. This is the case for *Wunderlichia azulensis* Maguire & G.M. Barroso, which is only found in four states: Bahia, Espírito Santo, Minas Gerais, and Rio de Janeiro. This

shrubby species stands out in the vegetation islands found on the Itaoca massif inselberg (MAUAD et al., 2014; SOUZA-BUTURI, 2022) and is classified as Data Deficient (DD) by the List of Threatened Plant Species of Brazil (CNCFLORA, 2012).

Studies of *Wunderlichia* species are restricted to taxonomic treatments and the isolation of chemical compounds. The internal structure of *Wunderlichia* vegetative parts still needs more elucidation (SOUZA-BUTURI, 2013), such as the subterranean system that has not been investigated. Anatomical analyses may suggest either adaptation or acclimatization traits in response to the seasonal environment in northern Rio de Janeiro and typical high solar irradiance and low water availability on rocky outcrops. Considering the above, this study aimed to characterize the morphology and anatomy of *W. azulensis* vegetative parts. Emphasis was given to the subterranean system, leaf trichomes, and characterizing the major compound classes through histochemical tests of the plant organs and glandular trichome exudates.

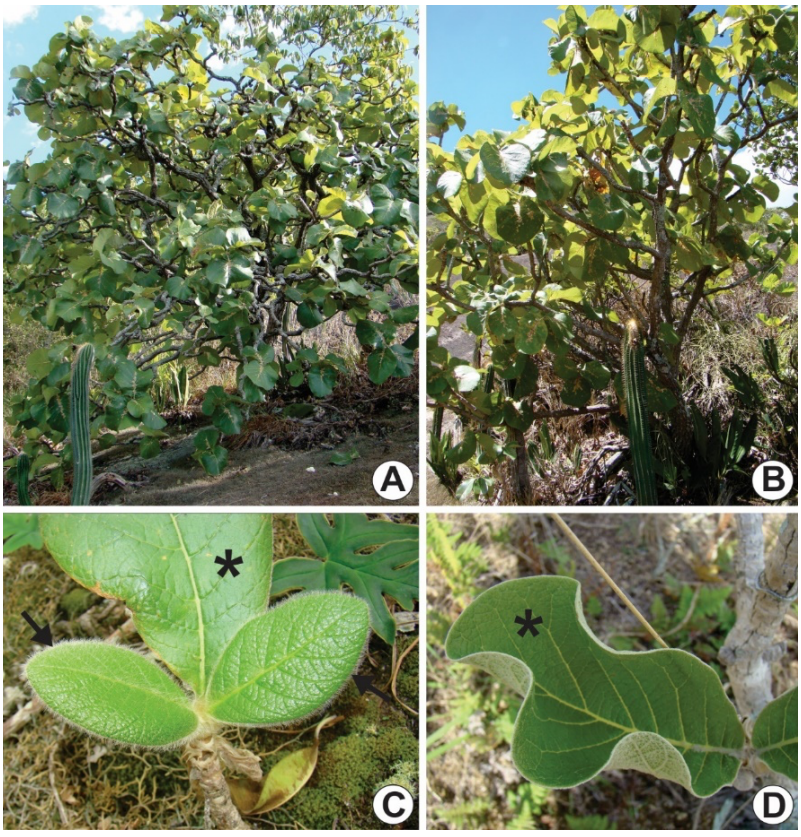
Material and Methods

Study area and plant material

This study was conducted on an inselberg known as the Itaoca massif, or Maciço do Itaoca (21°48'S 41°26'W), during summer (February 2014). This region is a rocky outcrop that reaches 420 m elevation and is 900 ha. It is occupied by Atlantic Forest and located in the Ibitioca district in the municipality of Campos dos Goytacazes, Rio de Janeiro, Brazil. The mean temperature, mean humidity, and mean radiation at noon next to the collection sites were 40.5°C, 44.2 $g.m^{-3}$, and 1982.42 $\mu m.m^{-2}.s^{-1}$, respectively.

Vegetative organs of *W. azulensis* were collected on the rocky walls of the inselberg between 40–150 m elevation (Figure 1). Fertile branches were collected, processed, and deposited in the Northern Rio de Janeiro State University (UENF) herbarium (HUENF) under the number H5580.

FIGURE 1: A-B. *Wunderlichia azulensis* from the Itaoca massif rocky outcrop, Campos dos Goytacazes, Brazil. C. Young pubescent leaf (arrow). Note the expanded leaf without pilosity (*). D. Fully expanded leaf (*).



Anatomical analysis

The underground system, aerial stems, and leaves (petiole and middle third of the leaf blade) were fixed in 2.5% glutaraldehyde, 4.0% paraformaldehyde, and a 0.05 M sodium cacodylate buffer (pH 7.2) for 2 h (KARNOVSKY, 1965, modified by DA CUNHA et al., 2000). The samples were washed three times in the same buffer, 1 h per time, before and after post-fixing the material with 1.0% OsO_4 in a 0.05 M sodium cacodylate buffer for 1 h at room temperature. After washing the post-fixed material, the samples were dehydrated using an increasing acetone series (50, 70, 90 and three times in 100%, for one hour each), embedded in gradual proportions of epoxy resin (Epon®) in acetone (1:3, 1:1, 3:1 v/v, for 12 h each), and finally embedded in pure epoxy resin for 72 h. Semithin (0.6–0.7 μm thick) sections were made using an ultramicrotome, stained with 1.0% toluidine blue for 1 min, and mounted on glass slides using Entellan® medium (O'BRIEN; MCCULLY, 1981). Sections were analyzed using a bright field microscope (Axioplan, Zeiss, Jena, Germany) coupled to an image capture system (Moticam Pro 282B, Motic, Kowloon, Hong Kong).

For the histochemical analysis, plant samples fixed as described above were dehydrated in a crescent ethanol series (50, 60, 70, 80, 90 and three times in 100%, for one hour each), embedded in a 100% ethanol/glycol metacrylate (Leica Historesin®) 1:1 v/v solution, and then in pure Historesin® (FEDER; O'BRIEN, 1968). Cross and longitudinal sections (3.0–6.0 μm thick) were made using a rotary microtome (Leica RM 2255, Leica, Wetzlar, Germany). Different chemical groups in the vegetative organs and trichomes were detected using the following histochemical procedures: Ruthenium red for pectins and mucilage, periodic acid-Schiff solution for polysaccharides, Lugol for starch grains, Auramine O for cuticle components, Sudan IV and Black B for lipid compounds, copper acetate and rubeanic acid for fatty acids, NADI reagent for terpenes and essential oils, phloroglucinol and HCl for lignin, ferric chloride for phenolic compounds, hydrochloric vanillin for tannins, 5–15% aluminum chloride and 5% magnesium acetate for flavonoids, Dragendorff reagent for alkaloids, and Xylidine Ponceau and coomassie blue for proteins (KRAUS; ARDUIN, 1997; ASCENSÃO et al., 2005).

The leaf epidermis was analyzed using paradermal sections from the leaf blade intercostal region. Fragments (1 cm^2) were submitted to the Franklin method to dissociate the epidermis. Staining was done using 1% safranin prior to the light microscopy analysis (FRANKLIN, 1945; JENSEN, 1962).

Scanning Electron Microscopy

Plant samples were dehydrated in an acetone series, as described above, and dried to the critical point using a Bal-Tec CPD 050 critical point dryer. Then, the dried samples were affixed to individual stubs with carbon adhesive tape and coated with 20 nm of gold (Bal-Tec Sputer Coater SCD 050) (KLEIN et al., 2004). The samples were analyzed using a DSEM 962 Scanning Electron Microscope (Zeiss, Jena, Germany) at 25 kV.

Results

Wunderlichia azulensis had a thickened, woody underground system with roots originating from the bottom region. The underground system is characterized by a fissured rhytidome, which is easily detachable. The aerial stems and leaves grow perennially from buds in the apical region.

Cross sections of roots show a typical structure of a primary root (Figure 2A) and the beginning of secondary root development (Figure 2B). The primary root had an epidermis formed by collapsed cells. The cortex was formed by parenchyma, which had idioblasts with stored compounds and thickened cell walls. The vascular cylinder had tetrarch primary xylem and small primary phloem groups of sieve tube elements and companion cells (Figure 2A). In the initial root secondary growth, the parenchyma cells were differentiating into phellogen and cambium. The latter produced secondary phloem and secondary xylem, and some vessel elements had occlusions (Figure 2B).

Cross sections of the thickened, woody underground system revealed a differentiated periderm with multiple cell layers (Figure 2C). The cortex was formed by

parenchymatous cells, of which most cells accumulated compounds. In this region, sclerenchyma cells were randomly distributed (Figure 2D). In the vascular cylinder, the secondary phloem surrounded the secondary xylem (Figure 2E), which was formed by isolated or paired vessel elements, fibers, and axial parenchyma (Figure 2F).

The aerial stem, in cross section, had a differentiating periderm (Figure 3A) as the outermost tissue. The stem cortex is similar to the woody root cortex, with parenchyma and sclerenchyma cells. The central cylinder is formed by collateral vascular bundles (Figure 3B). The phloem is formed by perivascular fibers with thick lignified cell walls, sieve tube elements and companion cells, and is separated from the xylem by 2–3 layers of cambium cells. Also, the stem medulla of *W. azulensis* is composed of parenchyma cells.

FIGURE 2: Cross sections of the *Wunderlichia azulensis* underground system. A-B. Herbaceous root. A. Primary root. B. Secondary root development, with cambium and phellogen in formation (arrows). C-F. Woody thickened portion. C. Detail of the periderm. D. Cortex. E. Secondary phloem (Ph) and xylem. F. Detail of the secondary xylem. (*) indicates compound accumulation in parenchymatous cells and vessel elements. Arrowheads indicate sclerenchymatous cells in the cortex. Ep = epidermal cells; Fi = fibers; Pa = parenchyma; Pe = periderm; Ph = primary phloem; Ra = ray parenchyma; Ve = vessel elements. Bars: 50 µm.

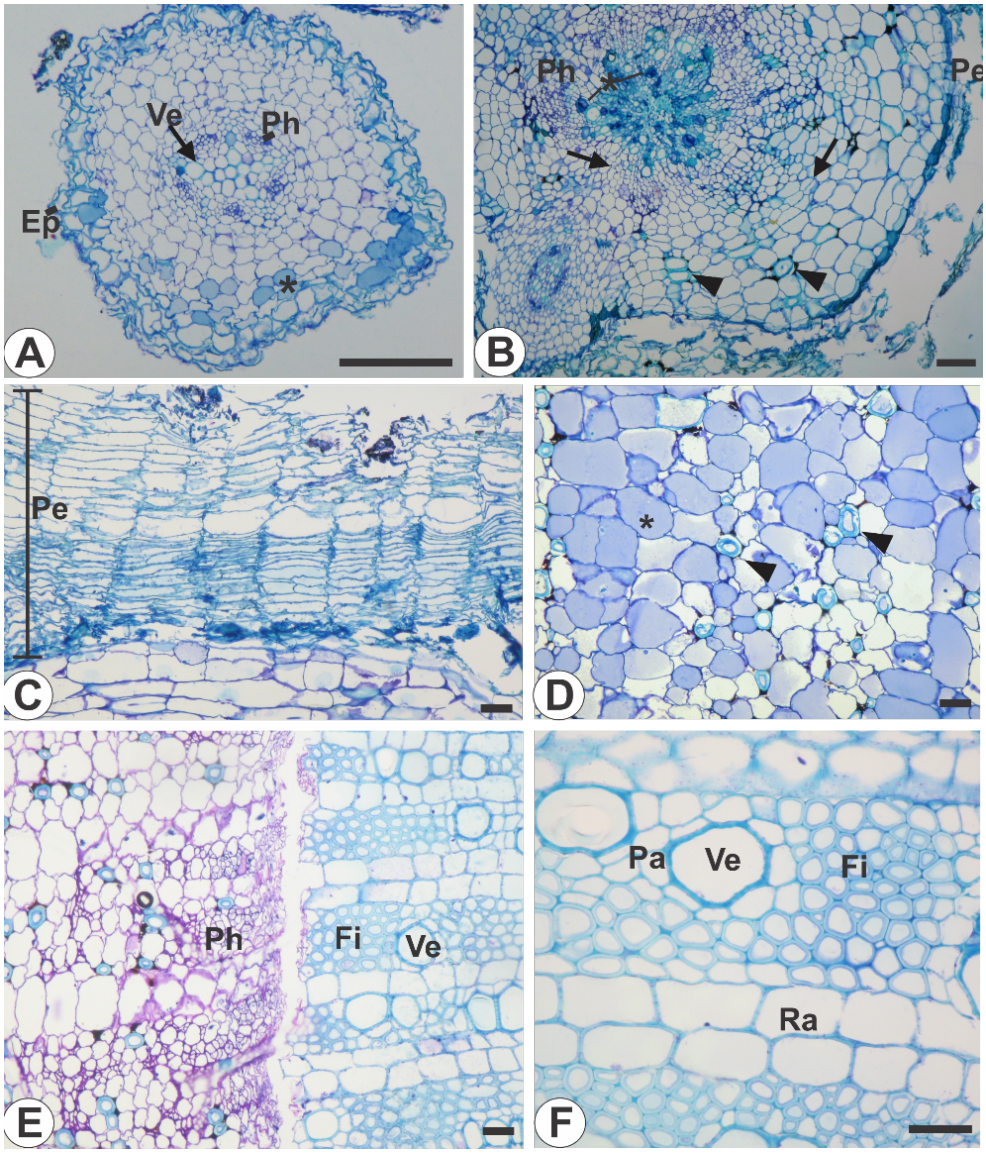
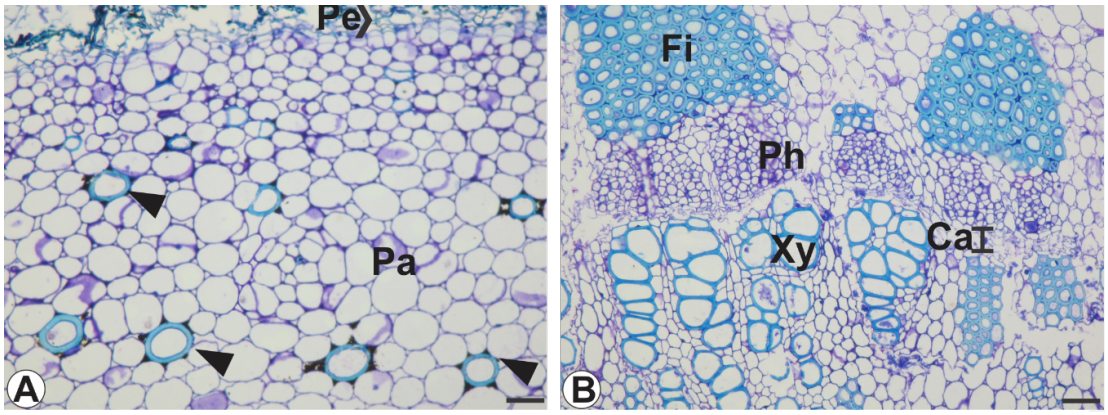
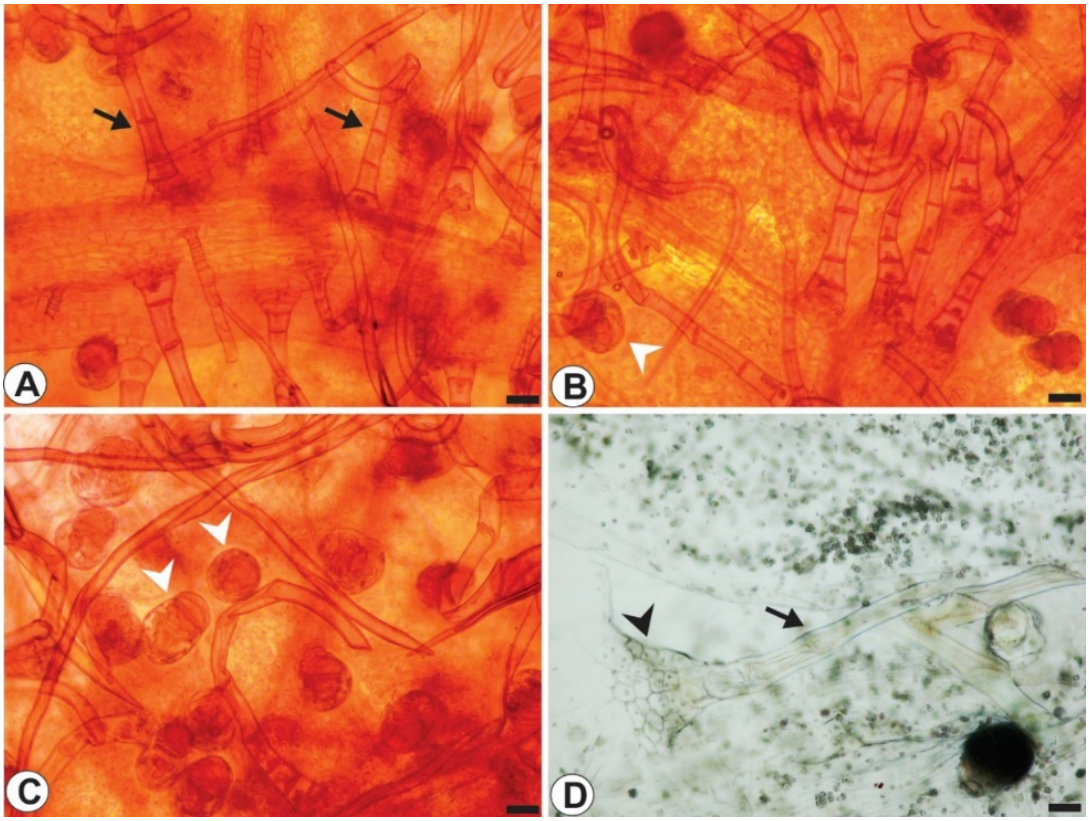


FIGURE 3: Cross sections of a *Wunderlichia azulensis* stem. A. General view of the periderm (Pe) and the cortex. Note the parenchyma (Pa) and sclerenchyma cells (arrowheads). B. Detail of the vascular bundles in the periphery of the vascular cylinder. Note the cambium cells (Ca), perivascular fibers (Fi), phloem (Ph), and xylem (Xy). Bars: 50µm.



Analysis of the leaf blade dissociated epidermis revealed a high density of glandular and tector trichomes on both the adaxial (Figure 4A-B) and abaxial (Figure 4C-D) surfaces. In frontal view, it was possible to observe long, multicellular, uniseriate tector trichomes, with a multicellular base (Figure 4A-D), whereas the glandular trichomes had a multicellular head (Figure 4B-C).

FIGURE 4: Frontal view of the *Wunderlichia azulensis* leaf epidermis using light microscopy. A-B. Adaxial surface. C-D. Abaxial surface. Arrows indicate uniseriate, multicellular tector trichomes. White arrowheads indicate glandular trichomes. Black arrowhead indicates the multicellular base of a tector trichome. Bars: 50 µm.



The scanning electron microscopy analysis revealed long tector trichomes (Figure 5A-B), epidermal cells, glandular trichomes, and stomata above the level of the other epidermal cells (Figure 5C-D). The epidermal cells varied in size and exhibited straight or slightly sinuous anticlinal walls. The leaves were hypostomatic, and the stomata were anomocytic (Figure 5C-D). The glandular trichomes were multicellular (Figure 5C-F), with bases formed by 2–4 cells. The peduncle was short (max. 4 cells long), and the head was formed by three globoid apical cells (Figure 5E-F).

Leaf blade cross sections revealed a uniseriate epidermis with a thick cuticle and one distinct subepidermal layer formed by rectangular cells on the adaxial surface (Figure 6A-C). The epidermal cells were similar in shape and size on both the adaxial and abaxial surfaces; they were oval or rectangular, although the stomatal guard cells protruded more (Figure 6D). The adaxial surface had a thick cuticle, as evidenced by the Sudan IV histochemical test (Figure 6C).

The mesophyll was dorsiventral, in which the palisade and spongy parenchyma were distinct (Figure 6E). However, the palisade parenchyma cells were not juxtaposed and exhibited intercellular spaces (Figure 6A, E). The spongy parenchyma was formed by a variable number of layers of oval cells (Figure 6E). The vascular bundle was surrounded by a sheath of perivascular cells, which extended to both epidermal surfaces (Figure 6E). The vascular system was composed of numerous collateral vascular bundles of different sizes that were surrounded by a parenchyma or sclerenchyma sheath (Figure 6E).

Druse crystals were detected in the palisade and spongy parenchyma (Figure 6F). Histochemical tests revealed phenolic compounds in the adaxial epidermis (Figure 6G), mucilage on both epidermal surfaces and the apical cells of glandular trichomes (Figure 6H-I), and essential oils or lipid bodies in epidermal and mesophyll cells (Figure 6J-K).

FIGURE 5: Leaf trichomes of *Wunderlichia azulensis* (A-D: scanning electron microscopy; E-F: light microscopy). A-B. Leaf abaxial surface, showing tector (*) and glandular (arrows) trichomes, and leaf veins (arrowheads). C. Detail of a glandular trichome, stomata, and protuberant epidermal cells. D. Detail of the glandular trichome. E. Glandular trichome with uniseriate, multicellular stem and multicellular head (arrowhead). F. Glandular trichome with multiseriate stem. Bars: A-B = 100 µm; C-F = 50 µm.

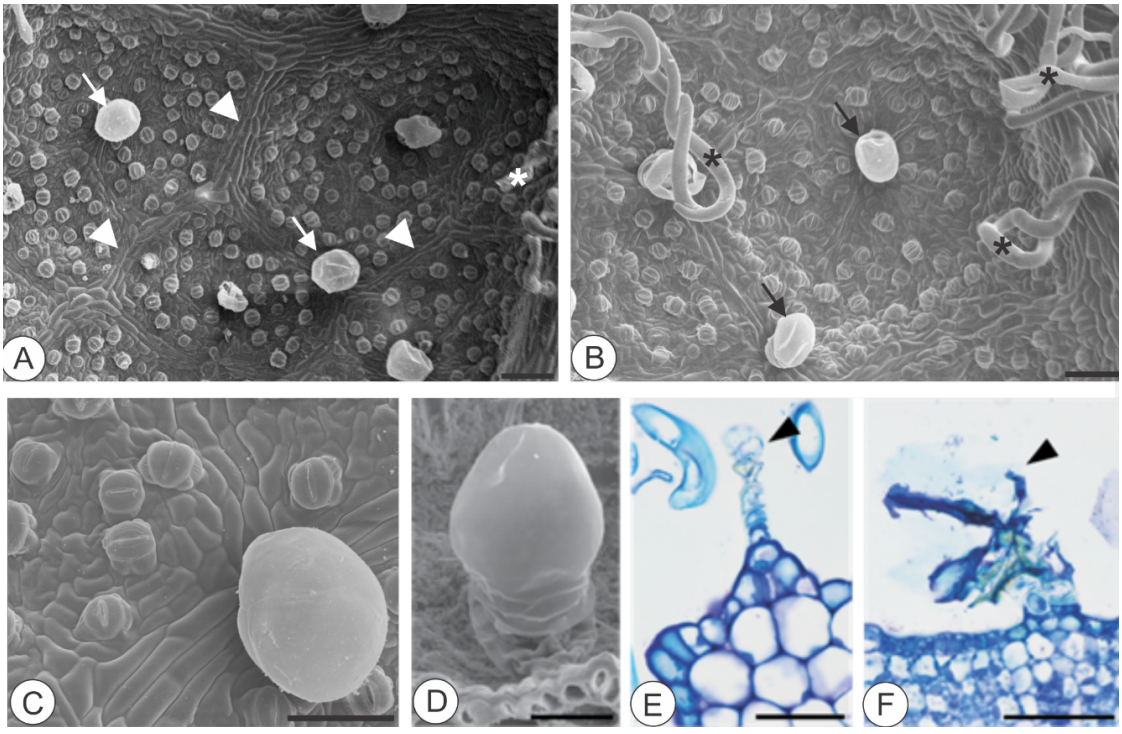
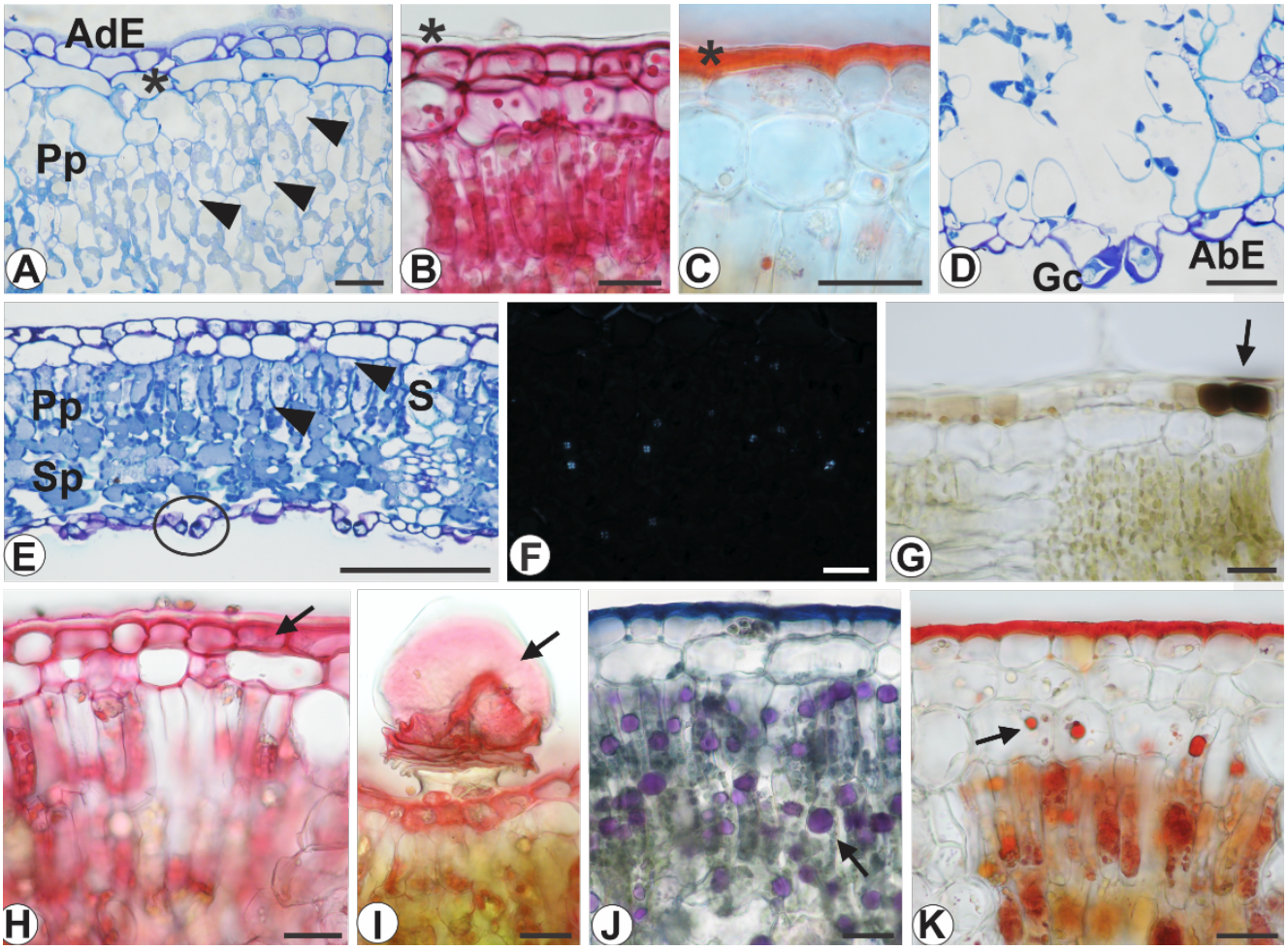


FIGURE 6: *Wunderlichia azulensis* leaf anatomy and histochemistry. A. Adaxial epidermis (AdE), one subepidermal layer (*) and palisade parenchyma (Pp) with intercellular spaces (arrowheads). B. Ruthenium red staining evidencing subcuticular strata. Note the negative reaction in the cuticle (*). C. Sudan IV positive reaction in the cuticle (*). D. Detail of the abaxial epidermis (AbE). E. General view of the leaf mesophyll. F. Druse-type crystals (using polarized light). G. Phenolic compounds (arrow) evidenced by ferric chloride. H-I. Mucilage (arrow) evidenced by Ruthenium red. J. Essential oils (arrow) evidenced by NADI reagent. K. Lipid bodies evidenced by Sudan IV (arrow). Pp = palisade parenchyma; Sp = spongy parenchyma; S = parenchymatous sheath. Bars: A-D, F-K = 10 **µm; E = 50 µm**.



Discussion

This study evaluated the anatomy of the *W. azulensis* subterranean system, which included herbaceous roots and a thickened, woody subterranean system, as well as the aerial stem. *Wunderlichia* species are morphologically characterized by a cylindric stem, thick and fissured rhytidome and perennial aerial branches (SOUZA-BUTURI, 2013), which were also observed in this study. Moreover, Souza-Buturi (2013) described the subterranean parts of *Wunderlichia* species as forming a possible xylopodium. Xylopodia are defined as rigid subterranean organs formed by the tuberization of the hypocotyl or the root-stem transition zone, with the high capacity for storage and bud formation (VILHALVA; APPEZZATO-DA-GLÓRIA, 2006; HAYASHI; APPEZZATO-DA-GLÓRIA, 2007; APPEZZATO-DA-GLÓRIA; CURY, 2011; PAUSAS et al., 2018).

Although the *W. azulensis* aerial branches were perennial, Appezzato-da-Gloria et al. (2008) observed that xylopodia of different Asteraceae species lose their stem branches in the dry season and resprout in the

rainy season. Xylopodia are common in species in the cerrado and campo rupestre regions and were previously described for other Asteraceae species by Machado et al. (2004), Vilhalva and Appezzato-da-Gloria (2006), Hayashi and Appezzato-da-Glória (2007), Silva et al. (2014), and Carbone et al. (2019).

Since xylopodia are formed from the tuberization of the hypocotyl or the proximal root zone (PAUSAS et al., 2018), their anatomy may exhibit either a secondary stem structure, especially in parts closer to the soil surface, or a secondary root structure. Xylopodia are covered by a periderm of subepidermal origin, and root buds differentiate from cortical parenchyma cells (HAYASHI; APPEZZATO-DA-GLÓRIA, 2007; APPEZZATO-DA-GLÓRIA et al., 2008; ABDALLA et al., 2016), as observed in this study. This indicates that the thickened, woody subterranean system of *W. azulensis* has a xylopodium-like structure, corroborating Souza-Buturi (2013). However, it is unclear whether the studied xylopodium region is derived from the hypocotyl, thus presenting a secondary stem structure, or from the proximal root zone. Thus, further studies are needed to elucidate both the morphology and anatomy of the secondary stem structure of *W. azulensis*, since it is difficult to collect and identify its parts.

The xylopodium is frequently mentioned as a storage structure for inulin, a fructan in the cortex parenchymatous cells or in the xylem, which is in the form of crystals and used for osmoregulation purposes (VILHALVA; APPEZZATO-DA-GLÓRIA, 2006). No inulin crystals were found in the thickened, woody subterranean system of *W. azulensis*, although its parenchyma cells accumulated compounds. Other studies of Asteraceae identified lipids (HAYASHI; APPEZZATO-DA-GLÓRIA, 2007; BOMBO et al., 2014) and phenolic compounds (APPEZZATO-DA-GLÓRIA; CURY, 2011; CARBONE et al., 2019). Moreover, secretory canals have also been described in roots and xylopodia of other Asteraceae species (CURY; APPEZZATO-DA-GLÓRIA, 2009). However, these structures were absent in *W. azulensis*. Further studies to evaluate the chemical composition of the subterranean system are recommended, as well as histolocalization of the chemical classes.

The cortex of the thickened, woody subterranean system of *W. azulensis* also had randomly distributed sclerenchymatous cells, which were previously described by Hayashi and Appezzato-da-Glória (2007) in xylopodia from *Lessingianthus grandiflorus* (Less.) H.Rob. and *L. brevifolius* (Less.) H.Rob. and by Abdalla et al. (2016) in tuberous roots of *Apopyrus warmingii* (Baker) G.L. Nelson and *Ichthyothere terminalis* (Spreng.) S.F. Blake. Appezzato-da-Glória and Cury (2011) classified such cells as brachysclereids.

Moreover, a xylopodium is considered an effective adaptation to xeromorphic and disturbed environments, including fire-prone ecosystems (PAUSAS et al., 2018). *Wunderlichia* Riedel ex Benth is endemic to Brazil, and the geographic distribution of its species is restricted to xeromorphic environments (BARROSO; MAGUIRE, 1973; ROQUE; PIRANI, 1997; HIND; SEMIR, 1998). This includes rocky outcrops, with high solar incidence and low water availability, such as the Itaoca massif (MAUAD et al., 2014) that is the studied site. Apart from the aforementioned tuberous, woody subterranean system, *W. azulensis* leaves had a thick cuticle, crystal idioblasts in the mesophyll, suprastomatal chambers, and specialized cells for water storage. According to Handro et al. (1970) and Simioni et al. (2017), these leaf characters are considered xeromorphic adaptations.

Both adaxial and abaxial surfaces of the leaf epidermis had a thick cuticle. Cutin and wax coverings minimize water loss through transpiration reduction (ZIV et al., 2018). Cuticle thickness is mainly a response to high light incidences (RÔÇAS et al., 2001) and lower water availability (PYYKKO, 1966). Ziv et al. (2018) correlated cuticle thickness to protection against pathogens.

Also, water loss depends on chemical and structural compositions of the cuticle, for example, associations with waxes. In this study, histochemical tests indicated the occurrence of a cutin matrix on the outer periclinal cell wall. Cutin is a lipophilic compound that may act as a barrier against the diffusion of water, solutes, and excess UV rays (NIKLAS et al., 2017). Thus, such compounds might act as additional barriers against high light incidence on rocky outcrops.

The epidermal cells of *W. azulensis* leaves varied in size and shape and had slightly sinuous walls in frontal view. Moreover, the adaxial epidermis is multiseriate. These characters were also observed by Smiljanic (2005) in other Asteraceae from a rocky outcrop in Minas Gerais State.

The sinuosity of the epidermal anticlinal cell walls might be affected by abiotic factors, such as luminosity and water availability. Leaves exposed to higher sun irradiation levels have straighter anticlinal cell walls because of cuticle thickening (MEDRI; LLEIRAS, 1980; KOLB et al., 2020). Moreover, the lower sinuosity of anticlinal cell walls is considered a xeromorphic adaptation against water loss (MEDRI; LLERAS, 1980; LEME; SCREMIN-DIAS, 2014). In this study, the anticlinal cell walls in the adaxial epidermis, which are directly exposed to light, were straighter.

This study did not evaluate the ontogeny of the leaf epidermis, such as whether it is multiseriate or uniseriate with a subepidermal layer. A subepidermal layer, which generally has parenchymatous, rectangular-shaped and chloroplast-free cells, may be present in species of Asteraceae genera, such as *Sheareria* S. Moore (LI et al., 2008) and *Mikania* Willd. (ALMEIDA et al., 2017). Souza-Buturi (2013) described this subepidermal layer as a hypodermis in *Wunderlichia* species, although the author did not conduct an ontogenetic study. A hypodermis is considered an adaptation for water storage (BIERAS; SAJO, 2009), a transpiration restrictor (ARAÚJO et al., 2010), and a secretory structure (CASTRO; DEMARCO, 2008).

The epidermis is a multifunctional tissue comprising different specialized cells, such as stoma and trichome cells, which structure and location influence water relationships and gas exchange in leaves. Stomata that are predominantly on the abaxial leaf surface and usually covered by trichomes, or the formation of a suprastomatal chamber that is generally found in epiphytes in dry environments, are strategies to reduce transpiration (SIMIONI et al., 2017). Although *W. azulensis* stomata were located above the distribution line of other epidermal cells, which is typical of plants from more humid environments, their function for *W. azulensis* might be linked to optimizing CO_2 capture (FERREIRA et al., 2013).

A dense indument of tector or glandular trichomes covered the abaxial and adaxial leaf epidermis of *W. azulensis*. Uni- or multiseriate trichomes are common in the tribe Mutisieae s.l. (METCALFE ; CHALK, 1950), whereas depressed, spatulate glandular trichomes are also common on the abaxial surface of the tribe Wunderlichieae (PANERO; FUNK, 2007; MARTÍNEZ-QUEZADA et al., 2022). Although trichome type is a taxonomic feature, the high trichome density creates a microclimate in this region that is an important xeromorphic adaptation, since these structures increase the reflection of sun irradiation (HOLMES; KEILER, 2002), which contributes to decreasing the temperature of leaves (EHLERINGER, 1983; WAGNER et al., 2004). The presence of trichomes on the epidermis also contributes to increasing the air layer thickness. This covers the leaf surface and consequently restricts water vapor loss from stomata to the atmosphere (FAHN; CUTLER, 1992), which is relevant due to the low water availability and high sun irradiance found on the rocky outcrop in this study. Moreover, trichomes may protect against herbivores (WOODMAN; FERNANDES, 1991) or pathogens (VALKAMA et al., 2005).

The mesophyll in *W. azulensis* leaves had loose cells and irregular intercellular spaces in both the palisade and spongy parenchyma. Both tissues were formed by more than one cell layer. Besides specialized epidermal structures, mesophyll influences plant water use efficiency, which is expressed by the stomatal conductance and the water loss speed. The development of stomatal pores and intercellular spaces is coordinated to optimize gas exchange efficiency, and water vapor might play a regulator role in the development of such spaces. Still, porosity of palisade parenchyma is a greater contributor than stomatal density to stomatal conductance (BAILLIE; FLEMING, 2020). This information is relevant because *W. azulensis* is exposed to high sun irradiation and tends to have greater evapotranspiration.

Druse-like crystals were observed in the mesophyll. According to Macnish et al. (2003) and Lersten and Honer (2008), calcium oxalate crystals and druses in

plant cells originate mostly from calcium precipitation as a result of evapotranspiration. According to Smiljanic (2005), druses were also found in large quantities in the mesophyll of *Vernonanthura discolor* (Less.) H.Rob. (Asteraceae). Since *V. discolor* is a pioneer plant in *Araucaria* Juss. (Araucariaceae) forests, Smiljanic (2005) suggested druses are a strategy against herbivory. Moreover, calcium oxalate crystals may affect leaf optical properties by redirecting the light due to the reflectance and transmittance properties of crystals, leading to increased photosynthetic activity and decreased photoinhibition of the palisade parenchyma (GOLOB et al., 2018).

The histochemical tests for the *W. azulensis* leaf blade indicated, for example, phenolic compounds in epidermal cells and lipid bodies and essential oils in the mesophyll parenchyma cells. Phenolic compounds are related to protection against ultraviolet irradiation and assist in the maintenance of the protoplast integrity under water stress (CHEYNIER et al., 2013). The chemical composition of essential oils is usually complex, although terpenes predominate (EL ASBAHANI et al., 2015). Terpenes and essential oils are common in Asteraceae and were previously described for *Wunderlichia* (NUÑEZ, 2000). Essential oils provide a chemical barrier by producing either toxic or repellant compounds (NERIO et al., 2010; NINKUU et al., 2021), and they play a pivotal role in adaptation to xeric environments (FAHN, 1986; FAHN; CUTLER, 1992) by releasing exudates, which constitute a continuous layer on the leaf surface. This type of layer may increase light refraction and decrease the temperature, influencing the water economy of the plant (FAHN, 1986; SALATINO et al., 1986).

In conclusion, the leaf and root anatomical analysis produced important data about *W. azulensis* and Asteraceae. The vegetative parts exhibited adaptations that correlate the success of the species to its xeromorphic environment. Traits such as a thick cuticle, suprastomatal chambers, and crystals in the mesophyll parenchyma are important strategies to optimize and protect the water relationships and gas exchange in leaves. Although the stomata were emergent, the tector and glandular trichomes, as well as the differentiated growth of the root cortical cells, might play roles against water loss. The histochemical results revealed the presence of lipids, oils, and phenolic compounds. These might be related to protection against water deficit and high irradiance, suggesting a correlation between secondary metabolites and adaptation to the xeromorphic environment. However, further analyses of the roots and stems are needed, especially anatomical and histochemical studies. This study contributed significantly to what is known about *Wunderlichia* and *W. azulensis*.

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