Article

Anatomy and histochemistry of the vegetative organs of *Brachystele guayanensis* (Lindl.) Schltr. (Orchidaceae), a potential medicinal species

Igor Soares dos Santos [1,*] and Marcos José da Silva [2]

[1] Post-graduate Program in Plant Biology, Department of Botany, Institute of Biosciences, São Paulo State University (Unesp), Botucatu, São Paulo, 18618-970, Brazil; I.S.S., is.santos@unesp.br
[2] Laboratory of Molecular Systematics and Plant Taxonomy, Department of Botany, Institute of Biological Science, Federal University of Goiás (UFG), Goiânia, Goiás, 74001-970, Brazil; M.J.S., marcos_agrorural@hotmail.com
* Correspondence: I.S.S., is.santos@unesp.br or igorsoares@discente.ufg.br; Tel.: +55-(62)98179-5337

Abstract: The orchid genus *Brachystele* Schltr. comprises 20 species distributed from Mexico to Argentina; 10 are found in Brazil. Anatomical studies of Orchidoideae Lindl. have been scarce, and the anatomy and histochemistry of *Brachystele* are still largely unknown. We characterized the vegetative organs of *B. guayanensis* (Lindl.) Schltr. using standard anatomical and histochemical microtechniques. *Brachystele guayanensis* was observed to display the anatomical characters commonly found in the vegetative organs of representatives of the Orchidaceae and Orchidoideae (including a uniseriate epidermis, thin cuticle, amphistomatic leaves, anomocytic, diacytic and tetracytic stomata, a homogeneous mesophyll, collateral vascular bundles, rhizomes with pericyclic fibers, roots with velamen, uniseriate exodermis, endodermis and pericycle). Histochemical tests confirmed the presence of lignin, proteins, and alkaloids, the lipidic nature of the cuticle, starch grains stored in spiranthosomes in the roots, and the composition of the raphides. Alkaloids were observed in great abundance, especially in the roots, and may have potentially useful medicinal activities, as has been observed in groups phylogenetically related to *Brachystele*.

Keywords: Alkaloids; Cells; Cranichideae; Ergastic substances; Leaf; Micromorphology; Orchidoideae; Rhizomes; Roots; Spiranthinae

1. Introduction

Brachystele Schltr. comprises 20 species distributed from southern Mexico to northern Argentina [1]; 10 species occur in Brazil, of which three are endemic [2]. The taxon is terrestrial, herbaceous, with short rhizomes, fleshy roots, leaves arranged in rosettes, lateral racemes with small flowers, resupinate, usually indumented, with a bilobed stigma [1, 2].

Anatomical descriptions of the vegetative organs of Orchidoideae Lindl., the subfamily in which *Brachystele* is included, are still incipient and have mainly focused on species not occurring in Brazil [e.g., 3–20]. Anatomical studies of *Brachystele* are currently limited to the work of Bernal et al. [18], who examined the root trichomes of representatives of Spiranthinae Lindl. ex Meisn. as well as *B. widgrenii* (Rchb.f.) Schltr. There have been no histochemical studies of any *Brachystele* species, although some medicinal properties of *B. dilatata* (Lindl.) Schltr. and *B. unilateralis* (Poir.) Schltr. were mentioned (e.g., their diuretic and carminative property) in ethnobotanical studies [21, 22].

We provide here anatomical and histochemical characterizations of the vegetative organs of *B. guayanensis* (Lindl.) Schltr. species (Figure 1) to expand available knowledge of that diversity in Orchidaceae, especially in *Brachystele*, and subsidize phylogenetic and micromorphological studies of that genus.

 doi:10.20944/preprints202306.0349.v1

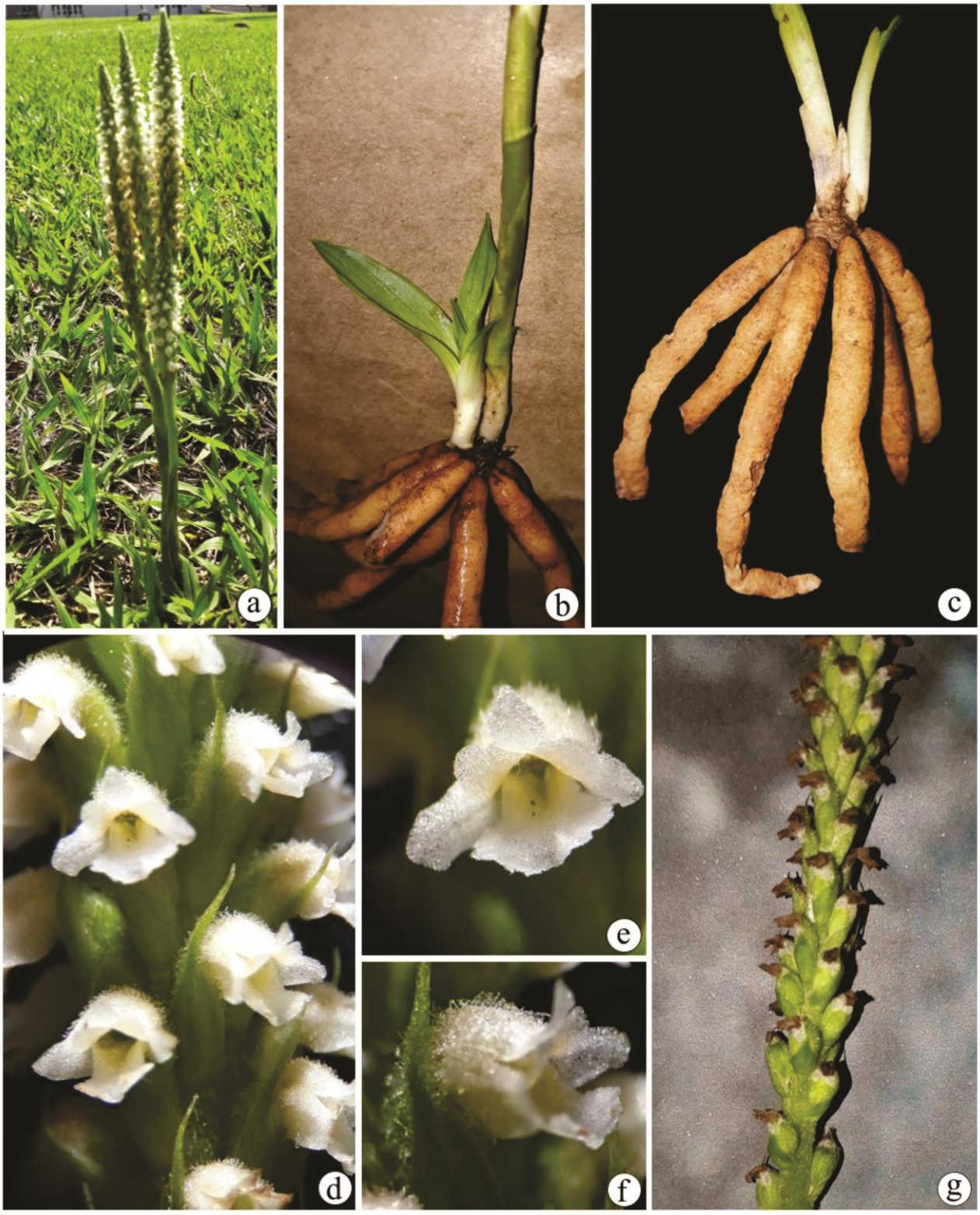


Figure 1. *Brachystele guayanensis* (Lindl.) Schltr. (**a**) Habit; (**b, c**) Details of leaf, rhizome, roots, and base of the inflorescence axis; (**d**) Detail of the inflorescence; (**e**) Flower frontal view; (**f**) Flower lateral view; (**g**) Capsules. Photographs by Igor Soares dos Santos.

2. Results and Discussion

2.1. Anatomical data

2.1.1. Leaf anatomy

 doi:10.20944/preprints202306.0349.v1

In frontal view, the leaf epidermis of *B. guayanensis* is covered by a striated cuticle and is composed of polygonal cells with both straight and curved walls (Figure 2a, b) – characters that have also been reported for *Microchilus arietinus* (Rchb. f. & Warm.) Ormerod and *Zeuxine strateumatica* (L.) Schltr.) by Andreota et al. [17] and Bona et al. [20] and described for *Aa paleacea* (Kunth) Rchb. f. and *Pterichis multiflora* (Lindl.) Schltr. by Corredor and Arias [15]. Anomocytic, diacytic and tetracytic stomata were observed on both faces of the leaf blade of *B. guayanensis* (Figure 2a, b), as seen in other species of Orchidaceae [3, 14, 16, 19].

In cross-section, the epidermis of *B. guayanensis* is covered by a thin, striated cuticle and is uniseriate and composed of rounded, oblong cells with slightly thickened external periclinal walls (Figure 2c, d) – aspects reported for other members of Orchidoideae [e.g., 6, 9, 10, 13–15, 17, 20]. The leaves are amphistomatic (Figure 2c-e), with stomata at the same level as common cells of the epidermis. The substomatal chambers are wider than the suprastomatal chambers (Figure 2c, d) – characteristics related to reducing water losses and evapotranspiration [23, 24]. Amphistomatic leaves are commonly observed in plants having both high photosynthetic capacities and high stomatal conductance, especially those that grow in open and sunny environments [25, 26], as studied here.

The mesophyll of *B. guayanensis* is homogeneous and consists of 6–11 layers of rounded cells having varying dimensions (Figure 2e). Those cells are interspersed with crystalliferous idioblasts containing raphides (Figure 2f) – a pattern cited for different groups of Orchidaceae [e.g., 6–10, 13–17, 19, 20, 27–29]. Collateral vascular bundles surrounded by a parenchyma sheath were observed in the median portion of the mesophyll (Figure 2e). The bundle corresponding to the midrib is flat-convex (Figure 2g) and has the largest caliber; the elements of that vessel are arranged in a "V" (Figure 2h); smaller bundles (Figure 2i), with smaller calibers are interspersed. This same pattern has been reported in other taxa by the authors cited above. The leaf margins of *B. guayanensis* are straight and rounded (Figure 2j).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 June 2023 doi:10.20944/preprints202306.0349.v1

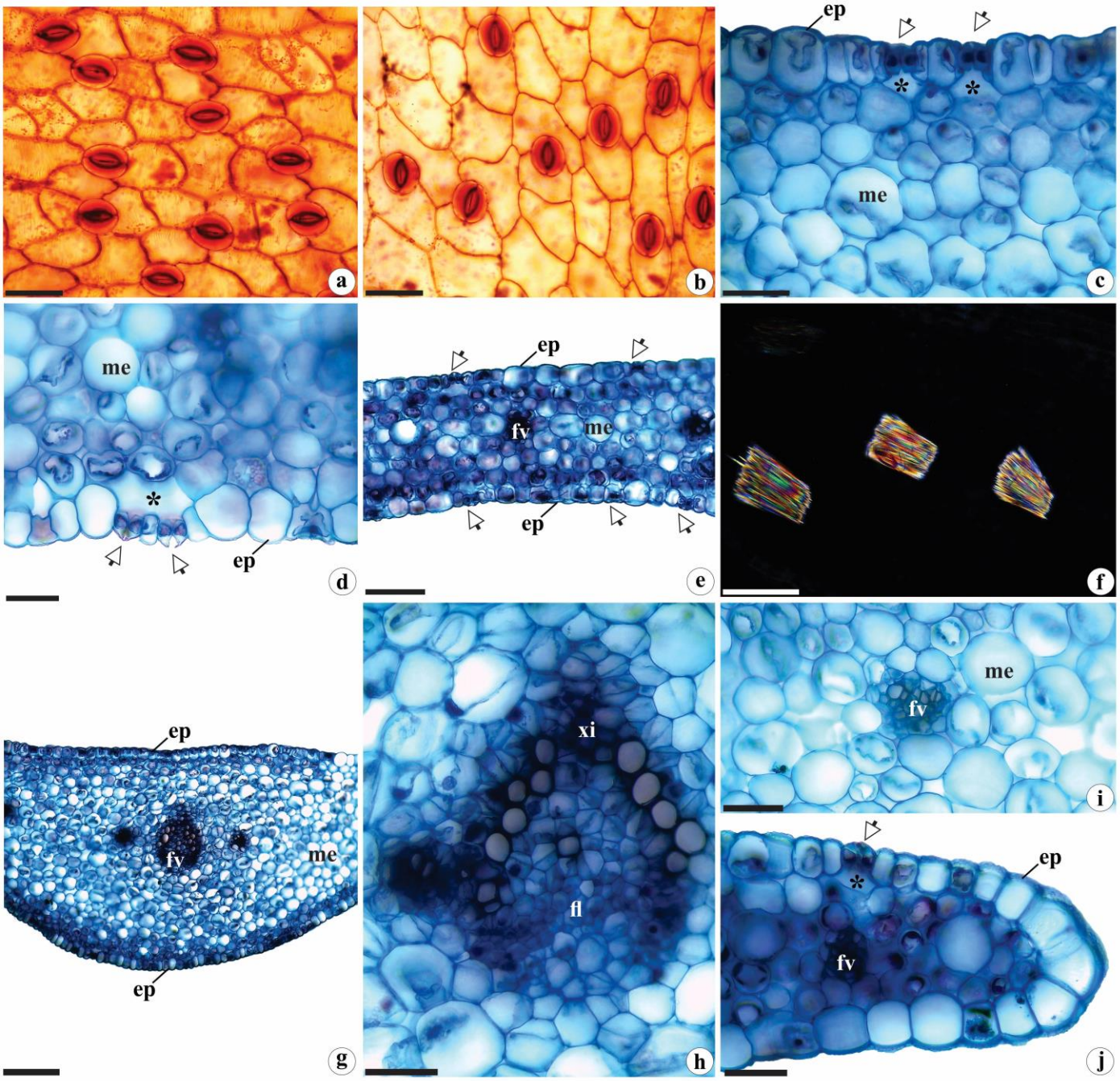


Figure 2. Leaf anatomy of *Brachystele guayanensis* (Lindl.) Schltr.; (**a, b**) Epidermis in frontal view; (**c-j**) Cross sections; (**a, c**) Detail of the adaxial surface; (**b, d**) Detail of the abaxial surface; (**e**) Leaf blade; (**f**) Detail of raphides under polarized light; (**g**) Midrib; (**h**) Detail of the vascular bundle of the midrib; (**i**) Detail of the smaller-caliber vascular bundle; (**j**) Margin. ep = Epidermis; fl = Phloem; fv = Vascular bundle; me = Mesophyll; xi = Xylem; * = Substomatic chamber; Arrows = Stomata. Scales: a-d, f, h-j = 50 μm; e, g = 200 μm. Photomicrographs by Igor Soares dos Santos.

2.1.2. Rhizome anatomy

The anatomy of the rhizomes of *B. guayanensis* was observed to be similar to other representatives of Orchidoideae [e.g., 6, 9, 10, 13–16, 19] in having rounded outlines and variable calibers (Figure 3a, b), with cataphylls in nodal regions or protecting the buds (Figure 3b, c). The epidermis is covered by a smooth, thin cuticle, is uniseriate, and composed of oblong, or occasionally, rounded common cells with thin cell walls (Figure 3d). Stomata were only observed on exposed portions of the rhizome and were arranged at

 doi:10.20944/preprints202306.0349.v1

the same level as the other common cells, with only tiny substomatal and suprastomatal chambers (Figure 3d).

The cortex of *B. guayanensis* is surrounded by a ring of pericyclic fibers (Figure 3h) and consists of 16–22 layers of rounded parenchyma cells of varying sizes (Figure 3a, b) with small triangular intercellular spaces; some of the parenchyma cells contain raphides (Figure 3e) and starch grains (Figure 3f). The arrangement is similar to that described by Stern [16] and by Stern and Judd [27, 28] for the aerial stems of other Orchidaceae groups (such as Vanilloideae [27, 28]; Orchidoideae, tribes Diseae Dressler and Ochideae Small; and Epidendroideae Lindl., tribes Sobralieae Pfitzer and Triphoreae Dressler [16, 29]), as well as other groups of Monocotyledons [e.g., 30]. The endodermis and/or pericycle, as described here (Figure 3b), may participate in the genesis of adventitious roots, and therefore exhibit meristematic activity [30].

Fungal hyphae and pelotons were observed to be concentrated mainly in the more peripheral portions of the cortical parenchyma (Figure 3g), as was reported by Pereira et al. [31] for the roots of *Bulbophyllum* sp., *Campylocentrum organense* (Rchb.f.) Rolfe and *Gomesa crispa* (Lindl.) Klotzsch ex Rchb. f. According to these authors, the strategic positions of those structures contribute to the maintenance and (re)colonization of internal tissues and thus constitute important sources of inoculum for adventitious roots that will extend from the rhizome, corroborating the findings of Bougoure et al. [32] for the terrestrial orchids *Rhizanthella gardneri* R.S. Rogers and Suetsugu et al. [33] and *Oreorchis indica* (Lindl.) Hook. f.

From 28 to 45 collateral vascular bundles can be observed internally, surrounded by a parenchyma sheath, in an atactostelic arrangement (Figure 3i) typical of Monocots [25, 34, 35]. Those bundles are observed in the central portion of the organ analyzed (Figure 3a, b, i), and have been observed in other groups of that family [e.g., 9, 10, 16, 27–29]. The vessel elements of the xylem are arranged in “V” formations in the collateral vascular bundles (Figure 3j), consistent with the findings of Andreota [36] for the tribe Cranichideae.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 June 2023 doi:10.20944/preprints202306.0349.v1

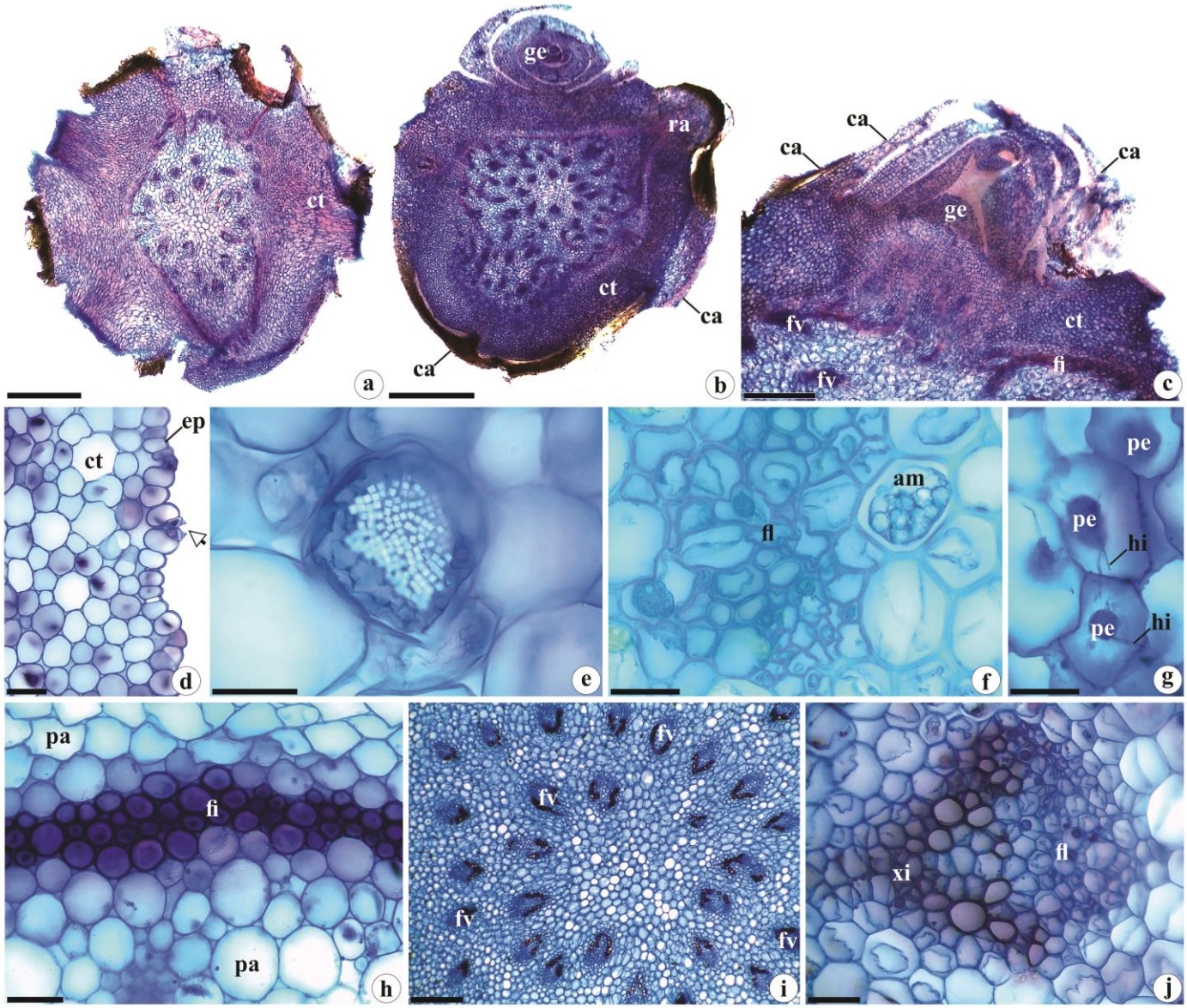


Figure 3. Rhizome anatomy of *Brachystele guayanensis* (Lindl.) Schltr.; (**a, b, d-j**) Cross-sections; (**c**) Longitudinal sections; (**a, b**) General aspect; (**c**) Detail of the buds and cataphylls; (**d**) Detail of the epidermis and portion of the cortical parenchyma; (**e**) Raphides; (**f**) Starch grains; (**g**) Detail of hyphae and pelotons; (**h**) Detail of pericyclic fibers; (**i**) Detail of the central portion of the rhizomes and arrangement of the vascular bundles; (**j**) Detail of the vascular bundle. am = Starch grains; ca = Cataphylls; ct = Cortex; ep = Epidermis; fl = Phloem; fi = Pericyclic fibers; fv = Vascular bundles; ge = buds; hi = Hyphae; pa = Parenchyma; pe = Pelotons; ra = Adventitious root; xi = Xylem; arrows = Stomata. Scales: a, b = 1000 μm; c = 500 μm; i = 200 μm; d, f-h, j = 50 μm; e = 20 μm. Photomicrographs by Igor Soares dos Santos.

2.1.3. Root anatomy

Brachystele guayanensis has transversely circular roots of variable calibers (Figure 4a) that are anatomically similar to those found in other taxa of Orchidoideae [e.g., 5, 6, 11, 12, 15–17], with uniseriate velamen of the "Spiranthes type". The component cells are irregular, elliptic, and thin, with thin cell walls with helicoidal thickenings and small pores in the non-thickened regions (Figure 4b, c). Our observations were similar to those of Porembski and Barthlott [4] for *Pelexia dolichorhiza* Schltr. (= *Pachygenium pteryganthum* (Rchb. f. & Warm.) Szlach., R. González & Rutk.), *Prescottia colorans* Lindl. (= *P. stachyoides*

 doi:10.20944/preprints202306.0349.v1

(Sw.) Lindl.), and *Sauroglossum elatum* Lindl. Moreira and Isaias [12] reported that terrestrial orchids generally have thinner velamen than epiphytic or rupicolous species in terms of their numbers of layers. Pridgeon [37], Moreira and Isaias [12], and Chomicki et al. [38] noted that velamen protects the roots from high solar radiation levels and excessive evapotranspiration losses, helps prevent overheating, and provides mechanical support to internal tissues, among other functions.

Simple, unicellular trichomes, such as those observed here (Figure 4c), were reported for *Brachystele widgrenii*, *Lankesterella caespitosa* (Lindl.) Hoehne, *L. ceracifolia* (Barb. Rodr.) Mansf., *Pelexia orthosepala* (Rchb.f. & Warm.) Schltr, and *Sacoila lanceolata* (Aubl.) Garay. by Bernal et al. [18], for *Cranichis candida* (Barb.Rodr.) Cogn. by Andreota et al. [17], and for 11 species of *Aspidogyne* Garay and *Microchilus* C. Presl. by Bona et al. [20]. According to Stern et al. [6], Andreota et al. [17] and Bernal et al. [18], simple trichomes provide better attachment to the substrate and increase contact with that surface – thus facilitating the absorption of both water and mineral salts.

The root cortex is composed of 18–20 layers of rounded parenchyma cells of varying sizes with innumerable small, triangular intercellular spaces (Figure 4d); hyphae and pelotons are also observed, mainly concentrated in more peripheral portions and underlying the exodermis (Figure 4d, g, h). This pattern has been reported in the roots of other Orchidaceae taxa by Pereira et al. [31], who noted that the presence of such symbiotic microorganisms is of great importance to the germination of orchid seeds as they increase the surface area of the roots and thus facilitate water and nutrient absorption [31, 39].

These characters are frequently observed in Orchidoideae [e.g., 11, 15, 16], with terrestrial members of the family (as was observed in the species studied here) generally having thick, fleshy roots with a more expressive cortex (in terms the numbers of layers) that is responsible for holding reserves of water, starch grains, and other nutrients, with a less expressive velamen. Some of the cells in the cortical parenchyma contain raphides (Figure 4e), as observed in different groups of Monocotyledons [e.g., 34], with polyhedral starch grains gathered in spiranthosomes (Figure 4f) – spherical specialized amyloplasts found in Cranichideae and interpreted as a synapomorphy of the tribe [See 5, 17, 40, 41].

The exodermis (Figure 4b, c), endodermis and pericycle (Figure 4k) of *B. guayanensis* are uniseriate, and are composed of rounded, elliptic cells with thin walls; the cells of the exodermis are slightly thickened (Figure 4b, c), and endodermis cells have evident Casparian strips (Figure 4k). Those patterns are repeated in other terrestrial orchids, especially in the subfamily Orchidoideae [See 13, 15, 17, 19, 20]. Benzing et al. [42] and Sanford and Adanlawo [43] noted that the thickening of the exodermal cells, as well as presence of a Casparian strip, aid in minimizing water losses to the external environment and provide mechanical support to root tissues.

The vascular cylinders in the roots of *B. guayanensis* are of variable calibers, have circular or elliptic shapes, and 12–14 protoxylem poles (Figure 4i, j), categorizing those roots as polyarchs, which are common in Monocotyledons [25, 35]. The xylem and phloem are interspersed (Figure 4i-k); the central portion of the vascular cylinder is composed of parenchyma cells of varying sizes and shapes with tiny triangular intercellular spaces (Figure 4i, j), similar to those observed in other groups of Orchidaceae [e.g., 11, 13, 15–17, 19, 20].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 June 2023 doi:10.20944/preprints202306.0349.v1

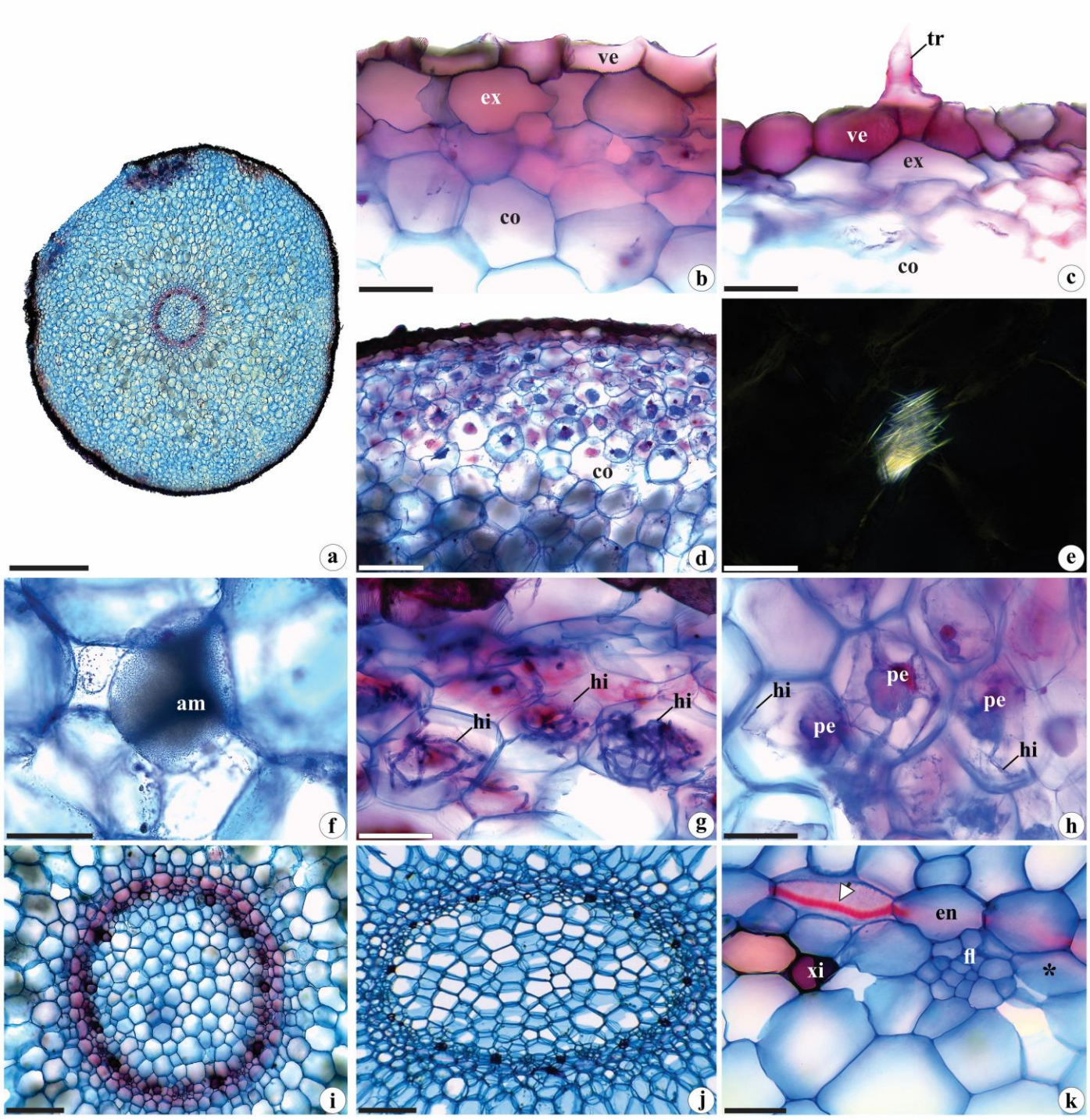


Figure 4. Root anatomy of *Brachystele guayanensis* (Lindl.) Schltr.; (**a-k**) Cross sections; (**a**) General aspect; (**b, c**) Detail of the velamen, exodermis and trichomes; (**d**) Detail of the cortex. Note the presence of hyphae and pelotons in more peripheral portions; (**e**) Raphides under polarized light; (**f**) Starch grains gathered in spiranthosomes; (**g, h**) Detail of hyphae and pelotons in the cortical parenchyma; (**i, j**) Vascular cylinder; (**k**) Detail of the endodermis, pericycle, xylem and phloem. am = Starch grains gathered in spiranthosomes; ct = Cortex; en = Endodermis; ex = Exodermis; fl = Phloem; hi = Hyphae; pe = Pelotons; tr = Trichomes; ve = Velamen; xi = Xylem; * = Pericycle; White arrows = Casparian strips. Scales: a = 1000 μm; b, c, e, g, h, k = 50 μm; f = 20 μm; d, i, j = 200 μm. Photomicrographs by Igor Soares dos Santos.

2.2. Histochemistry data

 doi:10.20944/preprints202306.0349.v1

Coomassie blue and Xylidine Ponceau strongly stained the pelotons and hyphae, indicating their protein contents (Figure 5a, b), and Sudan IV staining confirm the lipidic nature of the waxy cuticle (Figure 5c); the raphides are composed of calcium oxalate. Lignin was identified in the walls of xylem vessel elements (Figure 5d, f, g) and pericyclic fibers (Figure 5e), as well as in Casparian strips (Figure 5g). Starch grains and alkaloids were identified within the stomata guard cells (Figure 5h, o), epidermal cells, mesophyll cells (especially in the vicinity of vascular bundles) (Figure 5i, j, p), the cortical parenchyma of rhizomes (and occasionally in the central portion) (Figure 5k, l, q, r), as well as in roots (where they were most abundant) (Figure 5m, n, s-u). The histochemical tests were negative for reducing sugars, phenolic compounds, and tannins.

The waxy cuticle performs several functions, including protection against solar radiation, overheating, and water losses from internal tissues; it confers protection against the entry and the attacks of pathogens and herbivores [44–46].

Calcium oxalate raphides that are found in different groups of Orchidaceae, as well as other Angiosperms [e.g., 16, 47], serve as defenses against herbivores, act in osmoregulation and in other metabolic activities requiring calcium, and have a role in the detoxification of aluminum (an element very abundant savannas soils) [47, 48].

The lignin commonly deposited in vessel elements, pericyclic fibers, and the Casparian strip of the vegetative organs of the studied species confer stability, rigidity and mechanical support to the cell walls and internal tissues (25, 35). The Casparian strip, together with suberin deposits (both hydrophobic substances), contribute to solute selectivity in the root endodermis and act as barriers against apoplast movement – therefore preventing the influx of ions from the vascular cylinder to the cortical region [49–51].

Starch grains, especially those found in the roots and rhizomes, constitute some of the principal plant reserves are used (among other purposes) for vegetative growth and propagation [52–55]. Starch grains inside the stomata guard cells of the species studied here have also been reported in *Oxalis* L. (Oxalidaceae), *Sambucus australis* Cham. & Schltdl. (Adoxaceae), and *Socratea exorrhiza* (Mart.) H. Wendl. (Arecaceae) by Nunes et al. [56], Kikuchi et al. [57] and Reis and Alvim [58]. According to Appezzato-da-Glória and Carmello-Guerreiro [35], potassium levels in those structures appear to be associated with starch hydrolysis, which provides those organic anions. There is also evidence that malate, the regulator responsible for guard cell movements, can be synthesized through starch degradation [59, 60].

According to Bulpitt et al. [61] and Sut et al. [62], many of the secondary metabolic substances identified in representatives of the Orchidaceae (such as flavonoids, alkaloids, terpenes, glycosides) act in plant defenses against herbivores and pathogens, and show bioactive pharmacological effects [63, 64]. We believe that the presence of alkaloids in the stomata guard cells and in the cortical parenchyma of the rhizomes and roots together with the raphides identified in *B. guayanensis,* act to defend the species against attacks by pathogens and herbivores, as mentioned by Franceschi and Nakata [47] and Vizzotto et al. [65] for plants in general. Similar to what has been postulated by Li et al. [66] for the vegetative organs of other orchids, the abundant presence of raphides and secondary metabolites (e.g., alkaloids) in *B. guayanensis,* especially in the reserve organs of that species (e.g., the roots and rhizomes), are responsible for the renewal of their aerial portions, and help prevent pathogens and herbivores from reaching the vascular system (which is usually found in more internal regions) and causing local and/or systemic damage.

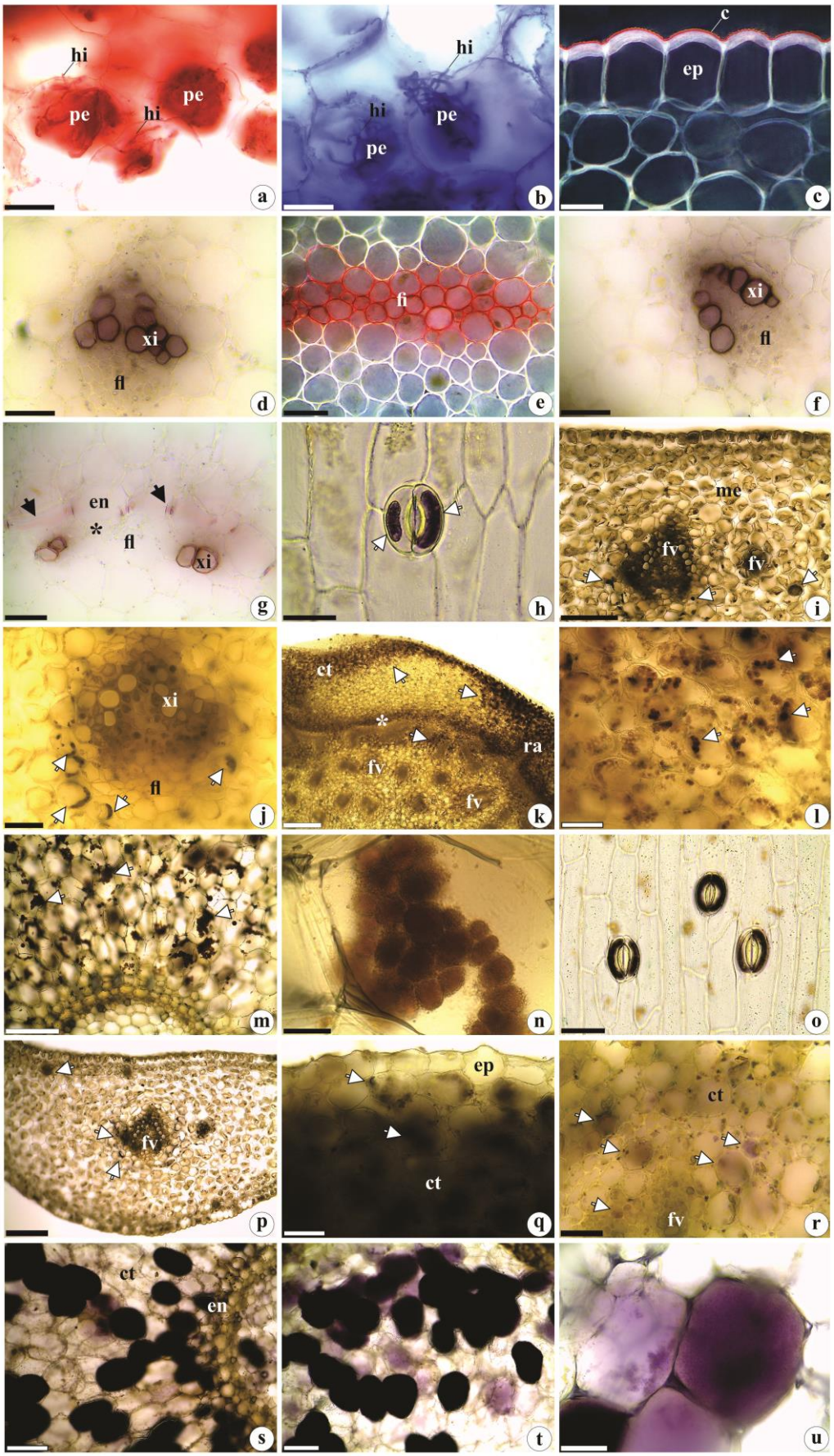
Among the bioactive substances found in Orchidaceae species are alkaloids (e.g., dendrobin, nobilonin, dendroxin) and nitrogenous heterocyclic organic molecules derived from the secondary metabolism of amino acids (e.g., phenylalanine, lysine, arginine, tyrosine, tryptophan). More than 100 types of bioactive alkaloids have been identified in more than 2,000 orchid species [67, 68]. Those alkaloids have been found to be effective for treating gastrointestinal disorders and cardiovascular diseases, and also evidence anti-inflammatory, diuretic, analgesic, antioxidant, immunomodulatory, antipyretic, and antitumor activities [69–71]. Within this context, the alkaloids found in *B. guayanensis* indicate

 doi:10.20944/preprints202306.0349.v1

it as a potential medicinal species. (Bio)phytochemical investigations could therefore be informative, with the subsequent isolation and testing of the toxicity of any bioactive substances, and the characterization of their chemical natures.

This study was designed to provide initial information concerning the anatomy and histochemistry of the vegetative organs of the genus *Brachystele* and its medicinal potential and indicates the importance of anatomical and histochemical studies focusing on neotropical Orchidaceae species, especially those that have been only poorly studied.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 June 2023 doi:10.20944/preprints202306.0349.v1



 doi:10.20944/preprints202306.0349.v1

Figure 5. Histochemical tests in the vegetative organs of *Brachystele guayanensis* (Lindl.) Schltr.; (**a-g, i-n, p-u**) Cross sections; (**h, o**) Epidermis in frontal view; (**a, b, g, m, n, s-u**) Root; (**c, d, h-j, o, p**) Leaf; (**e, f, k, l, q, r**) Rhizome; (**a, b**) Protein nature of fungal hyphae and pelotons; (**c**) Detail of the cuticle (total lipids) under polarized light; (**d-g**) Lignin (polarized light in **e**); (**h-n**) Starch grains. Note the spiranthosomes in **n**; (**o**) Total alkaloids. c = Cuticle; ct = Cortex; en = Endodermis; ep = Epidermis; fi = Pericyclic fibers; fl = Phloem; fv = Vascular bundle; hi = Hyphae; pe = Pelotons; xi = Xylem; * = Pericycle; Black arrows = Casparian strips; White arrows = starch grains and alkaloids. Scales: a, b, d-h, j, l, o, q-t = 50 μm; c, n, u = 20 μm; i, m, p = 200 μm; k = 500 μm. Photomicrographs by Igor Soares dos Santos.

3. Materials and Methods

For the anatomical studies, samples of vegetative organs (e.g., the mid-portions of the leaf blades, rhizomes, and roots) of five adult individuals of *B. guayanensis* (Figure 1) were collected in open fields, close to Bosque Auguste Saint-Hilaire, at *Campus* II (Samambaia) of the Federal University of Goiás (UFG), Goiânia, GO, Brazil. Collections of botanical material followed the recommendations of Mori et al. [72], and voucher specimens were deposited in the UFG Herbarium (registration numbers: *I.S. Santos 1160* and *1161*).

The samples collected for anatomical studies were fixed in 70% FAA (glacial acetic acid, formaldehyde, and 70% ethyl alcohol, 1:1:18) in hermetically sealed containers for 48 hours; after that time, they were preserved in 70% ethyl alcohol [73]. For the anatomical descriptions, cross sections were cut using a razor blade and clarified in a 20% aqueous solution of sodium hypochlorite (NaClO) (v/v) [74], stained with Astra Blue and Safranin (9:1) [75], and mounted in aqueous Glycerol solution (1:1). For analysis of the leaf surface in frontal view, the epidermis was dissociated using the Jeffrey method [73]. For the procedures mentioned above, the slides were sealed with a colorless sealant and subsequently photomicrographed using a Leica ICC50 HD® digital camera coupled to a Leica DM500® microscope, using Motic 2.0 Image Plus Software.

For histochemical studies, *in natura* samples obtained the time of collection were stained with the following reagents: Coomassie Blue and Xylidine Ponceau [76, 77] for detecting proteins; Ferric chloride for detecting phenolic compounds; acidified Phloroglucinol for lignin; Sudan IV [73] for total lipids; Dittmar [78] for alkaloids: Fehling [74] for reducing sugars, Lugol [79] for starch; and hydrochloric vanillin [80] to detect tannins. To verify the chemical constitution of the crystals, 10% hydrochloric acid was used, following Chamberlain [81]. The descriptions of the analyzed organs were based on the terminologies used in the specialized literature [15, 17, 18, 20].

Author Contributions: I.S.S. collected, processed, and identified the botanical material; M.J.S. supervised all laboratory work, and the analysis of data obtained by I.S.S.; M.J.S. guided the elaboration of the manuscript and provided a critical analysis of the data obtained; I.S.S and M.J.S. participated in the interpretation of the data, writing, conceptualization, theoretical basis, review and editing of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors would like to thank the Federal University of Goiás (UFG) and the São Paulo State University (Unesp) for the use of their facilities and logistical support, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the PhD and Productivity scholarships in Research provided to authors.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rutkowski, P.; Mytnik, J.; Szlachetko, D.L. New taxa and new combinations in Mesoamerican Spiranthinae (Orchidaceae, Spirantheae). *Ann. Bot. Fenn.* **2004**, *41*, 471–477. https://doi.org/10.2307/23727263.

2. Barros, F.; Vinhos, F.; Rodrigues, V.T.; Barberena, F.F.V.A.; Fraga, C.N.; Pessoa, E.M.; Forster, W.; Menini-Neto, L.; Furtado, S.G.; Nardy, C.; Azevedo, C.O.; Guimarães, L.R.S. Orchidaceae. In *Lista de Espécies da Flora do Brasil*. Jardim Botânico do Rio de Janeiro. 2015, Available online: http://servicos.jbrj.gov.br/flora/search/Brachystele_guayanensis (accessed on 18-X-2021).
3. Rasmussen, H. The diversity of stomatal development in Orchidaceae subfamily Orchidoideae. *Bot. J. Linn. Soc.* **1981**, *82*, 381–393. https://doi.org/10.1111/j.1095-8339.1981.tb00969.x.
4. Porembski, S.; Barthlott, W. Velamen radicum micromorphology and classification of Orchidaceae. *Nord. J. Bot.* **1988**, *8*, 117–137. https://doi.org/10.1111/j.1756-1051.1988.tb00491.x.
5. Stern, W.L.; Aldrich, H.C.; McDowell, L.M.; Morris, M.W.; Pridgeon, A.M. Amyloplasts from cortical root cells of Spiranthoideae (Orchidaceae). *Protoplasma* **1993a**, *172*, 49–55. https://doi.org/10.1007/BF01403721.
6. Stern, W.L.; Morris, M.W.; Judd, W.S.; Pridgeon, A.M.; Dressler, R.L. Comparative vegetative anatomy and systematics of Spiranthoideae (Orchidaceae). *Bot. J. Linn. Soc.* **1993b**, *113*, 161–197. https://doi.org/10.1111/j.1095-8339.1993.tb00336.x.
7. Pridgeon, A.M. Systematic leaf anatomy of Caladeniinae (Orchidaceae). *Bot. J. Linn. Soc.* **1994**, *114*, 31–48. https://doi.org/10.1111/j.1095-8339.1994.tb01922.x.
8. Kurzweil, H.; Linder, H.P.; Stern, W.L.; Pridgeon, A.M. Comparative vegetative anatomy and classification of Diseae (Orchidaceae). *Bot. J. Linn. Soc.* **1995**, *117*, 171–220. https://doi.org/10.1111/j.1095-8339.1995.tb00452.x.
9. Stern, W.L. Vegetative anatomy of subtribe Orchidinae (Orchidaceae). *Bot. J. Linn. Soc.* **1997a**, *124*, 121–136. https://doi.org/10.1111/j.1095-8339.1997.tb01786.x.
10. Stern, W.L. Vegetative anatomy of subtribe Habenariinae (Orchidaceae). *Bot. J. Linn. Soc.* **1997b**, *125*: 211–227. https://doi.org/10.1111/j.1095-8339.1997.tb02255.x.
11. Figueroa, C.; Salazar, G.A.; Zavaleta, H.A.; Engleman, E.M. Root character evolution and systematics in Cranichidinae, Prescottiinae and Spiranthinae (Orchidaceae, Cranichideae). *Ann. Bot.* **2008**, *101*, 509–520. https://doi.org/10.1093/aob/mcm328.
12. Moreira, A.S.F.P.; Isaias, R.M.S. Comparative anatomy of the absorption roots of terrestrial and epiphytic orchids. Braz. *Arch. Biol. Technol.* **2008**, *51*, 83–93. https://doi.org/10.1590/S1516-89132008000100011.
13. Aybeke, M.; Sezik, E.; Olgun, G. Vegetative anatomy of some *Ophrys*, *Orchis* and *Dactylorhiza* (Orchidaceae) taxa in Trakya region of Turkey. *Flora* **2010**, *205*, 73–89. https://doi.org/10.1016/j.flora.2008.11.009.
14. Aybeke, M. Comparative anatomy of selected rhizomatous and tuberous taxa of subfamilies Orchidoideae and Epidendroideae (Orchidaceae) as an aid to identification. *Plant Syst. Evol.* **2012**, *298*, 1643–1658. https://doi.org/10.1007/s00606-012-0666-9.
15. Corredor, B.A.D.; Arias, R.L. Morfoanatomía en Cranichideae (Orchidaceae) de la Estación Loma Redonda del Parque Nacional "Sierra Nevada", Mérida, Venezuela. *Lankesteriana* **2012**, *12*, 61–75. https://doi.org/10.15517/lank.v12i1.18267.
16. Stern, W.L. *Anatomy of the Monocotyledons: X. Orchidaceae*. 1st ed.; Oxford University Press: Oxford, England, 2014; pp. 1–288.
17. Andreota, R.C.; Barros, F.; Sajo, M.G. Root and leaf anatomy of some terrestrial representatives of the Cranichideae tribe (Orchidaceae). *Rev. Bras. Bot.* **2015**, *38*, 367–378. https://doi.org/10.1007/s40415-015-0133-2.
18. Bernal, A.A.; Smidt, E.C.; Bona, C. Spiral root hairs in Spiranthinae (Cranichideae: Orchidaceae). *Rev. Bras. Bot.* **2015**, *38*, 411–415. https://doi.org/10.1007/s40415-015-0141-2.
19. Şenel, G.; Akbulut, M.K.; Şeker, Ş.S. Comparative anatomical properties of some Epidendroideae and Orchidoideae species distributed in NE Turkey. *Protoplasma* **2019**, *256*, 655–668. https://doi.org/10.1007/s00709-018-1326-x.
20. Bona, C.; Engels, M.E.; Pieczak, F.S.; Smidt, E.C. Comparative vegetative anatomy of Neotropical Goodyerinae Klotzsch (Orchidaceae Juss.: Orchidoideae Lindl.). *Acta Bot. Bras.* **2020**, *34*, 530–539. https://doi.org/10.1590/0102-33062020abb0032.
21. Neumann, C. Orchideen als Arzneipflanzen: Ein Querschnitt durch ausgewählte medizinische und botanisch-pharmazeutische Literatur des 19. *Jahrhunderts. Z. fur Phytother*. **2009**, *30*, 1–29. https://doi.org/10.1055/s-0029-1239914.
22. Verettoni, H.N. *Contribución al conocimiesão bioativas de las plantas medicinales de la región de Bahía Blanca*. 1st ed.; Harris y Cia: Bahía Blanca, Argentina, 1985; pp. 1–374.
23. Oliveira, V.C.; Sajo, M.G. Leaf anatomy of epiphyte species of Orchidaceae. *Rev. Bras. Bot.* **1999**, *22*, 365–374. https://doi.org/10.1590/S0100-84041999000300003.
24. Silva, C.I.; Milaneze-Gutierre, M.A. Caracterização morfoanatômica dos órgãos vegetativos de *Cattleya walkeriana* Gardner (Orchidaceae). *Acta Sci.* **2004**, *26*, 91–100. https://doi.org/10.4025/actascibiolsci.v26i1.1664.
25. Fahn, A. *Plant anatomy*. 4th ed.; Pergamon Press: Oxford, England, 1990; pp. 1–530.
26. Smith, W.K.; Vogelmann, T.C.; DeLucia, E.H.; Bell, D.T.; Shepherd, K.A. Leaf form and photosynthesis. *Bioscience* **1997**, *47*, 785–793. https://doi.org/10.2307/1313100.
27. Stern, W.L.; Judd, W.S. Comparative vegetative anatomy and systematics of *Vanilla* (Orchidaceae). *Bot. J. Linn. Soc.* **1999**, *131*, 353–382. https://doi.org/10.1111/j.1095-8339.1999.tb01520.x.
28. Stern, W.L.; Judd, W.S. Comparative anatomy and systematics of the orchid tribe Vanilleae excluding *Vanilla*. *Bot. J. Linn. Soc.* **2000**, *134*, 179–202. https://doi.org/10.1006/bojl.2000.0369.
29. Carlsward, B.S.; Stern, W.L. Vegetative anatomy and systematics of Triphorinae (Orchidaceae). *Bot. J. Linn. Soc.* **2009**, *159*, 203–210. https://doi.org/10.1111/j.1095-8339.2008.00930.x.

30. Menezes, N.L.; Elbl, P.M.; Cury, G.; Appezzato-da-Glória, B.; Sasaki, K.L.; Silva, C.G.; Costa, G.R.; Lima, V.G. The meristematic activity of the endodermis and the pericycle and its role in the primary thickening of stems in monocotyledonous plants. *Plant Ecol. Divers.* **2012**, *5*, 153–165. https://doi.org/10.1080/17550874.2011.604925.
31. Pereira, O.L.; Kasuya, M.C.M.; Rollemberg, C.L.; Chaer, G.M. Isolamento e identificação de fungos micorrízicos rizoctonióides associados a três espécies de orquídeas epífitas neotropicais no Brasil. *Rev. Bras. Cienc. Solo* **2005**, *29*, 191–197. https://doi.org/10.1590/S0100-06832005000200004.
32. Bougoure, J.; Ludwig, M.; Brundrett, M.; Grierson, P. Identity and specificity of the fungi forming mycorrhizas with the rare mycoheterotrophic orchid *Rhizanthella gardneri*. *Mycol. Res.* **2009**, *113*, 1097–1106. https://doi.org/10.1016/j.mycres.2009.07.007.
33. Suetsugu, K.; Haraguchi, T.F.; Tanabe, A.S.; Tayasu, I. Specialized mycorrhizal association between a partially mycoheterotrophic orchid *Oreorchis indica* and a *Tomentella* taxon. *Mycorrhiza* **2021**, *31*, 243–250. https://doi.org/10.1007/s00572-020-00999-z.
34. Metcalfe, C.R. Comparative anatomy as a modern Botanical discipline. In *Advances in botanical research*, 1st ed.; Metcalfe, C.R., Ed.; Academic Press: New York, United States, 1963; Vol. VI, pp. 101–147.
35. Appezzato-da-Glória, B.; Carmello-Guerreiro, S.M. *Anatomia Vegetal*. 2rd ed.; Editora UFV: Viçosa, Bahia, Brazil, 2006; pp. 1–438.
36. Andreota, R.C. Anatomia dos órgãos vegetativos de representantes da tribo Cranichideae (Orchidoideae: Orchidaceae). Masters' thesis, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil, 2013.
37. Pridgeon, A.M. The velamen and exodermis of orchid roots. In *Orchid biology, reviews and perspectives*, 1st ed.; Arditti, J., Ed.; Cornell University Press: Ithaca, New York, United States, 1987; Vol. IV, pp. 139–192.
38. Chomicki, G.; Bidel, L.P.R.; Ming, F.; Coiro, M.; Zhang, X.; Wang, Y.; Baissac, Y.; Jay-Allemand, C.; Renner, S.S. The velamen protects photosynthetic orchid roots against UV-B damage, and a large dated phylogeny implies multiple gains and losses of this function during the Cenozoic. *New Phytol*. **2015**, *205*, 1330–1341. https://doi.org/10.1111/nph.13106.
39. Cameron, D.D.; Johnson, I.; Leake, J.R.; Read, D.J. Mycorrhizal acquisition of inorganic phosphorus by the green-leaved terrestrial orchid *Goodyera repens*. *Ann. Bot.* **2007**, *99*, 831–834. https://doi.org/10.1093/aob/mcm018.
40. Salazar, G.A.; Chase, M.W.; Arenas, M.S.; Ingrouille, M. Phylogenetics of Cranichideae with emphasis on Spiranthinae (Orchidaceae, Orchidoideae): evidence from plastid and nuclear DNA sequences. *Am. J. Bot.* **2003**, *90*, 777–795. https://doi.org/10.3732/ajb.90.5.777.
41. Şeker, Ş.S. What does the quantitative morphological diversity of starch grains in terrestrial orchids indicate? *Microsc. Res. Tech.* **2022**, *85*, 2931–2942. https://doi.org/10.1002/jemt.24143.
42. Benzing, D.H.; Friedman, W.E.; Peterson, G.; Renfrow, A. Shootlessness, velamentous roots, and the pre-eminence of Orchidaceae in the epiphytic biotype. *Am. J. Bot.* **1983**, *70*, 121–33. https://doi.org/10.1002/j.1537-2197.1983.tb12440.x.
43. Sanford, W.W.; Adanlawo, I. Velamen and exodermis characters of west african epiphytic orchids in relation to taxonomic grouping and habitat tolerance. *Bot. J. Linn. Soc.* **1973**, *66*, 307–321. https://doi.org/10.1111/j.1095-8339.1973.tb02178.x.
44. Juniper, B.E.; Jeffree, C.E. *Plant surfaces*. 1st ed.; Edward Arnold Publishers: London, England, 1983; pp. 1–93.
45. Dickison, W.C. *Integrative plant anatomy*. 1st ed.; Academic Press: San Diego, United States, 2000; pp. 1–533.
46. Evert, R.F. *Esau's plant anatomy: meristems, cells and tissues of the plant body: their structure, function and development*. 3rd ed.; John Wiley & Sons, Inc.: New Jersey, United States, 2006; pp. 1–601.
47. Franceschi, V.R.; Nakata, P.A. Calcium oxalate in plants: formation and function. *Annu. Rev. Plant Biol.* **2005**, *56*, 41-71. https://doi.org/10.1146/annurev.arplant.56.032604.144106.
48. Haridasan, M. Alumínio é um elemento tóxico para as plantas nativas do cerrado? In *Fisiologia Vegetal: práticas em relações hídricas, fotossíntese e nutrição mineral*, 1st ed.; Prado, C.H.B.A.; Casali, C.A., Eds.; Editora Manole: Barueri, São Paulo, Brazil, 2006; Vol. 1, pp. 1–10.
49. Peterson, C.A.; Murrmann, M.; Steudle, E. Location of the major barriers to water and ion movement in young roots of *Zea mays* L. *Planta* **1993**, *190*, 127–136. https://doi.org/10.1007/BF00195684.
50. Enstone, D.E.; Peterson, C.A.; Ma, F. Root endodermis and exodermis: structure, function, and responses to the environment. *J. Plant Growth Regul*. **2003**, *21*, 335–351. https://doi.org/10.1007/s00344-003-0002-2.
51. Lux, A.; Luxová, M. Growth and differentiation of root endodermis in *Primula acaulis* Jacq. *Biol. Plant.* **2003**, *47*, 91–97. https://doi.org/10.1023/A:1027389100479.
52. Holttum, R. Growth habitats of monocotyledons - variations on a theme. *Phytomorphology* **1955**, *5*, 399–413.
53. Beck, E.; Ziegler, P. Biosynthesis and degradation of starch in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1989**, *40*, 95–117. https://doi.org/10.1146/annurev.pp.40.060189.000523.
54. Oliveira, F.; Akisue, G. *Fundamentos de farmacobotânica*. 1st ed.; Atheneu: São Paulo, Brazil, 1989; pp. 1–216.
55. Salisbury, F.B.; Ross, C.W. *Plant physiology*. 4th ed.; Wadsworth: Belmont, United States, 1992; pp. 1–267.
56. Nunes, E.; Scopel, M.; Vignoli-Silva, M.; Vendruscolo, G.S.; Henriques, A.T.; Mentz, L.A. Caracterização farmacobotânica das espécies de *Sambucus* (Caprifoliaceae) utilizadas como medicinais no Brasil. Parte II. *Sambucus australis* Cham. & Schltdl. *Braz. J. Pharmacog*. **2007**, *17*, 414–425. https://doi.org/10.1590/S0102-695X2007000300017.
57. Kikuchi, T.Y.S.; Braga, Z.V.; Potiguara, R.C.V. Anatomia foliar de *Socratea exorrhiza* (Mart.) H. Wendl. (Arecaceae). *Biota Amazôn*. **2016**, *6*, 73–79. http://dx.doi.org/10.18561/2179-5746/biotaamazonia.v6n2p73-79.
58. Reis, R.E.; Alvim, M.N. Anatomia foliar comparada de três espécies do gênero *Oxalis* L. (Oxalidaceae). *NBC* **2013**, *3*, 59–72.

59. Vavasseur, A.; Raghavendra, A.S. Guard cell metabolism and CO_2 sensing. *New Phytol.* **2005**, *165*, 665–682. https://doi.org/10.1111/j.1469-8137.2004.01276.x.
60. Araújo, W.L.; Nunes-Nesi, A.; Osorio, S.; Usadel, B.; Fuentes, D.; Nagy, R.; Balbo, I.; Lehmann, M.; Studart-Witkowski, C.; Tohge, T.; Martinoia, E.; Jordana, X.; DaMatta, F.M.; Fernie, A.R. Antisense inhibition of the iron-sulphur subunit of succinate dehydrogenase enhances photosynthesis and growth in tomato via an organic acid mediated effect on stomatal aperture. *Plant Cell*. **2011**, *23*, 600–627. https://doi.org/10.1105/tpc.110.081224.
61. Bulpitt, C.J.; Li, Y.; Bulpitt, P.F.; Wang, J. The use of orchids in Chinese medicine. *J. R. Soc. Med.* **2007**, *100*, 558–563. https://doi.org/10.1177/0141076807100012014.
62. Sut, S.; Maggi, F.; Dall'Acqua, S. Bioactive secondary metabolites from orchids (Orchidaceae). *Chem. Biodivers*. **2017**, *14*, e1700172. https://doi.org/10.1002/cbdv.201700172.
63. Hartmann, T. Chemical ecology of pyrrolizidine alkaloids. *Planta* **1999**, *207*, 483–495. https://doi.org/10.1007/s004250050508.
64. Sepúlveda-Jiménez, G.; Porta-Ducoing, H.; Rocha-Sosa, M. La participación de los metabolitos secundarios en la defensa de las plantas. *Rev. Mex. Fitopatol.* **2003**, *21*, 355–363.
65. Vizzotto, M.; Krolow, A.C.R.; Weber, G.E.B. *Metabólitos secundários encontrados em plantas e sua importância*. 1st ed.; Embrapa Clima Temperado: Pelotas, Rio Grande do Sul, Brazil, 2010; pp. 1–17.
66. Li, J.W.; Zhang, Z.B.; Zhang, S.B. Widely targeted metabolic, physical and anatomical analyses reveal diverse defensive strategies for pseudobulbs and succulent roots of orchids with industrial value. *Ind. Crop. Prod.* **2022**, *177*, 114510. https://doi.org/10.1016/j.indcrop.2021.114510.
67. Gutierrez, R.M.P. Orchids: A review of uses in traditional medicine, its phytochemistry and pharmacology. *J. Med. Plant Res.* **2010**, *4*, 592–638. https://doi.org/10.5897/JMPR10.012.
68. Hossain, M.M. Therapeutic orchids: Traditional uses and recent advances—An overview. *Fitoterapia* **2011**, *82*, 102–140. https://doi.org/10.1016/j.fitote.2010.09.007.
69. Brito, H.O.; Noronha, E.P.; França, L.M. Phytochemical analysis composition from *Annona squamosa* (ATA) ethanolic extract leaves. *Rev. Bras. Farm*. **2008**, *89*, 180–184.
70. Ng, T.B.; Liu, J.; Wong, J.H.; Ye, X.; Sze, S.C.W.; Tong, Y.; Zhang, K.Y. Review of research on *Dendrobium*, a prized folk medicine. *Appl. Microbiol. Biotechnol.* **2012**, *93*, 1795–1803. https://doi.org/10.1007/s00253-011-3829-7.
71. Li, R.; Liu, T.; Liu, M.; Chen, F.; Liu, S.; Yang, J. Anti-influenza a virus activity of dendrobine and its mechanism of action. *J. Agric. Food Chem.* **2017**, *65*, 3665–3674. https://doi.org/10.1021/acs.jafc.7b00276.
72. Mori, S.A.; Silva, L.A.; Lisboa, G.; Coradin, L. *Manual de Manejo do Herbário Fanerogâmico*. 2nd. ed.; CEPLAC: Ilhéus, Bahia, Brazil, 1989; pp. 1–104.
73. Johansen, D.A. *Plant microtechnique*. 1st ed.; McGraw-Hill Book Company, Inc.: New York, United States, 1940; pp. 1–523.
74. Kraus, J.E.; Arduin, M. *Manual básico de métodos em morfologia vegetal*. 1st ed.; EDUR: Rio de Janeiro, Brazil, 1997; pp. 1–198.
75. Bukatsch, F. Bermerkungen zur Doppelfrbung Astrablau-Safranin. *Mikrokosmos* **1972**, *61*: 255.
76. Fisher, D.B. Protein staining of ribboned epon sections for light microscopy. *Histochemie* **1968**, *16*, 92–96. https://doi.org/10.1007/BF00306214.
77. O'Brien, T.P.; McCully, M.E. *The study of plant structure principles and selected methods*. 1st ed.; Termarcarphi: Melbourne, Australia, 1981; pp. 1–344.
78. Furr, M.; Mahlberg, P.G. Histochemical analyses of laticifers and glandular trichomes in *Cannabis sativa*. *J. Nat. Prod.* **1981**, *44*, 153–159. https://doi.org/10.1021/np50014a002.
79. Jensen, W.A. *Botanical histochemistry: principles and practice*. 1st ed.; W.H. Freeman: San Francisco, United States, 1962; pp. 1–408.
80. Mace, M.E.; Howell, C.R. Histological and identification of condensed tannin precursor in roots of cotton seedlings. *Canad. J. Bot.* **1974**, *52*, 2423–2426.
81. Chamberlain, C.J. *Methods in plant histology*. 5th ed.; University of Chicago Press: Chicago, United States, 1932; pp. 1–416.