



Plant anatomy: history and future directions

Anatomical and histochemical characterization of vegetative organs of Brazilian species of *Renealmia* (Zingiberaceae)

Kathlyn Vasconcelos Gevu^{1,3}, Helena Regina Pinto Lima², Camilla Ribeiro Alexandrino^{1,4}
& Maura da Cunha^{1,5,6}

Abstract

Renealmia comprises approximately 85 herbaceous and rhizomatous plants in Africa and America known for their metabolite diversity. This study characterized the anatomy and histochemistry of the vegetative organs of three native Brazilian *Renealmia* species, *R. breviscapa* and *R. nicolaioides*, collected from the Amazon Forest in Acre and *R. chrysotricha* from the Atlantic Forest in Rio de Janeiro. Samples were fixed and processed using conventional plant anatomy and micromorphology techniques, including histochemical tests to identify classes of compounds in vegetative organs. Characteristics including oil idioblasts, dorsiventral leaf structure, tetracytic stomata, unicellular trichomes, and prismatic crystals were common among the studied species of *Renealmia*, and thus significant for the family. Distinctive characteristics were identified in the vegetative organs of the three species, including the cellular distribution of root phloem, covering of rhizome tissue, different types and distributions of idioblasts and phenolic trichomes in rhizomes and leaves, presence or absence of petiole, and subepidermal layer in the intercostal region of leaves. Mineral content importance as a diagnostic characteristic of the genus is also emphasized. The results of this study advance the understanding of the distinctive anatomical characteristics of the three species of *Renealmia* in different environments, two in the Atlantic Forest and one in the Amazon Forest.

Key words: micromorphology, special metabolites, tribe Alpinieae, ultrastructure, Zingiberaceae.

Resumo

Renealmia abrange aproximadamente 75 espécies de plantas herbáceas e rizomatosas, oriundas de regiões africanas e neotropicais, conhecidas por sua diversidade de metabólitos. Este estudo visou caracterizar a anatomia e histoquímica dos órgãos vegetativos de três espécies nativas do Brasil: *Renealmia breviscapa* e *Renealmia nicolaioides*, coletadas na Floresta Amazônica, no Acre, e *Renealmia chrysotricha*, encontrada em uma floresta Atlântica, no Rio de Janeiro. Amostras foram fixadas e processadas por técnicas convencionais de anatomia vegetal, incluindo testes histoquímicos para identificação das classes de substâncias presentes nos órgãos vegetativos. Características como idioblastos oleíferos, estrutura foliar dorsiventral, estômatos tetracíticos, tricomas unicelulares e presença de cristais prismáticos foram comuns entre as espécies de *Renealmia* estudadas, revelando-se significativas para a família. Foram identificados caracteres distintivos nos órgãos vegetativos das três espécies, como distribuição celular do floema radicular, cobertura do tecido dos rizomas, diferentes tipos e distribuição de idioblastos e tricomas fenólicos tanto nos rizomas quanto nas folhas, presença ou ausência de pecíolo, além da camada subepidérmica na região intercostal das folhas. Destaca-se também a importância do conteúdo mineral como característica diagnóstica do gênero *Renealmia*. Este estudo proporciona um avanço no conhecimento das características anatômicas distintivas das três espécies de *Renealmia* em diferentes ambientes, tanto na Floresta Atlântica quanto na Floresta Amazônica.

Palavras-chave: micromorfologia, metabólitos especiais, tribo Alpinieae, ultraestrutura, Zingiberaceae.

¹ Universidade Estadual do Norte Fluminense Darcy Ribeiro - UENF, Centro de Biociências e Biotecnologia, Lab. Biologia Celular e Tecidual, Campos dos Goytacazes, RJ, Brazil.

² Universidade Federal Rural do Rio de Janeiro - UFRRJ, Inst. Ciências Biológicas e da Saúde, Depto. Botânica, Seropédica, RJ, Brazil. ORCID: <<https://orcid.org/0000-0002-7982-4146>>.

³ ORCID: <<https://orcid.org/0000-0002-3372-2144>>. ⁴ ORCID: <<https://orcid.org/0000-0003-1717-0447>>. ⁵ ORCID: <<https://orcid.org/0000-0003-1078-3742>>.

⁶ Author for correspondence: maurauenf@gmail.com

Introduction

The family Zingiberaceae encompasses 50 genera and over 1,000 species of herbaceous and rhizomatous plants with a global presence (Judd *et al.* 2009; Souza & Lorenzi 2012). Among these, the genus *Renealmia* L. fil. is classified within the tribe Alpinieae, subfamily Alpinioideae, along with *Aframomum* K. Schum., *Alpinia* L., *Amomum* L., and *Elettariopsis* Baker (Kress *et al.* 2002). Spanning across the Afrotropics and Neotropics, *Renealmia* is recognized for its ornamental, culinary, and medicinal uses (Negrelle 2015).

Renealmia chrysotricha Petersen, *R. breviscapa* Poepp. & Endland, and *R. nicolaioides* Loes. are native Brazilian species of the genus. *Renealmia chrysotricha*, commonly known as “caeté-fogo”, is found in the Atlantic Forest of Southeast and South Brazil, particularly in the states of Espírito Santo and Rio de Janeiro (Maas & Maas 2015), at elevations ranging from 600 to 1,000 meters. These plants thrive in hillside forests near river margins in well-preserved areas but can also be found in disturbed forest systems. *Renealmia breviscapa* is native to the rainforests of Colombia, Bolivia and Brazil, where it occurs at elevations from 800 to 1,660 meters (Maas 1977). In Brazil, it occurs in the states of Acre, Amazonas, Pará, Rondônia and Mato Grosso (Maas & Maas 2015). *Renealmia nicolaioides*, also known as “Matandrea-roja” and “Mishquipanga” (Vásquez *et al.* 2013; Rengifo-Salgado *et al.* 2017), is found in western South America, often growing in forests bordering streams or in open areas at elevations from 500 to 1,600 meters (Maas 1977). In Brazil, it occurs in the state of Acre (Maas & Maas 2015).

Anatomical characteristics have been regarded as diagnostic of Zingiberaceae after Tomlinson's (1956, 1962, 1969) studies of vegetative organs, as follows: unicellular trichomes, base often swollen or sunken; anticlinal walls of epidermal cells not sinuous in surface view; hypodermis commonly 1-layered below each surface, sometimes multiseriate or absent; leaf axis including a single abaxial arc of air-canals; chlorenchyma associated with tissue around main veins; stem divided into a distinct cortex and central cylinder by a smooth fibrous cylinder; root with a polyarchy structure; tannin and oil-cells frequent; silica-cells present; vessels either restricted to roots or more widely distributed. Subsequent investigations of leaf anatomy for the family focused on species of the genera *Alpinia* Roxb., *Amomum* Roxb., *Boesenbergia* Kuntze, *Kaempferia* L. and *Zingiber* Boehm., as well as *Hedychium coronarium*

J. Koenig, and *Curcuma zedoaria* (Christm.) Roscoe. These studies aimed to identify distinctive anatomical traits specific to the aforementioned species (Hussin *et al.* 2000, 2001; Albuquerque & Neves 2004; Talip *et al.* 2005; Boeger *et al.* 2007; Martins *et al.* 2010; Tang *et al.* 2010; Noberto-Irmão *et al.* 2013; Zhao *et al.* 2022).

Among anatomical examinations concerning underground structures of Zingiberaceae, Sherlija *et al.* (1998) offered a comparative analysis of rhizomes of four economically significant species of *Curcuma*. Similarly, Martins *et al.* (2010) reported on the anatomical features of the rhizomes of *H. coronarium*, while Gevú *et al.* (2013) contributed a comprehensive report on the morphoanatomy of subterranean organs across ten species of Zingiberaceae. With a broader scope, Uma & Muthukumar (2014) employed qualitative and quantitative parameters to thoroughly evaluate the root anatomy and morphology of 23 species of Zingiberaceae.

Still based on anatomical characteristics, Maas (1977) cited the indumenta as important for the classification of species of *Renealmia* and proposed a classification for trichomes firstly based on cell wall thickness. Accordingly, thick-walled trichomes, called prickles, are divided into the following four categories: simple, furcate, plurifurcate and stellate. Thin-walled trichomes were separated into the same categories.

Special metabolites represent chemical functional traits that are crucial for the survival and persistence of organisms (Gottlieb *et al.* 1996). Chemical investigations within Zingiberaceae have unveiled a remarkable diversity of these unique metabolites. This spectrum encompasses various chemical classes, including flavonoids, terpenoids, diarylheptanoids, coumarins, quinoids, and arylalkanooids, all of which have been identified in various species of the family. Particularly noteworthy among the phenolic compounds are flavonoids, which are distinguished within Zingiberaceae for their structural diversity and significance as chemical markers (Alarcón *et al.* 2008; Lima & Kaplan 2010; Martins *et al.* 2010; Gevú *et al.* 2013).

The accumulation of terpenoids in plant tissues would have occurred differently according to an evolutionary sequence from oil idioblasts, a cavity or canal, or glandular trichomes (Gottlieb & Salatino 1987). Monoterpenes and sesquiterpenes are commonly found in essential oils of species of Zingiberaceae and, together with phenylpropanoids, are the main constituents of these oils (Heinzmann *et*

al. 2017). The genus *Renealmia* has special relevance regarding biological activities. Gevú *et al.* (2019c) found that leaf oils from *R. chrysotricha* and *R. nicolaioides* inhibit the growth of *Candida buinensis* and *C. tropicalis* by about 50%. These authors also evaluated the activity of essential oils extracted from the rhizomes and leaves of *R. chrysotricha* and detected efficient antiparasitic activity against *Trypanosoma cruzi* epimastigotes.

Essential oils perform a multitude of ecological functions, including defense against herbivory and pathogens, pollinator attraction, moisture preservation, defense against oxidative stress, facilitation of communication between distinct plant organs, intraspecific signaling, and a range of allelopathic effects (Heinzmann *et al.* 2017). These micromolecules have demonstrated a myriad of biological activities, encompassing antibacterial, antifungal, anticancer, antiviral, anti-inflammatory, antiparasitic, anticonvulsant, analgesic, anxiolytic, antioxidant, antinociceptive, and antidepressant properties (Passos *et al.* 2009; Raut & Karuppaiyl 2014; Ud-Daula *et al.* 2016; Souza *et al.* 2023). *Renealmia* species have been used for medicinal purposes, being well succeeded in anticancer activity and snakebite treatment (Negrelle 2015).

Given Tomlinson's (1956; 1969) classification of tannin substances into two types based on occurrence, frequency, and coloration, the significance of proanthocyanidins, also called condensed tannins, has been widely acknowledged across plant species. Gevú *et al.* (2019a, b) identified different classes of phenolic compounds from the rhizomes of species of *Renealmia*, some of which presented antioxidant activities.

Previous histochemical and phytochemical studies have been effective at identifying these metabolites in various cell compartments, tissues and organs of Zingiberaceae plants (Gevú *et al.* 2019a, b; Silva *et al.* 2021; Mohanty *et al.* 2023).

Considering the botanical, economic, and biological properties of species of Zingiberaceae, as previously highlighted by their chemical importance, this study aimed to compare the anatomy and histochemistry of vegetative organs of *R. chrysotricha*, *R. breviscapa* and *R. nicolaioides*, to advance knowledge of the anatomy of Brazilian species of *Renealmia* and determine the location of their special metabolites.

Materials and Methods

Roots, rhizomes and completely expanded leaves of *Renealmia chrysotricha* were collected

at Parque Nacional de Itatiaia in southwest Rio de Janeiro state, between latitudes 22°15' and 22°30'S and longitudes 44°30' and 44°45'W (Itatiaia, RJ, Brazil). The same material of *R. breviscapa* and *R. nicolaioides* was collected in Fazenda Experimental Catuaba and Reserva Florestal Humaitá, respectively, both in Acre state, latitude 09°58' and longitude 67°48' (Rio Branco, Ac, Brazil). The collections were regulated by SISBIO, under authentication codes 38642-1 and 57784614. The material was identified and that for *R. chrysotricha* was deposited in the herbarium of the Universidade Federal Rural do Rio de Janeiro (RBR; voucher 33416), and that for *R. breviscapa* and *R. nicolaioides* in the herbarium of the Universidade Federal do Acre (UFACPZ; vouchers 6645 and 6646, respectively).

For light microscopy, fragments of vegetative organs of the three species were fixed in 70% FAA (Johansen 1940) and subsequently conserved in 70% alcohol (Jensen 1962). Rhizomes were embedded in Historesin® (Historesin Leica Instruments) and transverse sections (5 µm), made with a sliding microtome (SM2010 R, Leica, Germany), were stained with 0.05% toluidine blue (modification of O'Brien *et al.* 1964). Slides were sealed with Entellan and observed under a light microscope (Axioplan; Carl Zeiss, Oberkochen, Germany) with a Cannon Power Shot A640 camera. Other structural evaluations of vegetative organs were made on sections cut on a Ranvier microtome, which were bleached with 50% sodium hypochlorite, neutralized in 1% acetic acid solution, washed in distilled water, stained with a mixture of 2% Astra blue and 0.5% safranin in aqueous solution, and mounted as semipermanent slides (Bukatsch 1972).

For electron microscopy, fragments of vegetative organs were fixed in 2.5% glutaraldehyde, 4.0% formaldehyde and 0.05 M sodium cacodylate buffer of pH 7.2, then post-fixed in 1% osmium tetroxide in the same buffer for 1 h and dehydrated in an acetone series. For scanning electron microscopy (SEM), the material was subsequently submitted to critical point drying using a Bal-Tec CPD 030 Critical Point Dryer. The fragments were then affixed to supports using carbon adhesive tape, covered with a 20-nm layer of gold (Bal-Tec Sputter Coater SCD 050), and examined with a DSEM-ZEISS 962® scanning electron microscope. For transmission electron microscopy, dehydrated samples were infiltrated and embedded in epoxy resin (Epon®). Ultrathin sections (80 nm) were

then collected in copper grids (300 mesh) and stained with acetate with 1.0 % uranyl followed by 5.0 % lead citrate (Reynolds 1963). Observations were made at 80 kV using a JEOL 1400 Plus transmission electron microscope.

Histochemical tests were performed with fresh material to detect special metabolites. The following stain and reagents were used: NADI for essential oil and oil resin; Sudan IV for lipidic and cutinized cell walls; Lugol for starch grains; 10 % potassium dichromate and Hoephner-Vorsatz to detect phenolic compounds; and exposure to 10 % HCl to determine the nature of crystal inclusions (Kraus & Arduin 1997). Calcium oxalate crystal and starch grains were examined by polarized light

microscopy (Axioplan; Carl Zeiss, Oberkochen, Germany).

Leaf blades were dissociated according to Strittmater (1973) and stained with 1% safranin to determine stomatal frequency by examining a 1-mm² area of leaf epidermis. Twenty-five counts were averaged for each side of the leaf fragments. Stomata were classified according to Van Cotthem (1970).

Results

Root cross sections of the three studied species (Fig. 1) reveal a single-layered epidermis (Fig. 1a-c). The cortex comprises an exodermis of four to six layers, characterized by thin and

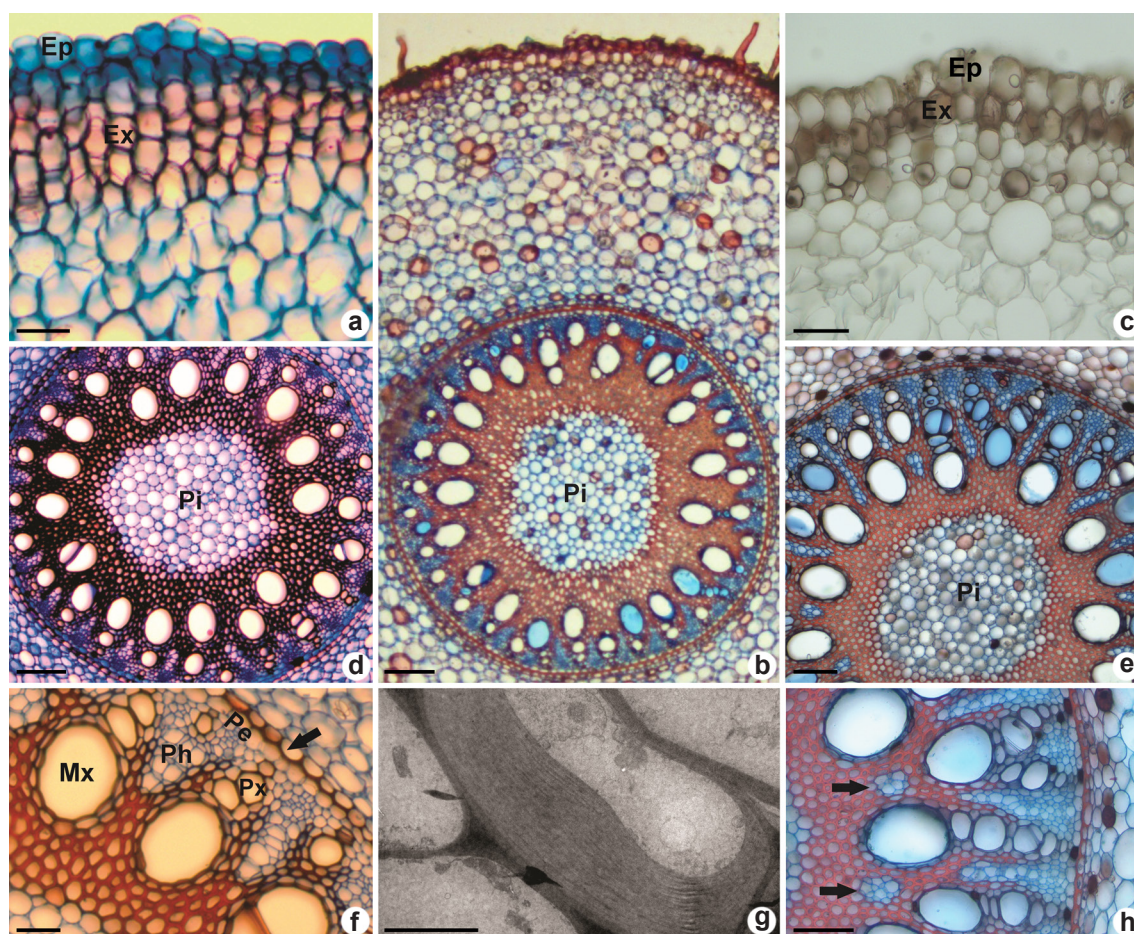


Figure 1 – a-h. Root anatomy of *Renealmia*, in cross section – a, d, f. *R. chrysotricha*; b, g. *R. breviscapa*; c, e, h. *R. nicolaioides* – a, c. details of epidermis, exodermis and cortex (LM); b. overview of roots (LM); d-e. details of central cylinder (LM); f. endodermal cell wall (arrow) with U-shaped thickening, pericycle, xylem and phloem (LM); g. detail of U-shaped thickening of endodermal cell wall (TEM); h. detail of phloem islands (arrows). (Ep = epidermis; Ex = exodermis; Mx = metaxylem; Pe = pericycle; Ph = phloem; Pi = pith; Px = protoxylem). LM = light microscopy, TEM = transmission electron microscopy. Scale bars: a, c, f, h = 50 μ m; b = 125 μ m; d-e = 100 μ m; g = 5 μ m.

lightly suberized cell walls (Fig. 1a,c). Directly beneath the exodermis lies a parenchymatous zone consisting of two regions: an outer region comprised of compact isodiametric cells with small intercellular spaces; and a well-defined internal layer. The cortex is bordered by a single-layered endodermis comprised of cells with U-shaped thickening as well as passage cells (Fig. 1f-g).

The vascular cylinder involves one layer of cells of the pericycle, which limits the vascular tissues (Fig. 1f). The number of xylem ridges varies among the three species as follows: *Renealmia chrysotricha*, 19–23 ridges; *R. breviscapa*, 30–32 ridges; and *R. nicolaioides* 38–39 ridges (Tab. 1).

In all three species, the phloem consists of small groups of perforated elements, companion cells, and phenolic idioblasts. Phloem strands extend radially inwards, also forming conspicuous

phloem islands only in *R. nicolaioides* (Fig. 1h). On the other hand, isolated phloem groups were found in *R. breviscapa* but were notably absent in *R. chrysotricha*. The pith is composed of fibers and central parenchymatous cells (Fig. 1b,d-e).

In cross sections of the species studied (Fig. 2a-f), the rhizome of *R. chrysotricha* possesses a suber with approximately six layers of compact and slightly thickened cells originating from the parenchymatous cell division of the subepidermal cells (Fig. 2a). Conversely, *R. breviscapa* and *R. nicolaioides* exhibit a single-layered epidermis (Fig. 2b-c). Additionally, unicellular, simple, and furcate trichomes were exclusively found in the epidermis of *R. nicolaioides* (Fig. 2g-h; Tab. 1).

The cortical region, when viewed in cross section, displays 10- to 15-layers of ground parenchyma and collateral bundles. A fibrous sheath encircles these vascular bundles in *R. breviscapa* and *R. nicolaioides*. The endodermis

Table 1 – Morphological and anatomical characteristics diagnostic identified in the vegetative organs of *Renealmia* species studied.

Organs / Species		<i>R. chrysotricha</i>	<i>R. breviscapa</i>	<i>R. nicolaioides</i>
Root	The number of xylem ridges	19–23 ridges	30–32 ridges	38–39 ridges
	Phloem	absent	some isolated phloem groups	conspicuous phloem islands present
Rhizome	Dermal tissue	six layers of compact and slightly thickened cells	a single-layered epidermis	a single-layered epidermis
	Phenolic compounds location	suber	subepidermal layer	subepidermal layer
Leaf	Sheath	without this feature	without this feature	reticulated sheath with grooves and projections on its abaxial surface
	Petiole	lacks one	short - 25 mm lenght	short - 40 mm lenght
	Trichomes	simple and furcate prickles	simple and furcate prickles; plurifurcate trichomes solely on the abaxial surface	simple and thin-walled furcate often have arms parallel to the surface
	Leaf blade with subepidermal layer	occur it on both surfaces	without this feature	occur it on both surfaces
	Phenolic compounds location	present in the epidermis and subepidermal layer; in the spongy parenchyma	without this feature	present in the epidermis and subepidermal layer; in the guard cells of the stoma
	Mean value and standard deviation of stomata density (mm2)	Ad 2,0 ± 1,1	6,5 ± 1,5	8 ± 1,7
		Ab 60,0 ± 4,0	49 ± 4,5	72,0 ± 4,8

Ad = adaxial surface; Ab = abaxial surface.

serves as the boundary layer of the cortex, with endodermal cells containing suberin in their radial and transverse walls (Fig. 2d-f).

The vascular cylinder consists of a parenchymatous uniseriate pericycle and with diffuse and randomly arranged collateral bundles. Vascular bundles of the vascular cylinder are more developed and abundant than those of the cortex.

Various cell contents were identified in underground organs of *Renealmia* (Fig. 3). Starch grains, both simple and enveloped, are present in the cortex and pith of roots of all three studied species. Rhizomes exhibit a remarkable concentration of starch within parenchyma cells

(Fig. 2i; 3c-f); nevertheless, the outer cortical layers of all three species lack starch.

Phenolic idioblasts are observed across the cortical cells and the vascular cylinder, and in association with the vascular bundles, within the roots and rhizomes of all examined species. The type and distribution of these compounds vary slightly among the subterranean organs of the three species. In the case of *R. chrysotricha*, phenolic compounds occur in the protoplasm, giving a dark- or golden-brown homogeneous appearance (Fig. 3i), and are notably present in the layered suberin (Fig. 3g). Phenolic compounds appear with greater frequency in *R. nicolaioides* compared to the other

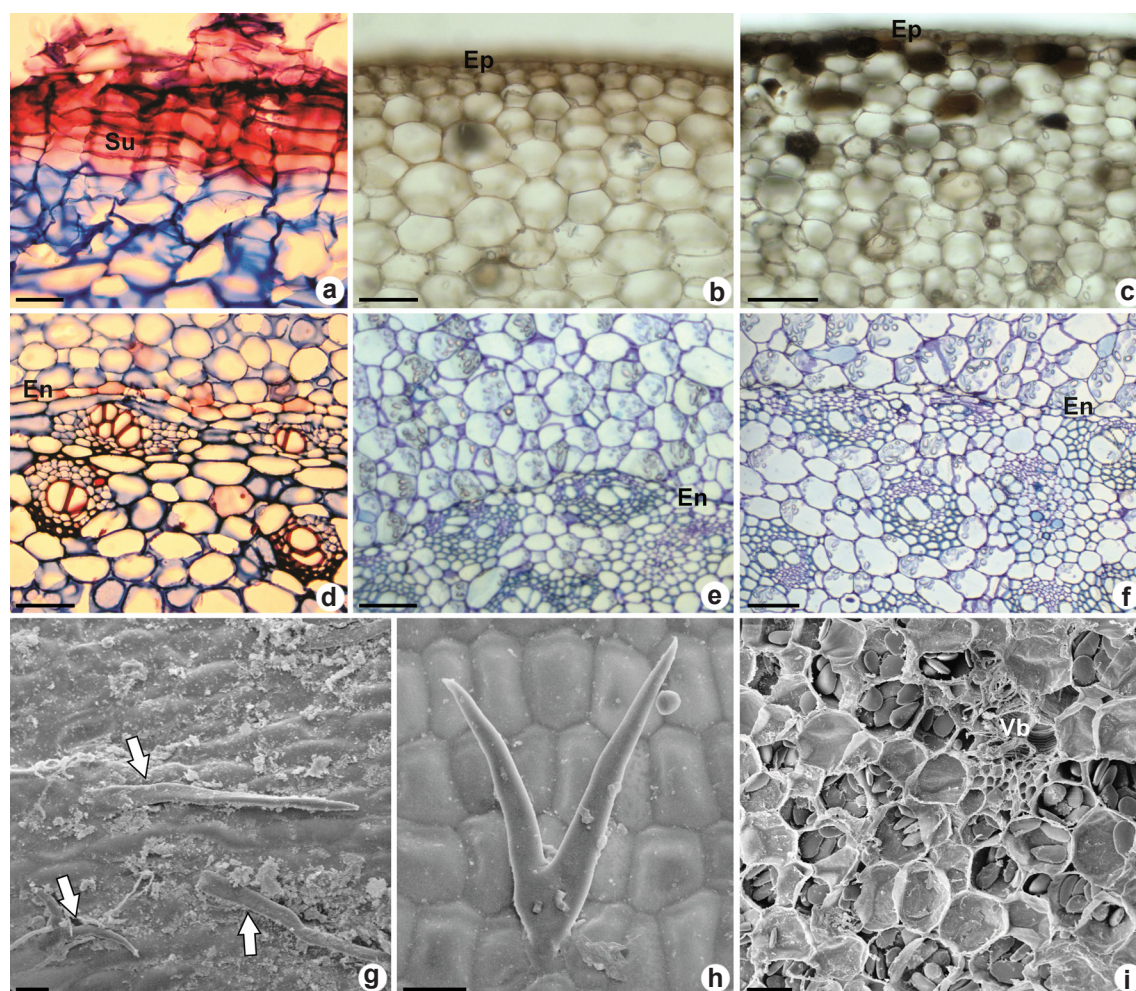


Figure 2 – a-i. Rhizome anatomy of *Renealmia*, in cross section – a, d, i. *R. chrysotricha*; b, e. *R. breviscapa*; c, f-h. *R. nicolaioides* – a. details of stratified suber and cortex (LM); b-c. details of epidermis and cortex (LM); d-f. details of endodermis and vascular bundles (LM); g. simple and furcate trichomes (SEM); h. detail of furcate trichome (SEM); i. storage parenchyma (SEM). (Ep = epidermis; En = endodermis; Vb = vascular bundle; Su = stratified suber). LM = light microscopy; SEM = scanning electron microscopy. Scale bars: a = 50 µm; b-f = 100 µm; g-h = 20 µm; i = 50 µm.

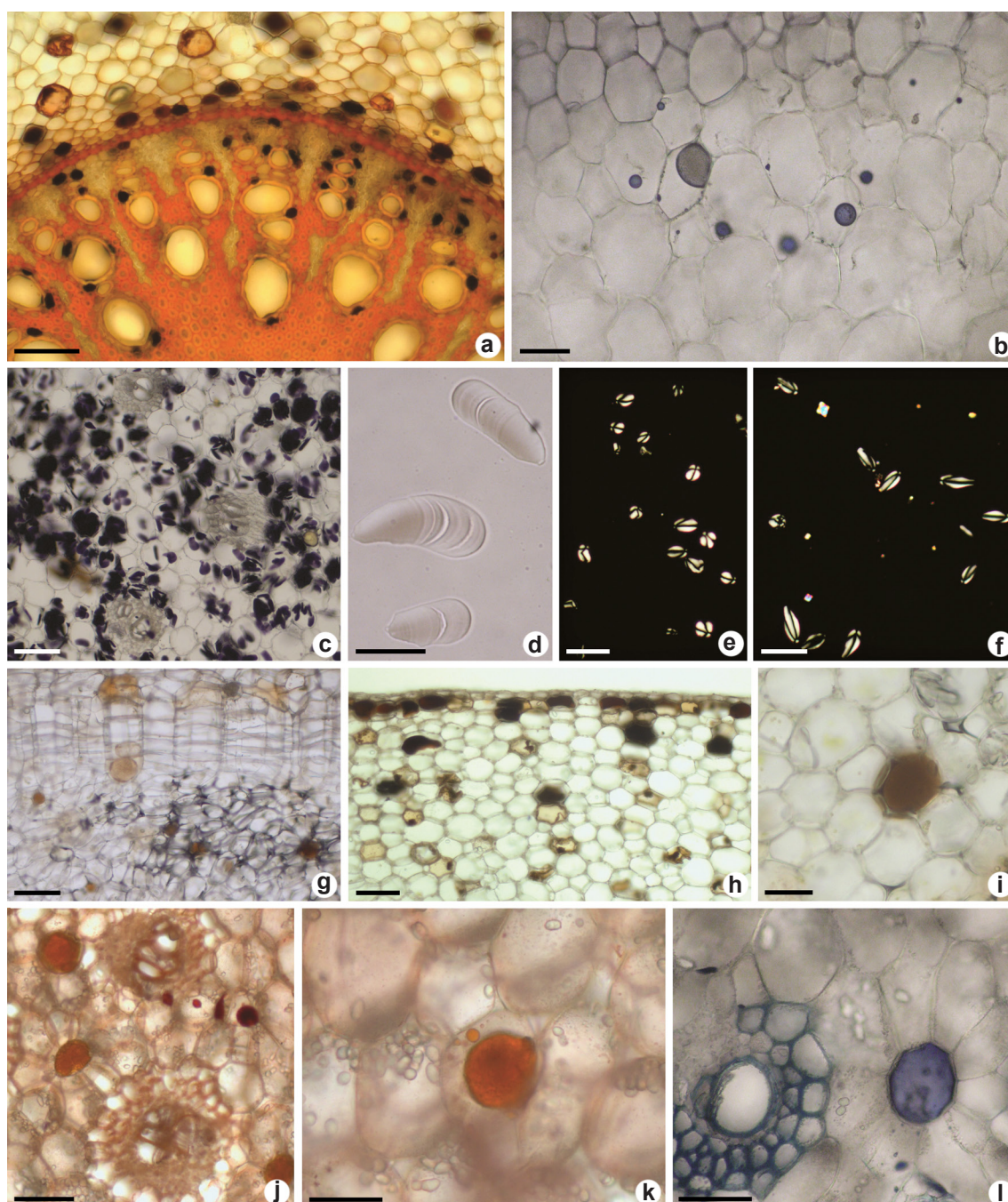


Figure 3 – a-l. Cell contents identified in underground organs of *Renalmia*, in cross section – a, b. roots; c-l. rhizomes – a. phenolic idioblasts of *R. nicolaioides*, Hoephner-Vorsatz; b. essential oil droplets in the cortex of *R. chrysotricha*, revealed by NADI; c-d. ellipsoid starch grains in *R. chrysotricha* – c. revealed by Lugol; e. envelope-layer type of starch grain in *R. breviscapa* under polarized light; f. starch grains and prismatic crystals in *R. nicolaioides* under polarized light; g-i. phenolic idioblasts, revealed by 10 % potassium dichromate – g. suber and cortex of *R. chrysotricha*; h. epidermis and cortex of *R. nicolaioides*; i. cortex of *R. chrysotricha*; j-l. oil idioblasts of *R. breviscapa* – j-k. revealed by Sudan IV; l. revealed by NADI. Scale bars: a, e-h, j = 100 μ m; b = 25 μ m; c-d = 150 μ m; i, k-l = 50 μ m.

species. They are also identified within the cortical layers proximal to the endodermis and cells of the pericycle situated adjacent to the xylem in the root, and within the subepidermal layers of the rhizome, of *R. breviscapa* and *R. nicolaioides* (Fig. 3a,h).

Oil idioblasts were found distributed across the cortex and vascular cylinder of both root (Fig. 3b) and rhizome (Fig. 3g,j-l) in all three species. These idioblasts are typically isodiametric and often encircled by cells arranged radially within the rhizomes in both cross and longitudinal sections. Additionally, prismatic crystals of calcium oxalate

were observed in the cortical region of the rhizome of *R. chrysotricha* and *R. nicolaioides* (Fig. 3f).

Several characteristics were noted in the cross-sections of the sheath and petiole (Fig. 4). The leaf sheaths of all three species exhibit a concave-convex contour. *Renalmia nicolaioides* notably showcases a reticulated sheath with grooves and projections on its abaxial surface (Fig. 4b) (Tab. 1). Cross section of the leaf sheath reveals that both adaxial and abaxial epidermis comprise a single layer covered by a thin cuticle (Fig. 4a-b). Simple unicellular trichomes are ubiquitous

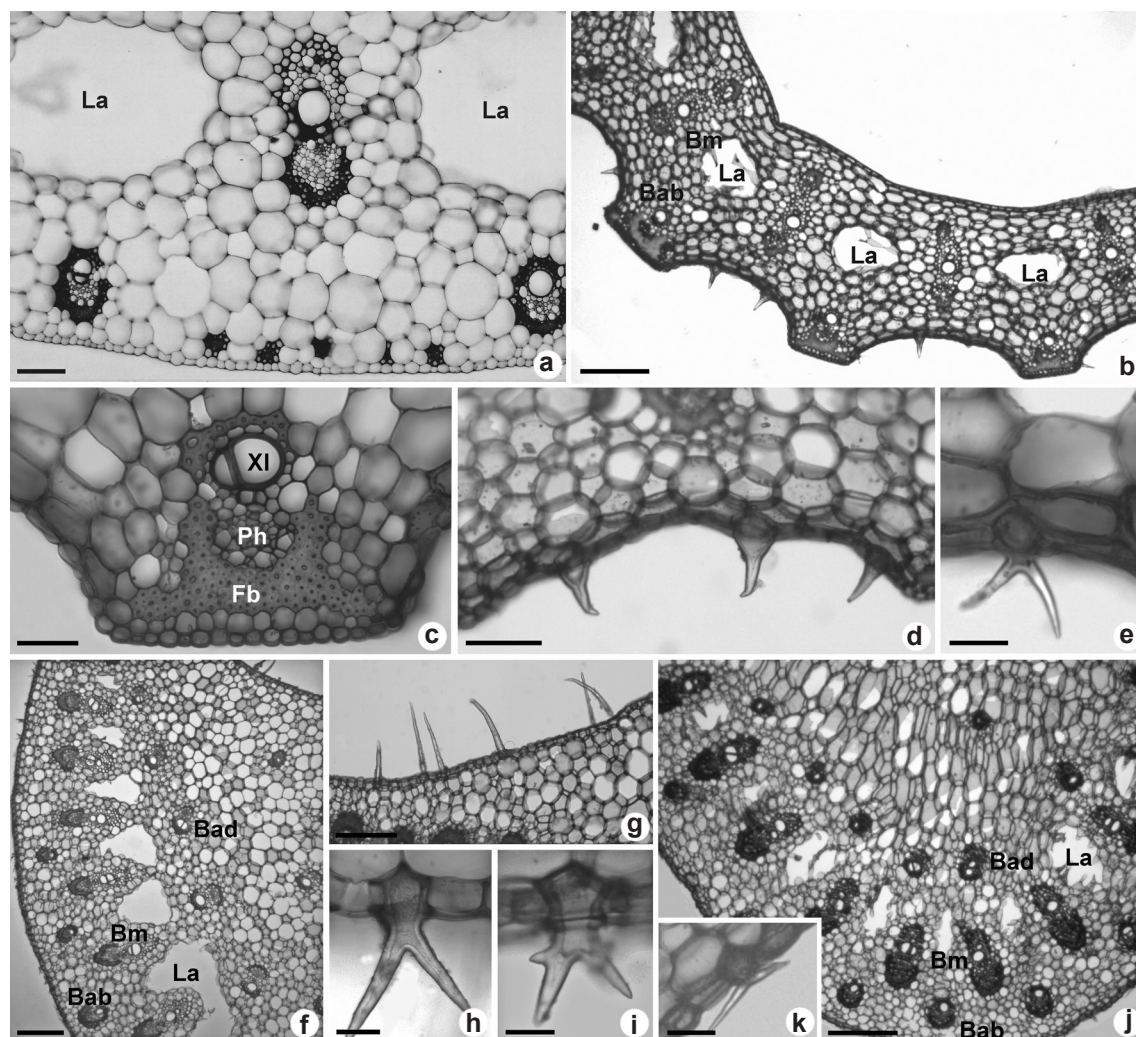


Figure 4 – a-k. Cross section of sheath and petiole – a. sheath of *R. chrysotricha*; b-e. sheath of *R. nicolaioides* – b. grooves and projections; c. detail of vascular bundle and fibers; d. prickle trichomes; e. detail of furcate trichome; f-i. petiole of *R. breviscapa* – f. general aspect; g. long simple trichomes; h. detail of furcate trichome; i. detail of plurifurcate trichome; j. petiole of *R. nicolaioides*; k. detail of furcate trichome. (Bab = abaxial bundle; Bad = adaxial bundle; Bm = main bundles; Fb = fiber; La = lacune; Ph = phloem; Xl = xylem). Scale bars: a = 200 μ m; b, f, j = 250 μ m; c = 50 μ m; d-g = 100 μ m; e, h-i, k = 25 μ m.

on the abaxial surface of all three species. These trichomes are characterized by their thick-walled, short, and sharply pointed structure, and are often found interspersed among the subepidermal cells (Fig. 4d). Additionally, *R. breviscapa* and *R. nicolaioides* exhibit furcate unicellular trichomes on their abaxial leaf surfaces (Fig. 4e). Notably, all trichomes of *R. nicolaioides* are concentrated within the region of the grooves (Tab. 1).

Isodiametric parenchyma cells are present below the adaxial surface, and 2–3 layers of thin-walled, colorless expansion cells are most commonly above the larger sheath veins. Adjacently there are air-canals segmented by transverse diaphragms of lobed cells. Main bundles alternate with air-canals (Fig. 4a–b) and are embedded in an abaxial chlorenchyma with isodiametric cells.

The vascular bundles are arranged in three systems: adaxial, main and abaxial (Fig. 4b). Within the adaxial system, a few vascular bundles of intermediate size are enclosed by a fibrous sheath. Main vascular bundles have larger veins with fibrous strands close to them (Fig. 4a–b), whereas the abaxial system comprises smaller vascular bundles that present fibrous strands in contact with the abaxial surface, forming a rigid peripheral zone (Fig. 4a,c).

Renealmia chrysotricha lacks a petiole, while *R. breviscapa* and *R. nicolaioides* possess short petioles, measuring 25 mm and 40 mm, respectively (Tab. 1). Cross section of the petiole shows concave-convex contours (Fig. 4f,j). Cross-section of the epidermis displays smaller ground cells and various types of trichomes. Thick-walled simple and furcate prickles were observed on the adaxial and abaxial surfaces of all the species (Fig. 4g). The thin-walled furcate trichomes of *R. nicolaioides* often have arms parallel to the surface (Fig. 4k). Furthermore, two other trichome variants were identified: thin-walled and long simple trichomes present on the adaxial side of all three species; and plurifurcate trichomes solely on the abaxial surface of *R. breviscapa* (Fig. 4g–i).

In frontal view, the epidermal cells of the leaf blade of all three species (Fig. 5) present anticlinal cell walls with straight to slightly sinuous contours (Fig. 5a–f). Epidermal cells with striate cuticular ornamentation are restricted to the abaxial surface of *R. nicolaioides* (Fig. 5h–i).

Prickle trichomes are observed in the intercostal region and midrib of *R. chrysotricha* (Fig. 5a), and distinct parts of the leaf blade of *R. breviscapa* and *R. nicolaioides*. This type is thick-

walled and lignified, with its base sunken among subepidermal cells. Thin-walled and long simple and furcate trichomes were found only on the leaf blade epidermis of *R. nicolaioides* (Fig. 5g–h).

The leaves of all three of the studied species are amphistomatic with tetracytic stomata (Fig. 5a–f, h–i) (Tab. 1). These stomata are situated at the same level as the other epidermal cells and have substomatal spaces that extend one or two layers into the spongy parenchyma. The three studied species of *Renealmia* vary in mean stomata density of the leaf surfaces, which is higher on the abaxial than the adaxial surface (Tab. 1).

In transversal sections, the midrib displays small epidermal cells and assumes a circular shape on both surfaces of all three species. Adjacent to the adaxial epidermis lies ground parenchyma with isodiametric cells. Expansion cells are observed within the petiole, consistent with previous descriptions. Beneath the abaxial surface, the main vascular bundles are enveloped by chlorenchyma.

Collateral vascular bundles extend towards the midrib, mirroring the organizational pattern observed in leaf sheaths. The small vascular systems are close to the abaxial surface and present a fibrous sheath. The main vascular bundles are intertwined with air-canals in all three species. The abaxial bundles present lignified and thick-walled phloem fibers and thin-walled xylem fibers (Fig. 6a–c).

The mesophyll structure in all three species exhibits dorsiventral characteristics, featuring a single layer of palisade parenchyma and two to four layers of spongy parenchyma (Fig. 6d–f). *Renealmia breviscapa* lacks a subepidermal layer, whereas *R. chrysotricha* and *R. nicolaioides* possess it on both surfaces (Fig. 6d–e) (Tab. 1). In *R. chrysotricha*, subepidermal cells display a slight thickening of the walls, a lack of color, and a larger appearance than those of the epidermis (Fig. 7e), whereas the cells of the epidermis and subepidermal layer are the same size in *R. nicolaioides*.

Histochemical analyses showed different accumulations of special metabolites in the tissues (Fig. 7). The subepidermal layer likely contains oleoresin (Fig. 7d), prismatic crystals, and phenolic compounds (Fig. 7d).

Oil droplets, starch grains and prismatic crystal idioblasts (Fig. 7f) were found in the parenchyma cells of leaves, alongside phenolic idioblasts. Phenolic compounds were detected in the leaf-sheath, petiole, and midrib of the three

species (Fig. 7c). Phenolic compounds were identified in the epidermis and subepidermal layer of the intercostal region in *R. chrysotricha* and *R. nicolaioides* (Fig. 7d-e), exhibiting a golden-brown color in the former and dark in the latter. Phenolic compounds were also identified in the guard cells of stomata in *R. nicolaioides* (Fig. 7d) (Tab. 1) In the vacuole of spongy parenchyma cells, with an homogenous and granular appearance, in *Renealmia chrysotricha* (Fig. 8a-b).

The transverse section of the petiole of *R. breviscapa* revealed phenolic-storage cells surrounded by an arrangement of radial cells next to the lacunae (Fig. 7a) and were found next to veins and chlorenchyma (Fig. 7c), and

inside the trichomes (Fig. 7b). Phenolic idioblasts consistently exhibited a black or brown color.

Calcium oxalate prismatic crystals were observed in all leaf levels but were more abundant in the petiole. Starch grains were found in the parenchymatic cells associated with vascular bundles in *R. nicolaioides* (Fig. 7f).

Discussion

Many of the anatomical characteristics observed across the three studied species have been identified as traits for the family Zingiberaceae and diagnostic of the genus *Renealmia*. These characteristics include the presence of oil cells in all organs, predominantly single-celled and

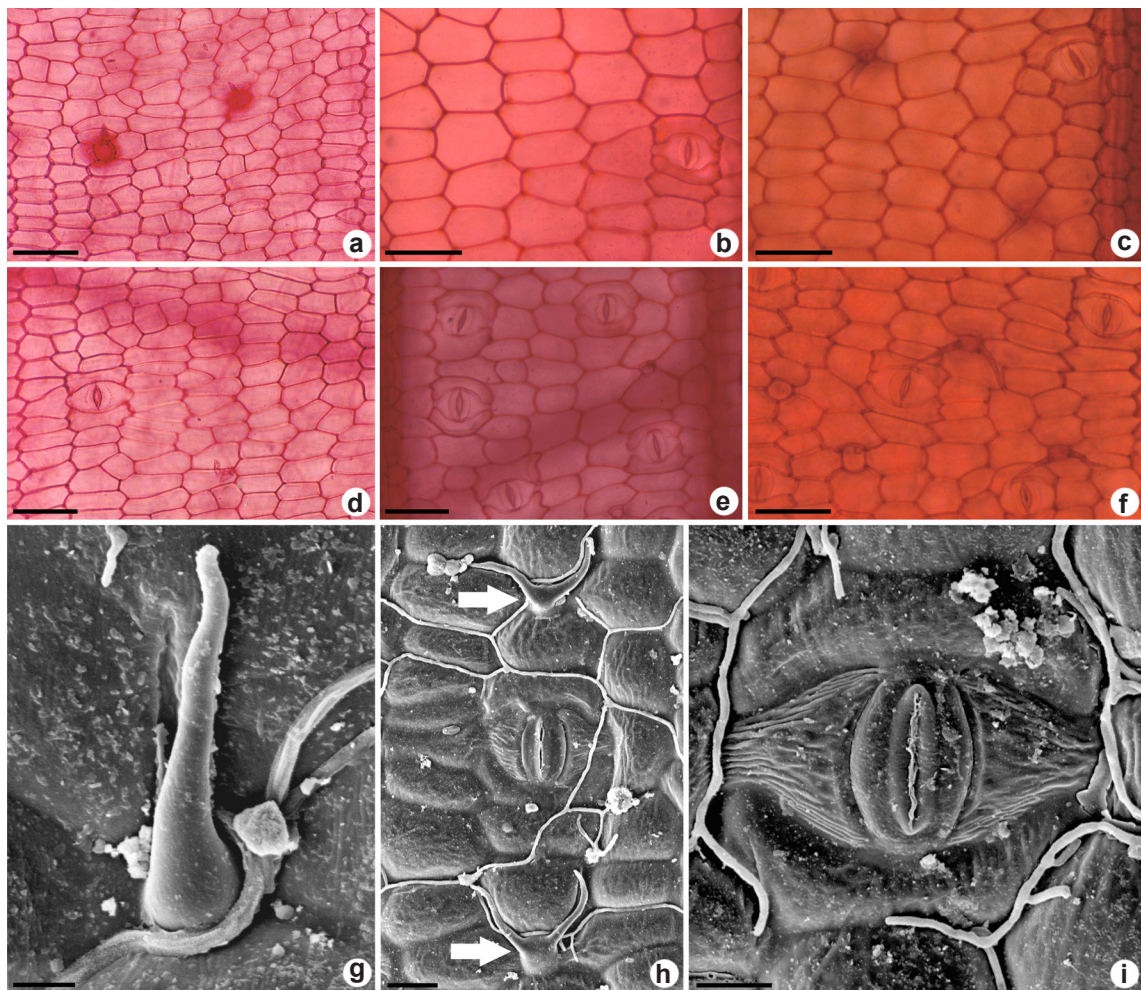


Figure 5 – a-i. General aspects of leaf epidermis of *Renealmia*, in frontal view – a-c. adaxial surfaces; d-f. abaxial surfaces – a, d. *R. chrysotricha* – a. simple prickly trichome; b, e. *R. breviscapa*; c, f-i. *R. nicolaioides* – g. long simple trichome; h. furcate trichomes (arrows) and stomata; i. detail of striated cuticle. (a-f. LM = light microscopy; g-i. SEM = scanning electron microscopy). Scale bars: a-f = 100 µm; g = 5 µm; h = 20 µm; i = 10 µm.

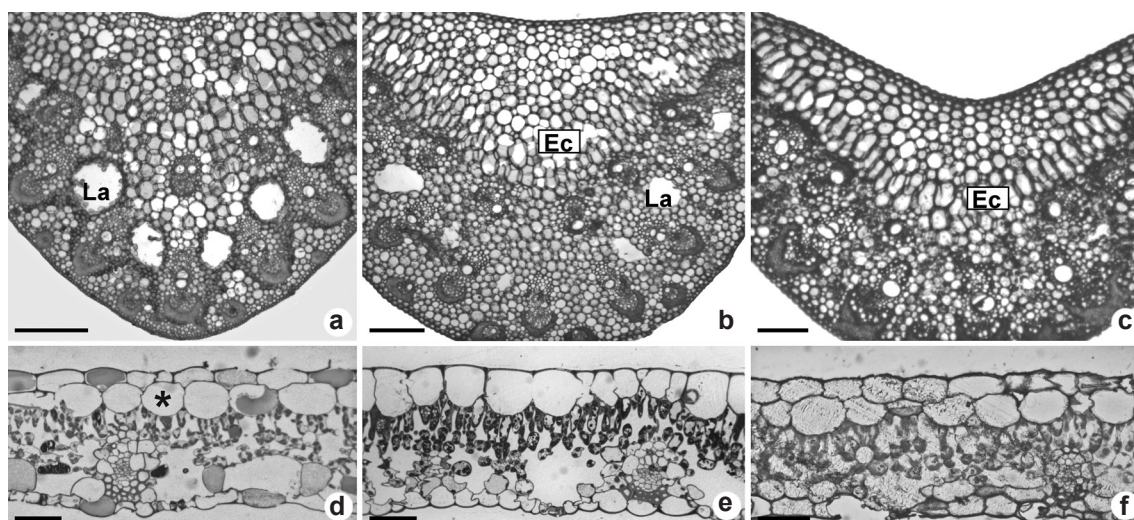


Figure 6 – a-f. Cross sections of leaf blade – a-c. midrib; d-f. dorsiventral mesophyll – a, d. *R. chrysotricha*; b, e. *R. breviscapa*; c, f. *R. nicolaioides*. (Ec = Expansion cells; La = lacune), subepidermal layer (*). Scale bars: a-c = 250 µm; d-f = 50 µm.

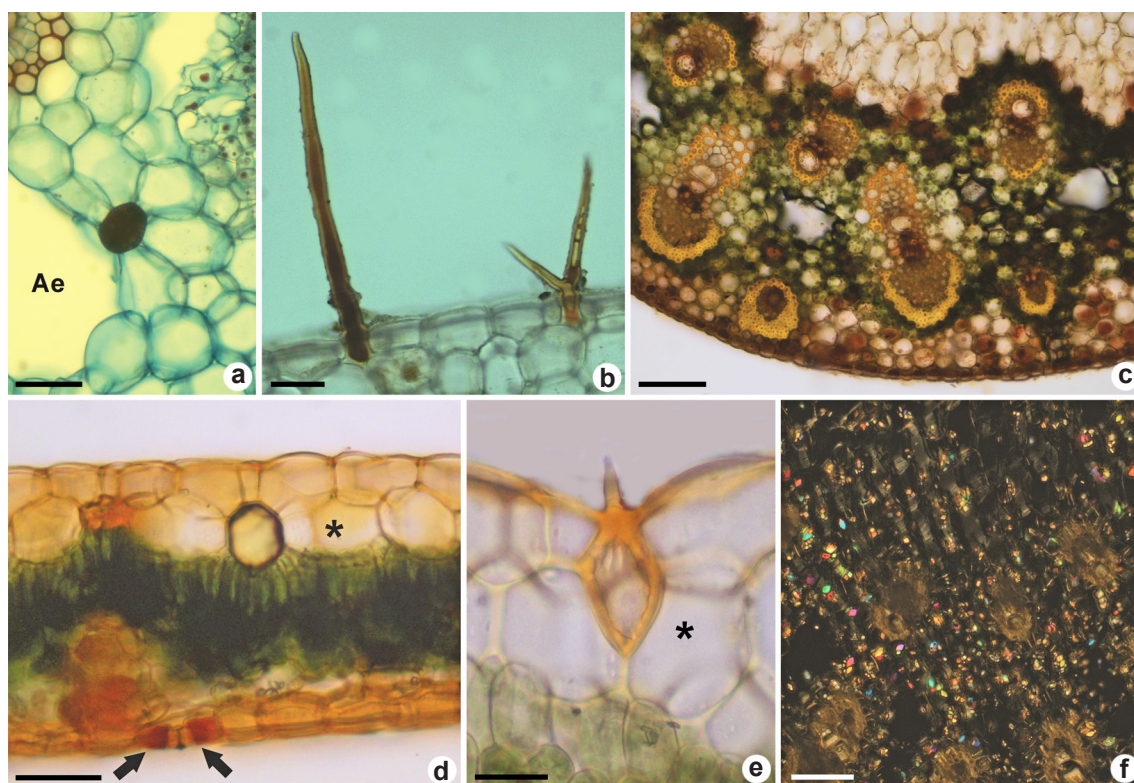


Figure 7 – a-f. Cell contents of leaves of *Renealmia*, in cross section – a-c. phenolic compounds in *R. breviscapa* – a-b. petiole – b. phenolic compounds in trichome, revealed by 10 % potassium dichromate; c-e. revealed by Hoephner-Vorsatz – c. leaf blade; d-e. mesophyll of *R. nicolaioides* – d. oil idioblasts in subepidermal cell, phenolic compounds in subsidiary cells; e. detail of prickly trichome; f. prismatic crystals on the petiole of *R. nicolaioides* evidenced by polarized light. Scale bars: a, b, d = 50 µm; c, f = 100 µm; e = 20 µm.

infrequently branched hairs, an aerial stem typically divided into cortex and a central cylinder encircled by perivascular fibers, starch grains that are commonly flattened, air canals that often extend from the sheath base to the blade midrib, dorsiventral mesophyll, and a hypodermis comprised of a single layer. Additionally, large veins are commonly situated directly beneath an autonomous strand of hypodermal fibers, while the abaxial system presents numerous arcs of variously sized vascular bundles (Tomlinson 1956, 1962, 1969).

Renalmia nicolaioides exhibited several distinctions in its root vascular system, compared to the two other studied species. These differences include radially extended phloem strands, the presence of phloem islands, and a greater number of xylem poles. Interestingly, isolated phloem groups were identified in *R. breviscapa* but were notably absent in *R. chrysotricha*. Similar root phloem islands have been documented in *Alpinia purpurata* (Vieill.) K. Schum. and *Etlingera fulgens* (Ridl.) C. K. Lim. (Gevú et al. 2013).

In contrast to the other species, the rhizome of *R. chrysotricha* displayed an outermost layer consisting of cork cells, resulting from the suberification of external cortical cells within the family Zingiberaceae (Tomlinson 1956).

Conversely, *R. breviscapa* and *R. nicolaioides* feature a singular layer of cells in their rhizome structure, as previously described by Gevú et al. (2019b) for rhizomes of *Renalmia* species. These anatomical distinctions not only contribute to understanding species-specific adaptations, but also shed light on the diversity and evolution of root structures within Zingiberaceae. Continued research in this area promises to unveil further insights into the intricate complexities of plant anatomy and evolution.

Various types of trichomes, including simple prickles and thin-walled furcate and plurifurcate trichomes, were observed across different parts of the leaves. The present findings support the observations made by Maas (1977), who noted the rarity of stellate trichomes within the genus *Renalmia*. Additionally, Dahlgren & Clifford (1982) demonstrated that, while most species of Zingiberales are typically glabrous, unicellular trichomes can still be found in some families such as Marantaceae and Zingiberaceae. Moreover, the contours of epidermal cells exhibited variation when viewed from the front, suggesting a potential role in stress sensing, as discussed by Sapala et al. (2018). This observation underscores the intricate relationship between plant morphology and environmental adaptation, highlighting the dynamic



Figure 8 – a-b. Phenolic compounds in the leaf vacuole of *R. chrysotricha* (TEM) – a. homogeneous appearance; b. granular appearance. TEM = transmission electron microscopy. Scale bars: a = 1,5 µm; b = 2,5 µm.

nature of plant responses to external stimuli. Further exploration of trichome diversity and epidermal cell characteristics promises to deepen our understanding of plant-environment interactions within evolutionary and ecological dynamics.

Renealmia chrysotricha and *R. nicolaioides*, but not *R. breviscapa*, possess a subepidermal layer on both leaf surfaces. The term hypodermis was omitted in this study due to the necessity for ontogenetic research to ascertain the nature of this layer, however, other researchers of species of Zingiberaceae have used this term. The hypodermis was used to segregate species of *Alpinia* (Hussin *et al.* 2000; Talip *et al.* 2005) and its absence was reported as a primitive character (Tomlinson 1962).

The present study observed lacunes that extend from the leaf sheath to the midrib, serving important roles in both mechanical support and oxygen distribution within the plant. Tomlinson (1962) proposed two hypotheses to explain the occurrence of lacunes in all families of Zingiberales. The first suggested that lacunes may be a consequence of the scarcity of ground tissues in large, rapidly developing organs. The second arises from speculation regarding the potential influence of aquatic ancestry on the development of lacunes in Zingiberales. These hypotheses shed light on the evolutionary origins and functional significance of lacunes in the context of plant morphology and adaptation within the order Zingiberales.

In Zingiberaceae, starch grains are abundant in the rhizome and tuberous roots and common throughout the leaf axis in the region close to the vascular bundles (Tomlinson 1969). The present study found starch grains in roots and rhizomes. *Renealmia nicolaioides* showed abundant starch content in the leaf blade. The observed grains (simple and ellipsoid) are like those described by Czaja (1978) for some monocotyledon families.

The present findings reveal the presence of oil and phenolic idioblasts across all vegetative organs of the three studied species. Tomlinson (1962, 1969) and Pugiagli (1991) noted that the occurrence of oils is a diagnostic feature for Zingiberaceae. Various species of *Renealmia* have been investigated for their oil compositions and associated bioactivities. For instance, the essential oils extracted from the leaves of *R. thyrsoides* (Ruiz & Pav.) and *R. alpinia* has demonstrated antimicrobial and antioxidant properties. Furthermore, oils extracted from the leaves of *R. chrysotricha* and *R. nicolaioides* have exhibited antifungal activity, while oil from the leaf and rhizome of *R. chrysotricha* have shown

antiparasitic effects, suggesting that this species is a potential source of a compound useful in the control of trypanosomiasis (Noriega *et al.* 2016; Gevú *et al.* 2019c; Souza *et al.* 2023). These findings underscore the pharmacological potential of oils derived from species of *Renealmia*, highlighting their importance in traditional and modern medicine.

Polyphenolic secondary metabolites are related to plant defense against microbial pathogens in subterranean organs and protection against predation and UV damage in the leaf blade (Dixon *et al.* 2005; Mierziak *et al.* 2014). Plant polyphenols are even multifunctional, acting as antioxidant agents as well as in chronic disease prevention, such as cancer and cardiovascular disease (Rice-Evans *et al.* 1996; Bravo 1998). Gevú *et al.* (2019a, b) investigated histochemical and phytochemical aspects of the rhizomes of species of *Renealmia* and identified two classes of phenolic compounds - diarylheptanoid and flavonoid - both presenting chemotaxonomic markers with relevant biological properties.

In conclusion, the present analysis of species of *Renealmia* revealed the first description of all studies species anatomical structure: prismatic-type calcium oxalate crystals across various organs, while silica bodies or sand silica were absent. The mineral content, particularly calcium oxalate crystals, emerges as a significant diagnostic feature for the genus *Renealmia*.

Common anatomical features of the examined species of *Renealmia* include oil idioblasts, dorsiventral leaf structure, amphistomatic leaves, tetracytic stomata, unicellular trichomes, and prismatic crystals. These features are characteristic of the genus *Renealmia* and hold broader significance within the family Zingiberaceae.

Moreover, the distinctive vegetative characteristics that contribute to distinguishing the three studied species include the cell distribution of root phloem, the covering tissue of rhizomes, the types and distribution of phenolic idioblasts and trichomes in both rhizomes and leaves, the presence or absence of a petiole, and a subepidermal layer in the intercostal region of leaves.

By elucidating these anatomical characteristics, the present study contributes to a deeper understanding of the morphological diversity and taxonomic relationships within the genus *Renealmia*. These findings provide valuable insights for future research to unravel the evolutionary and ecological significance of these plant traits.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); and Fundação de Amparo à Pesquisa do Rio de Janeiro (FAPERJ). This study was part of the thesis of K.V.G. at the Programa de Pós-graduação em Biociências e Biotecnologia da Universidade Estadual do Norte Fluminense Darcy Ribeiro (UNEF).

Data availability statement

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

References

- Alarcón JC, Martínez DM, Quintana JC, Jiménez S, Díaz A & Jiménez I (2008) Propagación in vitro de *Renalmia alpinia* (ROTTB), planta com actividad antiofídica. VITAE 15: 61-69.
- Albuquerque ESB & Neves LJ (2004) Anatomia foliar de *Alpinia zerumbet* (Pers.) Burt & Smith (Zingiberaceae). Acta Botanica Brasílica 18: 109-121. DOI: 10.1590/S0102-33062004000100010
- Boeger MRT, Pil MWB & Filho NB (2007) Arquitetura foliar comparativa de *Hedychium coronarium* J. Koenig (Zingiberaceae) e de *Typha domingensis* Pers. (Typhaceae). Iheringia 621: 113-20.
- Bukatsch F (1972) Bemerkungen zur Doppelfärbung Astrablau-Safranin. Mikrokosmos 61: 255.
- Czaja AT (1978) Structure of starch grains and the classification of vascular plant families. Taxon 27: 463-470. DOI: 10.2307/1219895
- Dahlgren RMT & Clifford HT (1982) The monocotyledons. A comparative study. Academic Press, London. 378p.
- Dixon RA, Xie D & Sharma SB (2005) Proanthocyanidins - a final frontier in flavonoid research? New Phytologist 165: 9-28. DOI: 10.1111/j.1469-8137.2004.01217.x
- Gevú KV, Cunha M, Barros CF, Pereira SM & Lima HRP (2013) Structural analysis of subterranean organs in Zingiberaceae. Plant Systematics and Evolution 300: 1089-1098. DOI: 10.1007/s00606-013-947-y
- Gevú KV, Carvalho MG, Silva IG, Lima HRP, Castro RN & Cunha M (2019a) Phenolic compounds from the rhizome of *Renalmia nicolaoides* Loes.: a new diarylheptanoid. Anais da Academia Brasileira de Ciências 91: e20180312. DOI: 10.1590/0001-3765201920180312
- Gevú KV, Castro RN, Souza JPLLS, Silva IG, Lima HRP, Cunha M & Carvalho MG (2019b) Avaliação das substâncias fenólicas em rizomas de três espécies de *Renalmia* L.f.: quantificação, atividade antioxidante e histolocalização. Revista Virtual de Química 11: 1625-1634.
- Gevú KV, Lima HRP, Neves IA, Mello EO, Taveira GB, Carvalho LP, Carvalho MG, Gomes VM, Melo EJT, & Cunha M (2019c) Chemical composition and anti-*Candida* and anti-*Trypanosoma cruzi* activities of essential oils from the rhizomes and leaves of Brazilian species of *Renalmia* L. fil. Records of Natural Products 13: 268-280. DOI: 10.25135/rnp.105.18.08.125
- Gottlieb OR & Salatino A (1987) Função e evolução de óleos essenciais e de estruturas secretoras. Ciência e Cultura 3: 707-716.
- Gottlieb OR, Kaplan MAC & Borin MRMB (1996) Biodiversidade: um enfoque químico-biológico. Editora UFRJ, Rio de Janeiro. 268p.
- Heinzmann BM, Spitzer V & Simões CMO (2017) Óleos voláteis. In: Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, & Petrovick PR (eds.) Farmacognosia: do produto natural ao medicamento. Artmed, Porto Alegre. 464p.
- Hussin KH, Chua TS, Ibrahim I, Wu QG, Ping LJ & Liu N (2000) Comparative leaf anatomy of *Alpinia* Roxb. species (Zingiberaceae) from China. Botanical Journal of the Linnean Society 133: 161-180. DOI: 10.1006/bojl.1999.0308
- Hussin KH, Ibrahim H, Ali DAHA, Liao J & Liu N (2001) Anatomical variations in leaves of *Boesenbergia* O. Kuntze and *Kaempferia* L. species (Zingiberaceae). Journal of Tropical and Subtropical Botany 9: 49-54. DOI: 10.3969/j.issn.1005-3395.2001.1.008
- Judd WS, Campbell CS, Kellogg EA, Stevens PF & Donoghue MJ (2009) Sistemática vegetal: um enfoque filogenético. 3rd ed. Artmed, Porto Alegre. 632p.
- Kraus JE & Arduin M (1997) Manual básico de métodos em morfologia vegetal. Ed. UFRRJ, Seropédica. 198p.
- Kress WJ, Prince LM & Williams KJ (2002) The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. American Journal of Botany 89: 1682-1696. DOI: 10.3732/ajb.89.10.1682
- Lima HRP & Kaplan MAC (2010) Quimiosistemática micromolecular e tendências evolutivas da superordem Zingiberiflorae (*sensu* Dahlgren). In: Kaplan MAC, Abreu HS, Lima HRP & Soares GLG (eds.) Abordagem quimiosistemática e evolução química das fanerógamas. Ed. UFRRJ, Seropédica. Pp. 231-260.
- Maas PJM (1977) *Renalmia* (Zingiberaceae-Zingiberoideae), Costoideae (Zingiberaceae). Flora Neotropica. The New York Botanical Garden, New York. 218p.
- Maas P & Maas H (2015) Costaceae in Lista de Espécies da Flora do Brasil. Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. Available at <http://

- floradobrasil2015.jbrj.gov.br/jabot/floradobrasil/FB110639>.
- Martins MBG, Caravante ALC, Apezatto-da-Glória B, Soares MKM, Moreira RRD & Santos LE (2010) Caracterização anatômica e fitoquímica de folhas e rizomas de *Hedychium coronarium* J. König (Zingiberaceae). *Revista Brasileira de Plantas Mediciniais* 12: 179-187. DOI: 10.1590/S1516-05722010000200009
- Mierziak J, Kostyn K & Kulma A (2014) Flavonoids as important molecules of plant interactions with the environment. *Molecules* 19: 16240-16265. DOI: 10.3390/molecules191016240
- Mohanty S, Ray A, Jena S, Sahoo A, Sahoo T, Kamila PK, Panda PC & Nayak S (2023) Chemical composition and antioxidant activity of rhizome essential oil of *Hedychium griffithianum*. *Chemistry of Natural Compounds* 59: 568-570. DOI: 10.1007/s10600-023-04055-y
- Negrelle RRB (2015) *Renealmia* L.F.: aspectos botânicos, ecológicos, farmacológicos e agrônômicos. *Revista Brasileira de Plantas Mediciniais* 17: 274-290. DOI: 10.1590/1983-084X/13_049
- Noberto-Irmão V, Silva IV, Pessoa MJG & Rossi AB (2013) Anatomia foliar como ferramenta na identificação de *Curcuma zedoaria* (Zingiberaceae) utilizada medicinalmente e cultivada em quintais no município de Alta Floresta-MT. *Enciclopédia Biosfera* 9: 2669-2685.
- Noriega PF, Paredes EA, Mosqueira TD, Diaz EE, Lueckhoff A, Basantes JE & Trujillo AL (2016) Chemical composition antimicrobial and free radical scavenging activity of essential oil from leaves of *Renealmia thyrsoides* (Ruiz Pav.) Poepp. Endl. *Journal of Medicinal Plants Research* 10: 553-558. DOI: 10.5897/JMPR
- O'Brien TP, Feder N & McCully ME (1964) Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma* 59: 368-373. DOI: 10.1007/BF01248568
- Pugialli HRL (1991) Quimiotaxonomia da superordem Zingiberiflorae (*sensu* Dahlgren). Dissertação de Mestrado. Universidade Federal do Rio de Janeiro, Rio de Janeiro. 99p.
- Raut JS & Karuppayil SM (2014) A status review on the medicinal properties of essential oils. *Industrial Crops and Products* 62: 250-264. DOI: 10.1016/j.indcrop.2014.05.055
- Rengifo-Salgado E, Rios-Torres S, Malaveri LF & Vargas-Arana G (2017) Saberes ancestrales sobre el uso de flora y fauna en la comunidad indígena Tikuna de Cushillo Cocha, zona fronteriza Perú-Colombia-Brazil. *Revista Peruana de Biología* 24: 67-78. DOI: 10.15381/rpb.v24i1.13108
- Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electronmicroscopy. *Journal of Cell Biology* 17: 208-212. DOI: 10.1083/jcb.17.1.208
- Rice-Evans CA, Miller NJ & Paganga G (1996) Structure-antioxidant activity relations of flavonoids and phenolic acids. *Free Radical Biology and Medicine* 20: 933-956. DOI: 10.1016/0891-5849(95)02227-9
- Sapala A, Runions A & Smith RS (2018) Mechanics, geometry and genetics of epidermal cell shape regulation: different pieces of the same puzzle. *Current Opinion in Plant Biology* 47: 1-8. DOI: 10.1016/j.pbi.2018.07.017
- Sherlija KK, Remashree AB, Unnikrishnan K & Ravindran PN (1998) Comparative rhizome anatomy of four species of *Curcuma*. *Journal of Spices and Aromatic Crops* 7: 103-109.
- Silva CF, Petró RR, Almeida RN, Cassel E & Vargas RMF (2021) On the production and release of *Hedychium coronarium* essential oil from nanoformulations. *Industrial Crops and Products* 171: 113984.
- Souza ECA, Flach A & Costa LAMA (2023) Chemical composition and antioxidant activity of the essential oil of *Renealmia alpinia* (Rottb.) Maas. *Natural Product Research* 37: 4042-4048 DOI: 10.1080/14786419.2022.2164578
- Souza VC & Lorenzi H (2012) Botânica sistemática: guia ilustrado para identificação das famílias de Fanerógamas nativas e exóticas no Brasil, baseado em APG III. 3ª ed. Instituto Plantarum de Estudos da Flora, Nova Odessa. Pp. 203-204.
- Strittmatter CGD (1973) Nueva técnica de diafanización. *Boletín de la Sociedad Argentina de Botánica* 15: 126-129.
- Talip N, Hussin KH & Ibrahim H (2005) Comparative leaf anatomy of *Alpinia* species (Zingiberaceae) in Malaysia. *Nordic Journal of Botany* 23: 463-483. DOI: 10.1111/j.1756-1051.2003.tb00420.x
- Tang Y, Liao J & Wu Q (2010) Comparative anatomy of the leaves of *Amomum* (Zingiberaceae). *Subtropical Plant Science* 39: 38-43.
- Tomlinson PB (1956) Studies in the systematic anatomy of the Zingiberaceae. *Botanical Journal of the Linnean Society* 55: 547-592. DOI: 10.1111/j.1095-8339.1956.tb00023.x
- Tomlinson PB (1962) Phylogeny of the Scitamineae - morphological and anatomical considerations. *Evolution* 16: 192-213. DOI: 10.1111/j.1558-5646.1962.tb03211.x
- Tomlinson PB (1969) Classification of the Zingiberales (Scitamineae) with special reference to anatomical evidences. In: Metcalfe CR (ed.) *Anatomy of the monocotyledons*. Vol. 3. Clarendon Press, Oxford. Pp. 224-302.
- Ud-Daula AFMS, Demirci F, Abu Salim K, Demirci B, Lim LBL, Baser KHC & Ahmad N (2016) Chemical composition, antioxidant and antimicrobial activities of essential oils from leaves, aerial stems, basal stems, and rhizomes of *Etilingera fimbriobraceata* (K. Schum.) R.M.Sm. *Industrial Crops and Products*

- 84: 189-198. DOI: 10.1016/j.indcrop.2015.12.034
- Uma E & Muthukumar T (2014) Comparative root morphological anatomy of Zingiberaceae. *Systematics and Biodiversity* 12: 195-209. DOI: 10.1080/14772000.2014.894593
- Van Cotthem WRJ (1970) A classification of stomatal types. *Botanical Journal of the Linnean Society* 63: 235-246. DOI: 10.1111/j.1095-8339.1970.tb02321.x
- Vásquez J, Jiménez SL, Gómez IC, Rey JP, Henao AM, Marín DM, Romero JO & Alarcón JC (2013) Snake bites and ethnobotany in the Eastern of Antioquia, Colombia - the traditional use of plants. *Journal of Ethnopharmacology* 146: 449-455. DOI: 10.1016/j.jep.2012.12.043
- Zhao H, Xiao M, Zhong Y & Wang Y (2022) Leaf epidermal micromorphology of Zingiber (Zingiberaceae) from China and its systematic significance. *PhytoKeys* 190: 131-146. DOI: 10.3897/phytokeys.190.77526

Area Editor: Dr. João Paulo Basso-Alves

Received on August 30, 2023. Accepted on April 29, 2024.



This is an open-access article distributed under the terms of the Creative Commons Attribution License.