



ECOSYSTEMS

Leaf anatomy of seven endemic species of *Glicophyllum* (Malpighiaceae) in Brazil

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Abstract: *Glicophyllum* is well supported, presenting four homoplasies, easily differentiated from the other genera of the clade due to characteristics related to the reproductive organs, which can make the identification of their species difficult when they are not in the reproductive phase. Therefore, there are provided the leaf anatomical and morphological description of the leaf glands of seven species of *Glicophyllum* to assist in the identification of their species. The samples for the study were obtained from several national and international herbaria, sectioned freehand, stained with basic fuchsin – astra blue and compared through a binary matrix using the Sorensen's coefficient in the MVSP software. Among the leaf anatomical characters found, the following stand out: presence/absence of trichomes; petiole contour; contour of the main vein; organization of the mesophyll, presence/absence of bundle sheath extension and the surface of the glands. In this study, an identification key with leaf anatomical data is presented for the first time, demonstrating the applicability of leaf anatomy for the taxonomy of *Glicophyllum*. In the multivariate analysis, it is observed that the characteristics of leaf venation and topology of the glands are more representative to differentiate the taxa. Therefore, the data obtained can support future taxonomic and phylogenetic studies of the genus.

Key words: anatomical description, leaf glands, taxonomy, Tetrapteroids.

INTRODUCTION

Several studies with molecular phylogenetic approaches were conducted in Malpighiaceae (Cameron et al. 2001, Davis et al. 2001, 2020, Davis & Anderson 2010, Almeida & van den Berg 2021). However, many of its genera were not considered monophyletic, among them *Tetrapteryx* Cav. (Davis & Anderson 2010). The *Tetrapteryx* species were distributed in two well supported subclades: the first subclade (*Tetrapteryx* s.s.) sister group of *Heteropteryx* Kunth with species mainly vines, found on the edge of forests; the second subclade (*Tetrapteryx* p.p.) sister group of *Niedenzuella* W. R. Anderson, represented by species of shrubs and subshrubs

found especially in open environments, such as the Cerrado (Davis & Anderson 2010, Francener et al. 2015). The two subclades belong to the Tetrapteroid Clade and may not be closely related due to morphological differences between species that have been assigned to the genus *Tetrapteryx* (Davis & Anderson 2010, Francener et al. 2015).

In order to solve the taxonomic problems of Malpighiaceae, new genera and combinations were proposed for example in Anderson (2006) and Anderson & Davis (2007). Almeida & van den Berg (2021) also proposed a new genus of the family, they performed the reconstruction of the molecular phylogeny of Tetrapteroids based on molecular and morphological data,

recovering the clade as monophyletic, along with all its groups. The new genus proposed by these authors was *Glicophyllum* R.F.Almeida comprising 27 spp., to accommodate the species of *Tetrapterys* p.p. *Glicophyllum* species are found in evergreen and seasonally dry forests and savannas of Central and South America (Almeida & van den Berg 2021).

Glicophyllum is well supported, presenting four homoplasies (inflorescences and bracteoles, expanded into a leaf-like structure with an elliptical shape; presence of glands at the base of inflorescences, bracts and bracteoles; absence of wings between dorsal and lateral wings in mericarp), easily distinguished from the other genera of the clade. Its species were segregated from *Tetrapterys* for presenting pseudoracemic inflorescences or rarely umbels; bracts and bracteoles expanded, with a pair of glands near the base; divergent slender stylets, with apexes usually curved inwards and mericarps usually with (2 -) 4 lateral wings of equal size (Almeida & van den Berg 2021). These characteristics together with the molecular data indicate the monophyly of *Glicophyllum*. When *Glicophyllum* species are not in the reproductive stage, identification may be difficult, since most of the characters used for recognition of these taxa are related to reproductive organs. Therefore, leaf anatomy can be useful for the identification of these species. Pace et al. (2019) demonstrate that the main character distinguishing *Tetrapterys* s.s. from *Glicophyllum* is the absence of laticifers in the latter.

Recently, Vilarinho et al. (2023) have showed that the outline of the petiole and main vein, type of mesophyll and extension of the bundle sheath are useful for the taxonomy of the Stigmaphylloid clade, a group close to the Tetrapteroid clade. These authors also point out characters that support the segregation of *Bronwenia* and *Diplopterys* from *Banisteriopsis*.

The relevance of anatomy for taxonomy is also pointed out in genera close to *Glicophyllum* as in *Heteropterys* HBK by Araújo et al. (2010), *Amorimia* W.R. Anderson by Mello et al. (2019) and *Banisteriopsis* C.B Rob ex Small by Araújo et al. (2010), Araújo et al. (2020).

The applicability of leaf anatomy for taxonomy is also seen in other genera such as *Byrsonima* Rich. ex Kunth, *Glandonia* Griseb., *Barnebya* W.R. and *Camarea* A. St-Hil (Araújo et al. 2010, Silva et al. 2011, Guesdon et al. 2018, Câmara et al. 2020, Santos et al. 2020, Matos et al. 2022). In general, these authors point out the relevance of the outline of the petiole and main vein, accessory vascular bundles in the petiole, trichomes, mesophyll type and sheath extension of the bundle for the delimitation of species. Confirming the relevance of plant anatomy as a subsidy for the taxonomy of species and genera of Malpighiaceae.

Thereby, considering the scarcity of anatomical studies of *Glicophyllum*, this study provides the leaf anatomical and morphological description of the leaf glands of seven species of *Glicophyllum* endemic to Brazil, in order to assist in the identification of their representatives and provide new data on the genus.

MATERIALS AND METHODS

The seven species of *Glicophyllum* analyzed (Table I) were obtained from exsiccates from some national and international herbaria. Data related to the number of species and individuals analyzed are described in Table I. Due to the unavailability of material, it was not possible to analyze more than one sample of some species.

The samples underwent the herborization reversal process based on the method of Smith & Smith (1942) (modified), which consists of boiling the material in distilled water until it submerges (on average 5 minutes), never letting

Table I. Species and vouchers analyzed.

Species	Identification number/ Collector	Collection location
<i>Glicophyllum chamaecerasifolium</i> (A.Juss.) R.F.Almeida	NMNH 7548/ Williams, L. O; Assis, V. SP 346366/Pompeu, M.	Morro das Pedras, Belo Horizonte, Minas Gerais, Brazil. Copasa Reserve - Mutuca, Belo Horizonte, Minas Gerais, Brazil.
<i>Glicophyllum humile</i> (A.Juss.) R.F.Almeida	US 1055675/Dusén,P. K.	St. Theresa. Rio de Janeiro, Brazil
<i>Glicophyllum jussieuanum</i> (A.Juss.) R.F.Almeida	US 1055675/Souza Silva, S; Pereira, JDA; Dambrós, LA	Caiapó river basin, Caiapônia, Goiás, Brazil.
<i>Glicophyllum microphyllum</i> (A.Juss.) R.F.Almeida	US 2752569/H. S. Irwin, R. R. Santos, R. Souza & R. Souza	15 km west of Grão Mogol. Minas Gerais, Brazil.
<i>Glicophyllum paludosum</i> (A.Juss.) R.F.Almeida	US 2771064/ R. M. Harley; Renvoize, S. A.; Erskine, C. M.; Brighton, C. A.; Pinheiro, R. SP 370680/Sasaki, D; Pavan, D	Monte Santo, Bahia, Brazil. Furnas do Bom Jesus State Park, close to the headquarters, below the dirt road, Pedregulho, São Paulo, Brazil.
<i>Glicophyllum salicifolium</i> (A.Juss.) R.F.Almeida	US 1199449/Dusén,P. K. UNOP 7901/Silva, J.M.; Poliquesi, C.B.; Maranhão, L.T.; Ristow, R.	Paraná, Brazil. Vila Velha State Park, Ponta Grossa, Paraná, Brazil.
<i>Glicophyllum turnerae</i> (A.Juss.) R.F.Almeida	SP 378764/Pott, A; Pott, V.J.	Terena Village, Córrego Seco, Aquidauana, Mato Grosso do Sul, Brazil.

it boil for more than fifteen minutes. After reaching room temperature for a period of 24 hours, they were immersed in a 2% KOH solution (an average 2 hours) and washed every twenty minutes for four times. Soon after, the leaves were dehydrated in an ethanol series and stored in 70% ethanol.

The leaves were analyzed for the presence/absence of glands, later these structures were morphologically examined using a stereoscopic microscope with a camera coupled with a Scopelmage 9.0 system. The classification of glands was based on Araújo & Meira (2016) and Araújo et al. (2020).

Freehand cross-sections were made with the aid of a razor blade, from the middle region of the leaf blade and petiole, then the material was clarified in 50% sodium hypochlorite,

stained with basic fuchsin-astra blue, mounted in glycerin gelatin and struggled with colorless varnish (Roeser 1972, Kaiser 1880, Kraus & Arduin 1997). The trichomes were analyzed based on the literature from Metcalfe & Chalk (1979).

Part of the samples were submitted to the clearing process based on the Foster (1950) technique (modified). The regions of the apex, middle, base and close to the margin of the leaf were analyzed. The selected material was kept in a 10% sodium hydroxide solution for 2 hours, washed in distilled water four times, and then placed in 50% sodium hypochlorite until the samples became clear. Then, the material was washed in distilled water four times, dehydrated in an ethanol series of 50% ethanol every twenty-minute, stained with alcoholic fuchsin diluted in 50% ethanol. The slides were mounted

in glycerin gelatin and sealed with nail varnish. Subsequently, they were analyzed based on the literature by Ellis et al. (2009).

After mounting the slides, the material was analyzed and compared through a binary matrix (Table II) using the Sorensen's coefficient in the MVSP software. The photographic records were performed in a *Coleman* photomicroscope with U-Photo system and the anatomical plates were mounted in the *CorelDraw 22* program.

Due to the unavailability of material, it was not possible to describe the petiole anatomy and the venation pattern of *Glicophyllum humile*

(A.Juss.) R.F.Almeida and *Glicophyllum turnerae* (A.Juss.) R.F.Almeida.

RESULTS

Leaf glands

External leaf glands are present in most species, varying in location: Present in the proximal region on the petiole (Fig. 1a) of *Glicophyllum chamaecerasifolium*; at the base of the leaf blade (Fig. 1b) of *G. chamaecerasifolium*, *G. microphyllum*, *G. paludosum* and *G. salicifolium*; at the margin of the median region of the leaf

Table II. Binary matrix of leaf anatomical characters of seven species of *Glicophyllum*.

Evaluated characteristics	Gcha	Ghum	Gjus	Gmic	Gpal	Gsal	Gtur
Outer leaf glands on the petiole	1	-	0	0	0	0	-
Outer leaf glands at the base of the leaf blade	1	0	0	1	1	1	0
Outer leaf glands at the edge of the leaf blade	1	0	0	0	0	0	1
External leaf glands dispersed in the leaf blade	0	0	1	1	1	0	0
Leaf glands with convex surface	1	0	0	0	1	1	0
Leaf glands with concave surface	1	0	1	1	1	0	0
Leaf glands with a flat surface	0	0	0	1	0	0	1
Malpighiaceae trichomes on petiole	1	-	0	1	1	1	-
Convex flat petiole outline	1	-	0	1	0	1	-
Concave convex petiole outline	0	-	1	0	1	0	-
Convex plane midrib contour	0	0	0	1	0	1	0
Biconvex midrib outline	1	1	1	0	1	0	1
Malpighiaceae trichomes on the leaf blade	1	1	0	1	1	1	1
Isobilateral mesophyll	0	1	0	1	1	1	1
Dorsiventral mesophyll	1	0	1	0	0	0	0
Sheath extension	1	1	1	1	0	1	1
Inconsistent angle variation between primary and secondary veins	0	-	1	0	1	0	-
Uniform angle variation between the primary and secondary veins	1	-	0	1	0	0	-
Angle variation between primary and secondary veins decreases smoothly towards apex and base	0	-	0	0	0	1	-
Quaternary veins	0	-	1	0	1	1	-
Quinternary veins	0	-	1	0	1	1	-
Venules that terminate freely	0	-	1	0	0	0	-

Caption: (0) absent; (1) present. Abbreviations: Gcha - *Glicophyllum chamaecerasifolium*; Ghum - *Glicophyllum humile*; Gjus - *Glicophyllum jussieuanum*; Gmic - *Glicophyllum microphyllum*; Gpal - *Glicophyllum paludosum*; Gsal - *Glicophyllum salicifolium*; Gtur - *Glicophyllum turnerae*.

blade (Fig. 1c) of *G. chamaecerasifolium* and *G. turnerae* and dispersed in the leaf blade (Fig. 1d) of *G. jussieuanum*, *G. microphyllum* and *G. paludosum*. Regarding the morphological pattern of the glands, in all species these structures are rounded and sessile (Fig. 1a-f). However, three surface variations are observed: Convex glands (Fig. 1e) in *Glicophyllum chamaecerasifolium*, *G. paludosum*, *G. salicifolium* and *G. turnerae*; flat (Fig. 1f) in *G. microphyllum* and *G. turnerae* and concave (Fig. 1a-c) in *G. chamaecerasifolium*, *G. jussieuanum*, *G. microphyllum* and *G. paludosum*. External leaf glands are absent in *G. humile*.

Anatomical description of the petiole

The species present two types of petiole contours, the convex plane (Fig. 2a) is observed in *Glicophyllum chamaecerasifolium*, *G. microphyllum* and *G. salicifolium* and the concave convex (Fig. 2b) is observed in *G.*

jussieuanum and *G. paludosum*. Malpighiaceae trichomes are present in most of the analyzed species. In *G. chamaecerasifolium*, morphotypes T (Fig. 2c), V (Fig. 2d) and Y (Fig. 2e) are observed. *G. paludosum* has T and V, while *G. salicifolium* and *G. microphyllum* have T and Y types. Only *G. jussieuanum* is glabrous.

All species have unistratified epidermis (Fig. 2f-g) and annular collenchyma (Fig. 2g). Accessory bundles are absent in all species. The conformation of the vascular system is of the open arch type (Fig. 2a-b) in all taxa. Druses (Fig. 3a) are present dispersed in the parenchyma tissue of the cortex in *G. chamaecerasifolium*, *G. jussieuanum* and *G. paludosum* while in *G. microphyllum* and *G. salicifolium* they are absent. Prismatic crystals (Fig. 3a) are present dispersed in parenchymatic and collenchymatic tissues of the cortex in *G. jussieuanum* and *G. paludosum*,

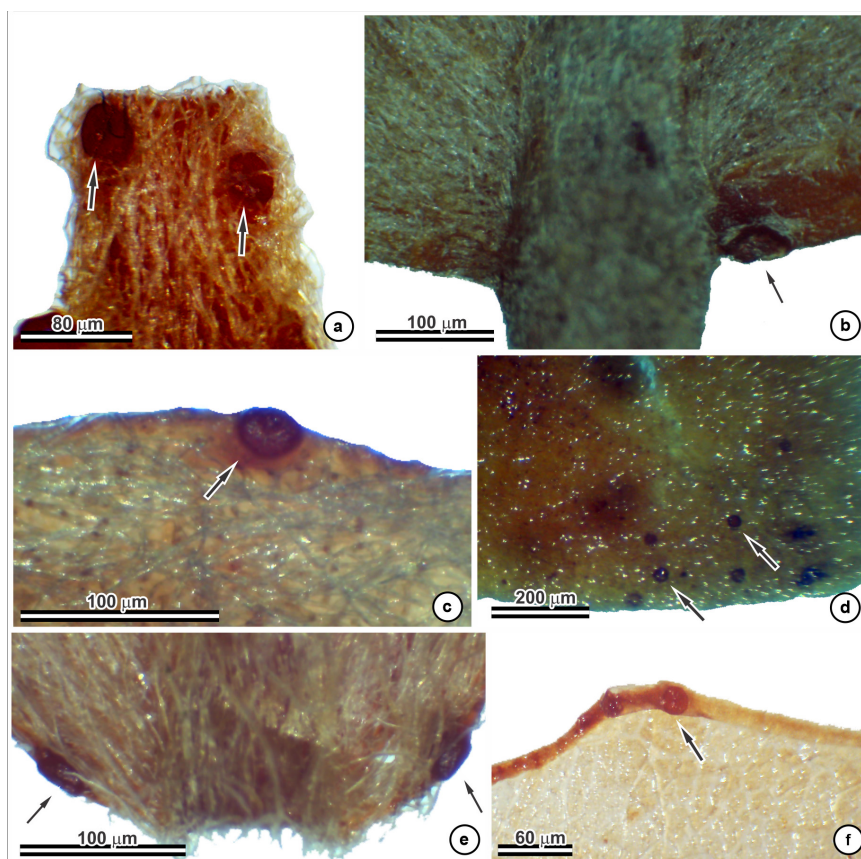


Figure 1. Location and morphology of leaf glands.
 a. Glands on the petiole of *Glicophyllum chamaecerasifolium*; b. Glands at the base of the leaf blade in *Glicophyllum microphyllum*; c. Glands at the edge of the leaf blade in *Glicophyllum chamaecerasifolium*; d. Dispersed leaf glands in the leaf blade in *G. microphyllum*. e. Glands with convex surfaces in *Glicophyllum salicifolium*; f. Flat-surfaced glands in *Glicophyllum turnerae*. Arrows = Glands.

and absent in *G. chamaecerasifolium*, *G. microphyllum* and *G. salicifolium*.

Anatomical description of the leaf blade

The species studied present two types of midrib contour: biconvex (Fig. 3b) in *Glicophyllum chamaecerasifolium*, *G. humile*, *G. jussieuanum*, *G. paludosum* and *G. turnerae*; plane-convex (Fig. 3c) in *G. microphyllum* and *G. salicifolium*. Malpighiaceaceous trichomes with T (Fig. 2c) and V (Fig. 2d) morphotype are observed in *G. paludosum*. On the other hand, *G. humile* and *G. microphyllum* present T (Fig. 2c) and Y (Fig. 2e),

while *G. salicifolium* and *G. turnerae* only present the T morphotype. Only *G. chamaecerasifolium* has the T, V and Y morphotypes, concomitantly. Malpighiaceaceous trichomes are absent in *G. jussieuanum*. The cuticle is thin (Fig. 3b) on the abaxial and adaxial faces in *Glicophyllum chamaecerasifolium*, *G. humile* and *G. jussieuanum*, while *G. microphyllum*, *G. paludosum*, *G. salicifolium* and *G. turnerae* is thick (Fig. 3c) in both faces.

In all species the epidermis is unistratified (Fig. 3b-3c). The epidermal cells of the midrib are smaller compared to those of the adaxial

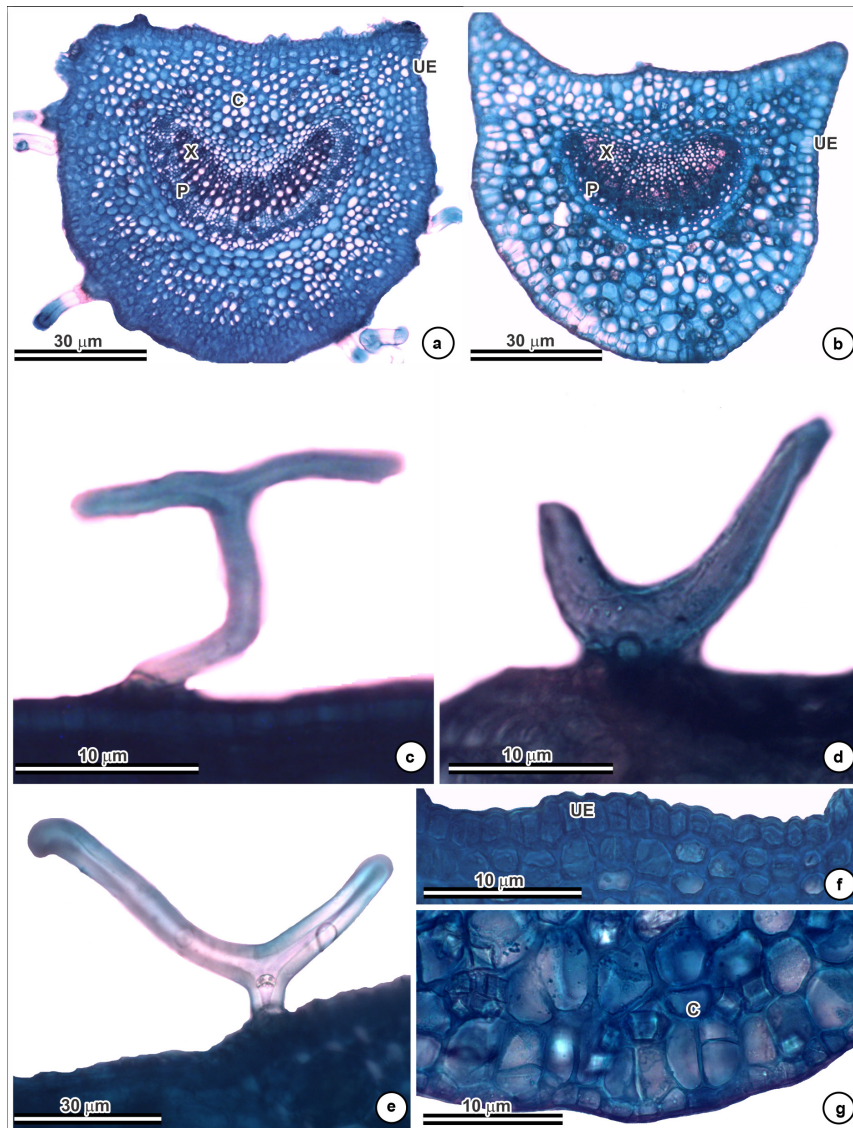


Figure 2. Cross sections of the petiole.

a. Convex plane petiole contour in *Glicophyllum chamaecerasifolium*; b. Convex concave petiole contour in *Glicophyllum jussieuanum*; c. T-shaped trichome on *Glicophyllum chamaecerasifolium*; d. “V” shaped trichome on *Glicophyllum paludosum*; e. “Y”-shaped trichome on *Glicophyllum chamaecerasifolium*; f. Thin cuticle in *Glicophyllum chamaecerasifolium*; g. Thick cuticle and annular collenchyma in *Glicophyllum jussieuanum*. Abbreviations: C = Annular Collenchyma; UE = Epidermis; P = Phloem; X = xylem.

surface of the mesophyll (Fig. 3d-3e), on the adaxial and abaxial sides of the mesophyll, the cells are the same size. Below the epidermis of the midrib region, the presence of angular collenchyma (Fig. 3f) is observed in *Glicophyllum chamaecerasifolium*, *G. salicifolium*, *G. paludosum* and *G. turnerae* and the annular collenchyma (Fig. 3d) is observed in *G. humile*, *G. jussieuanum* and *G. microphyllum*. The conformation of the open-arch vascular system (Fig. 3b-c) is common for all species sampled. Sclerenchymatic tissue (Fig. 3c-d) is present close to the vascular bundle of *G. jussieuanum*, *G. microphyllum*, *G. paludosum* and *G. salicifolium*, being absent in *G. chamaecerasifolium*, *G. humile* and *G. turnerae* (Fig. 3b).

Prismatic crystals (Fig. 4a) are present in the mesophyll and midrib in *Glicophyllum humile* and *G. jussieuanum*; only in the mesophyll in *G. chamaecerasifolium*, *G. paludosum* and *G.*

turnerae and absent in *G. microphyllum* and *G. salicifolium*. Druses (Fig. 4b) are present in the mesophyll and midrib in *G. chamaecerasifolium*, *G. paludosum* and *G. turnerae*; only in the midrib in *G. jussieuanum*, and only in the mesophyll in *G. salicifolium*. In *G. humile* and *G. microphyllum* druses are absent.

In cross-section, the mesophyll epidermis is unistratified with cells larger on the adaxial surface than on the abaxial surface in all species (Fig. 4c). Regarding the location of the stomata, all species are hypostomatic. The stomata are at the same level as the epidermal cells (Fig. 4d) in all proven species. Stomatal ridges are absent in all species sampled.

The mesophyll is isobilateral (Fig. 4e) in *Glicophyllum humile*, *G. microphyllum*, *G. paludosum*, *G. salicifolium* and *G. turnerae*, and dorsiventral (Fig. 4c) in *G. chamaecerasifolium* and *G. jussieuanum*. Parenchymal sheath extension

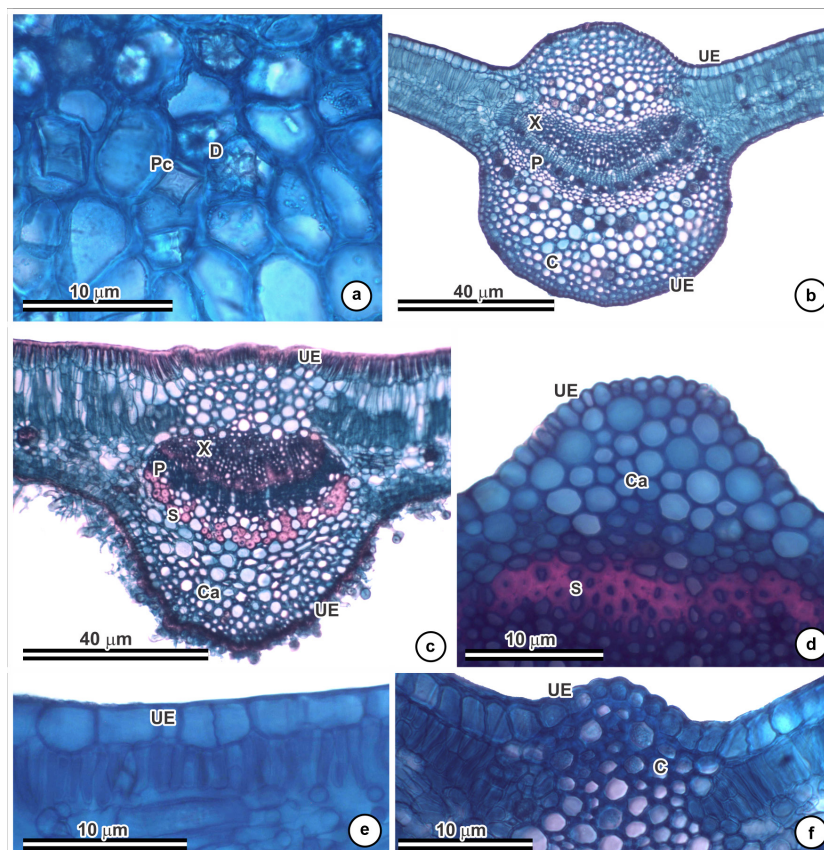


Figure 3. Cross sections of the leaf blade.

a. Druses and prismatic crystals in *Glicophyllum jussieuanum*. b. Contour of the biconvex midrib and conformation of the vascular system of the open-arch midrib in *Glicophyllum turnerae*; c. Convex plane midrib contour and vascular system conformation of the open arch midrib in *Glicophyllum microphyllum*; d. Adaxial epidermis of the mesophyll of *Glicophyllum jussieuanum*; e. Adaxial epidermis of midrib of *Glicophyllum chamaecerasifolium*; f. Angular collenchyma in *Glicophyllum chamaecerasifolium* g. Prismatic crystal in the mesophyll of *Glicophyllum humile*; h. Drusen in the mesophyll of *Glicophyllum chamaecerasifolium*. Abbreviations: C = Angular Collenchyma; Ca = Annular Collenchyma; D = Druse; Pc = Cristal prismatic; S = Sclerenchyma; UE = Epidermis; P = Phloem; X = xylem.

(Fig. 4e) is observed in *G. chamaecerasifolium*, *G. humile*, *G. jussieuanum*, *G. microphyllum*, *G. salicifolium* and *G. turnerae*, being absent only in *G. paludosum*.

Leaf venation

The venation pattern in *Glicophyllum chamaecerasifolium*, *G. jussieuanum*, *G. microphyllum*, *G. paludosum* and *G. salicifolium* is of the camptodromous and brochidodromous pinnate type (Fig. 5a). They do not have basal veins and have secondary veins that do not reach the margin and do not branch, but form loops of smaller caliber (Fig. 5b). In *Glicophyllum chamaecerasifolium* and *G. microphyllum* the variation in the angle between the primary and secondary veins is uniform (Fig. 5c), in *G. jussieuanum* and *G. paludosum* the variation is inconsistent (Fig. 5d). In *G. salicifolium*, the variation of the angle between the primary and secondary veins decreases smoothly towards the apex and the base (Fig. 5a). The development of the secondary vein in relation to the primary one is decurrent (Fig. 5d) in all species.

Perimarginal fimbrial veins (Fig. 5d) are present in most species, apart from *G. chamaecerasifolium* and *G. microphyllum* that do not have perimarginal veins (Fig. 5c). In *G. jussieuanum*, free-terminated venules are observed (Fig. 5e), in the other evaluated species, free-terminated venules are absent (Fig. 5d). The marginal terminal venation of all species forms loops with smaller gauge arches (Fig. 5b). Quaternary and quinary veins are not observed in *Glicophyllum chamaecerasifolium* and *G. microphyllum* (Fig. 5f), the other species have veins up to the fifth order (Fig. 5d). The areolation of *G. jussieuanum*, *G. salicifolium* and *G. paludosum* has moderate development (Fig. 5b) and in *G. chamaecerasifolium* and *G. microphyllum* there is no formation of areolas (Fig. 5c, f).

Identification key of seven *Glicophyllum* endemic species to Brazil

- 1a. Dorsiventral mesophyll (Fig. 4c)2
- 1b. Isobilateral mesophyll (Fig. 4e)3
- 2a. Trichomes present on the leaf (Fig.2c-2e); contour of the petiole plane-convex (Fig.2a);

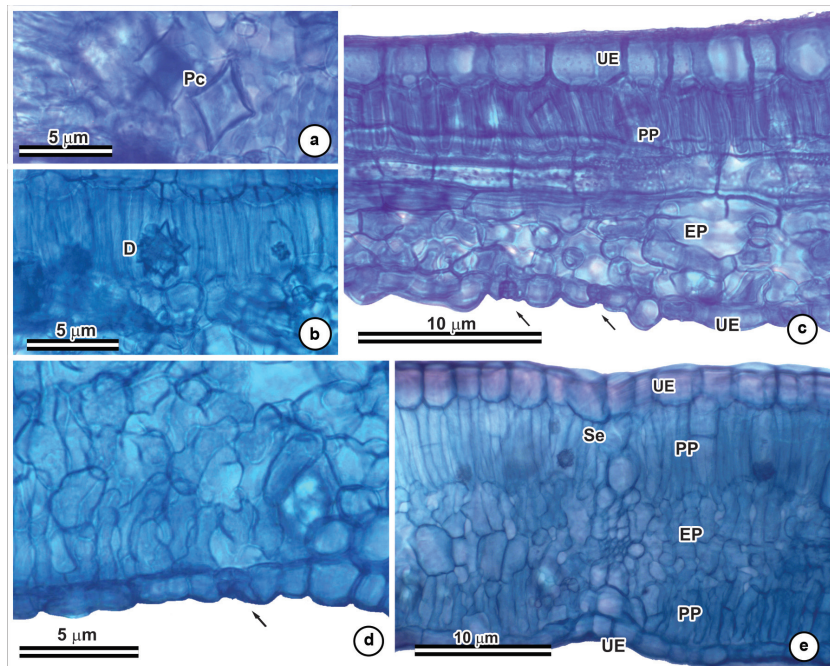


Figure 4. Cross sections of the mesophyll.
 a. Prismatic crystal in the mesophyll of *Glicophyllum humile*;
 b. Drusen in the mesophyll of *Glicophyllum chamaecerasifolium*;
 c. Dorsiventral mesophyll and stomata below the level of epidermal cells in *Glicophyllum jussieuanum*; d. Stomata at the same level as the epidermal cells in *Glicophyllum turnerae*;
 e. Isobilateral mesophyll *Glicophyllum turnerae*.
 Abbreviations: D = Druse; UE = Unistratified epidermis; Se = Sheath extension; EP = spongy parenchyma; Pc = Cristal prismatic; PP = Palisade parenchyma; Arrow = Stomata.

external leaf glands present on the petiole (Fig. 1a).....*Glicophyllum chamaecerasifolium*
 2b. Trichomes absent on the leaf; contour of the petiole concave-convex (Fig. 2b) o; external leaf glands absent on petiole.....*Glicophyllum jussieuanum*
 3a. External leaf glands absent on leaf blade.....*Glicophyllum humile*
 3b. External leaf glands present on the leaf blade (Fig. 1b-1d).....4
 4a. Biconvex midrib contour (Fig. 3b).....5
 4b. Convex plane midrib contour (Fig. 3c)6

5a. Sheath extension present in the vascular bundles of the mesophyll (Fig. 4e)..*Glicophyllum turnerae*
 5b. Sheath extension absent in mesophyll vascular bundles.....*Glicophyllum paludosum*
 6a. Glands with convex surface (Fig. 1e)
*Glicophyllum salicifolium*
 6b. Glands with a flat surface (Fig. 1f)
*Glicophyllum microphyllum*

Multivariate analysis

The similarity analysis (Figure 6) organizes the seven species of *Glicophyllum* into 3 groups. The first group is composed of *G. humile* and *G. turnerae*, which have the highest degree of

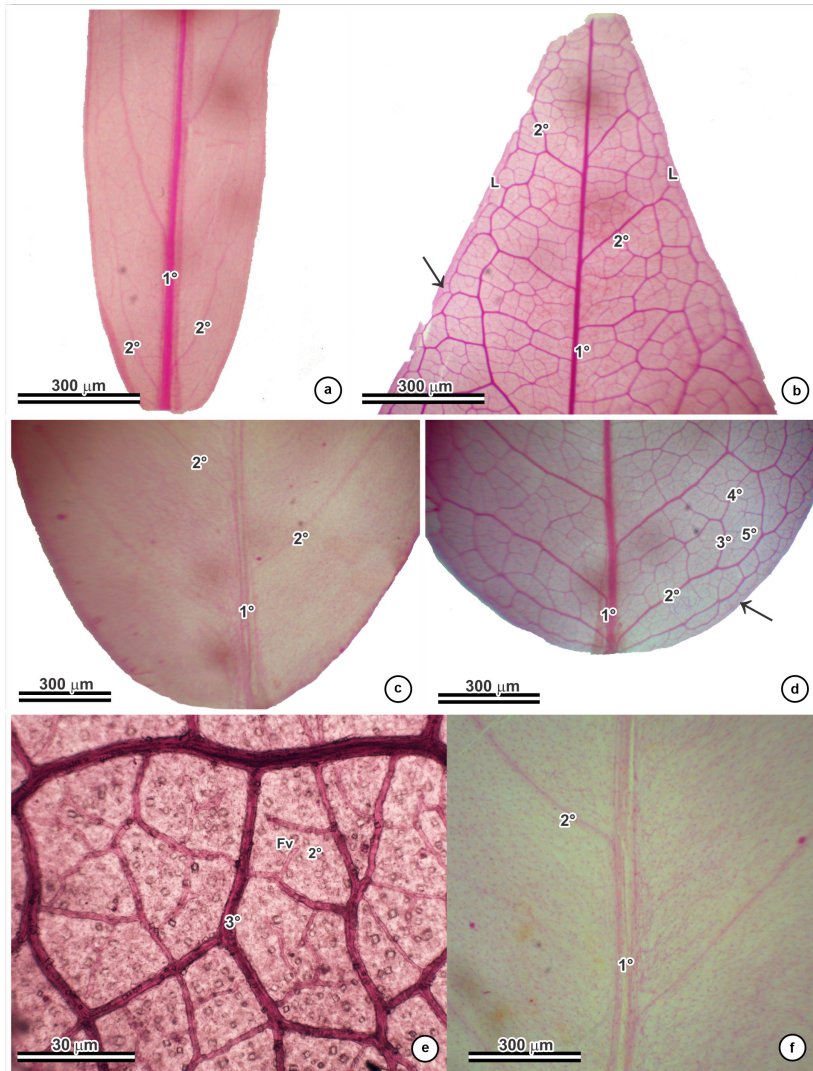


Figure 5. Leaf venation.
 a. Primary nerve in *Glicophyllum salicifolium*; b. Secondary veins in *Glicophyllum jussieuanum*; c. Uniform angle variation between primary and secondary veins in *Glicophyllum chamaecerasifolium*; d. Angle variation between primary and secondary vein inconsistent in *Glicophyllum paludosum*; e. Free-terminated venules in *Glicophyllum jussieuanum* f. Areolation in *Glicophyllum chamaecerasifolium*. Abbreviations: L = “Loops”; Fv = Free terminating veins; 1° = Primary vein; 2° = Secondary vein; 3° = Tertiary vein; 4° = Quaternary vein; 5° Quinternary vein; Arrow = fimbrial perimarginal vein.

similarity between the species because they have the highest number of characteristics in common. The second group is formed by *G. paludosum* and *G. jussieuanum* that share convex-concave petiole contour, inconsistent angle variation between the primary and secondary veins, quaternary and quinary veins present. The third group is composed of *G. chamaecerasifolium*, *G. microphyllum* and *G. salicifolium*, which are related because they have a convex flat petiole contour. Within this group, there can be highlighted a greater proximity between *G. chamaecerasifolium* and *G. microphyllum* for sharing characters such as: concave surface glands, uniform angle variation between the primary and secondary veins, quaternary and quinary veins and absent fimbrial perimarginal veins. On the other hand, the species *G. salicifolium* is found in a single branch because it presents the variation of the angle between the primary and secondary veins, which decreases smoothly towards the apex and the base.

DISCUSSION

A recent phylogenetic approach based on molecular and morphological data on the Clade Tetrapteroids has resulted in the recognition of *Glicophyllum* as a well-supported monophyletic genus of Malpighiaceae (Almeida & van den Berg 2021). Moreover, useful characters are identified

to recognize their representatives, providing support for future works on the genus.

The external leaf glands are reported in other genera of Malpighiaceae, being considered useful for the taxonomy of these taxa. Araújo et al. (2020) use morphology and presence/absence to identify *Banisteriopsis* species; Guesdon et al. (2018) the location of leaf glands to distinguish *Glandonia* species; Câmara et al. (2020) with *Camarea* and Matos & Araújo (2021) with *Stigmaphyllon* point out that the morphology is taxonomically relevant for the studied taxa. Furthermore, Guimarães et al. (2016) point out that the shape, number, and disposition of the glands can be diagnostic. These data are corroborated by the present study. where the presence/absence, location and morphological pattern contribute to the identification of the *Glicophyllum* species sampled.

Trichomes stand out in studies of anatomy applied to the taxonomy of Malpighiaceae, due to the variety of morphotypes observed among different genera, and in general this is a character considered useful to help distinguish species, as seen in Araújo et al. (2010) with *Banisteriopsis*, *Byrsonima* and *Heteropterys*; Guimarães et al. (2016) with *Stigmaphyllon*; Araújo et al. (2020) with *Banisteriopsis*; Lima et al. (2021) with *Dinemagonum*, *Dinemandra*, *Lasiocarpus* and *Ptilochaeta*. The trichomes are also significant to distinguish the *Glicophyllum* species evaluated, confirming the relevance taxonomic of these

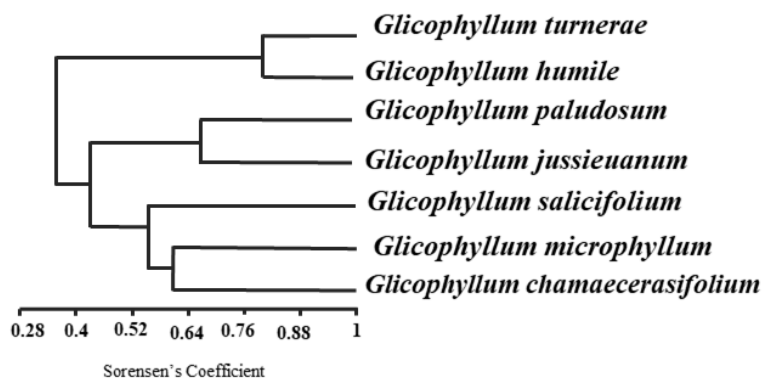


Figure 6. Similarity analysis of the seven species of *Glicophyllum*.

structures to the family. According to Metcalfe & Chalk (1979) the size and quantity of these structures can vary in response to environmental conditions, however the presence/absence and type of trichomes are useful to identify species. In the species of *Glicophyllum* analyzed, the presence of three trichome morphotypes was identified (T, V and Y). The observed distribution of these morphotypes varies between taxa, but there was a predominance of the T morphotype. The morphotypes are considered diagnostic for Malpighiaceae and are observed in genera already studied of the family, such as *Amorimia*, *Banisteriopsis* and *Heteropterys* (Metcalfe & Chalk 1979, Araújo et al. 2010, Mello et al. 2019).

Metcalfe & Chalk (1957) emphasize that the anatomical characters of the petiole are relevant to the taxonomy of species because they are not influenced by environmental factors. This relevance is also pointed out by several authors such as Guesdon et al. (2018) with *Glandoniai*; Mello et al. (2019) with *Amorimia* and Araújo et al. (2020) with *Banisteriopsis* who use the conformation of the petiole vascular system to distinguish the studied taxa. The *Glicophyllum* species analyzed have an open arch vascular system, which does not allow them to be distinguished. We point out here the relevance of this character to support future studies of the genre. This is the most common pattern in Malpighiaceae taxa, as observed in *Byrsonima*, *Camarea* and *Stigmaphyllon* (Guimarães et al. 2016, Câmara et al. 2020, Santos et al. 2020).

Accessory bundles are highlighted in studies of Malpighiaceae, as they allow the distinction of species in different genera, as seen in Araújo et al. (2010) who consider the presence and number of accessory bundles useful to distinguish sixteen species of Malpighiaceae and Santos et al. (2020) with *Byrsonima* that show the taxonomic value of the number of accessory bundles for the studied group. Although this character is

important and variable within the family, which makes it possible to distinguish species, there was no variation for specific *Glicophyllum* taxa, therefore, it does not help in the identification of the taxa under study.

Araújo et al. (2020) use the patterns of the midrib contour to determine species of *Banisteriopsis* and Lima et al. (2021) highlight the importance of this character for the description of taxa of Malpighiaceae. In the *Glicophyllum* species, the observed midrib contour patterns are significant and provide subsidies to differentiate them. Thus, we corroborate with the studies mentioned above that evidence the importance of the rib contour for the taxonomy of Malpighiaceae.

The stratification of epidermal cells in some regions of the leaf blade is pointed out as a relevant characteristic to distinguish Malpighiaceae species, as shown in the studies by de Araújo et al. (2020) with *Banisteriopsis*, de Santos et al. (2020) with *Byrsonima* and de Araújo et al. (2010) with *Banisteriopsis*, *Byrsonima* and *Heteropterys*. The *Glicophyllum* species presented have the same number of stratifications in the epidermal cells of the leaf blade, therefore this characteristic does not provide support for their identification.

Another relevant character in the taxonomy of several genera of the family is the conformation of the vascular system of the midrib, as in *Amorimia*, *Banisteriopsis*, *Byrsonima*, *Heteropterys*, *Diacidia* and *Stigmaphyllon* (Araújo et al. 2010, Guimarães et al. 2016, Mello et al. 2019, Santos et al. 2020). Although this character has been used to distinguish species from different genera of Malpighiaceae, in the analyzed species there was no variation. We emphasize here the importance of future studies to determine whether this character is common in other species of *Glicophyllum* and, thus, confirm its usefulness to assist in

the identification of the genus. However, the vascular bundle of the midrib in an open arch is highlighted in Malpighiaceae, according to Metcalfe & Chalk (1957) this character state is representative for the family.

Lima et al. (2021) mention that the organization of the mesophyll is significant for the taxonomy of Malpighiaceae, corroborating Araújo et al. (2020) who point out the importance of this character for *Banisteriopsis* and with Araújo et al. (2010) who use mesophyll organization to distinguish species of *Heteropterys*, *Banisteriopsis* and *Byrsonima*. Although this character is considered influenced by the environment, given the application of the mesophyll type to the taxonomy of species and genera in Malpighiaceae, we point out its relevance for the distinction of *Glicophyllum* species.

The sheath extension of the mesophyll vascular bundles in *Glycophyllum* it is shown significant for the distinction of its representatives, since most species have this characteristic. Other authors also point out the taxonomic importance of this character for Malpighiaceae, as seen in Araújo et al. (2010) with *Banisteriopsis*, *Byrsonima* and *Heteropterys*; Araújo et al. (2020) with *Banisteriopsis*; Santos et al. (2020) with *Byrsonima* and Lima et al. (2021) with *Dinemagonum*, *Dinemandra*, *Lasiocarpus* and *Ptilochaeta*.

The venation pattern shared between species is often found in other genera of Malpighiaceae, such as *Amorimia*, *Camarea*, *Dinemagonum*, *Dinemandra*, *Lasiocarpus* and *Ptilochaeta* (Mamede 1993, Mello et al. 2019, Lima et al. 2021). The absence of quaternary and quinternary veins is also reported for species of *Banisteriopsis* by Araújo et al. (2020). For the *Glicophyllum* representatives evaluated, the variation of the angle between the primary and secondary veins, the patterns

of the perimarginal veins and the presence of free-terminated veins can be considered as taxonomically useful characters, corroborating other studies of Malpighiaceae that show the importance taxonomy of characteristics related to leaf venation, as in Araújo et al. (2010), Santos et al. (2020) and Lima et al. (2021).

Based on the similarity analysis, group one (*Glicophyllum humile* and *Glicophyllum turnerae*) is distinguished from the other groups mainly by the characters related to the leaf glands. Therefore, leaf glands can provide important data for the identification of *Glicophyllum* species, confirming the taxonomic relevance of these structures as mentioned by Araújo et al. (2020). The characters contour of the petiole, contour of the midrib, variation of the angle between the primary and secondary veins, presence/absence of quaternary, quinternary and perimarginal veins justify the distinction between groups two (*G. jussieuanum* and *G. paludosum*) and three (*G. chamaecerasifolium* and *G. microphyllum*). Therefore, the leaf anatomical characters can help future studies of the genus. The species *Glicophyllum salicifolium* stands out for having the lowest degree of similarity among the seven species evaluated, which can be attributed to the presence of an exclusive characteristic in the pattern of leaf venation. This fact confirms the taxonomic value of leaf venation characters as mentioned by Mello et al. (2019), in addition, demonstrates the potential applicability of these characters for the identification of *Glicophyllum* species. The relevance of similarity analysis has already been pointed out for other genera of Malpighiaceae such as *Banisteriopsis* and *Byrsonima* (Araújo et al. 2010, 2020, Santos et al. 2020).

In this study, it is presented for the first time an identification key for *Glicophyllum* with leaf morphoanatomical data demonstrating the applicability of leaf anatomy for the taxonomy

of the genus. Among the characters used, the following stand out: presence/absence of trichomes; petiole contour; contour of the midrib; mesophyll type and presence/absence of sheath extension. As well as the morphological characteristics of the external leaf glands. In the multivariate analysis, it is possible to observe that the characteristics related to leaf venation and topology of the glands are the most representative characters for the differentiation of the formed groups. Therefore, the data obtained can support future taxonomic and phylogenetic studies of the genus.

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CSL applied standard anatomical techniques, performed material analysis, anatomical interpretation and manuscript editing. JSL performed the anatomical interpretation and manuscript editing. FSSF performed the anatomical interpretation, review and editing of the manuscript. JSA Performed material analysis, anatomical interpretation, editing and manuscript review.

