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ORIGINAL ARTICLE

Leaf anatomy as an additional taxonomy tool for 16 species of Malpighiaceae found in the Cerrado area (Brazil)

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Abstract This work describes the leaf anatomy of 16 species belonging to three genera of the Malpighiaceae family found in the Cerrado (Minas Gerais State, Brazil). The scope of this study was to support the generic delimitation by contributing to the identification of the species and constructing a dichotomous identification key that includes anatomical characters. The taxonomic characters that were considered to be the most important and used in the identification key for the studied Malpighiaceae species were as follows: the presence and location of glands; presence of phloem in the medullary region of the midrib; mesophyll type; presence and type of trichomes; and presence, quantity, and disposition of accessory bundles in the petiole. It was also possible to indicate promising characters for future taxonomic and phylogenetic studies in the Malpighiaceae family, especially for the *Banisteriopsis*, *Byrsonima*, and *Heteropterys* genera.

Keywords Taxonomy · *Byrsonima* · *Banisteriopsis* · *Heteropterys* · Cerrado

Introduction

The Malpighiaceae family has nearly 65 genera and 1,250 species occurring in tropical and subtropical areas in both hemispheres (Anderson 2001). Although this family is clearly monophyletic (Davis et al. 2001), the infrafamily classification based on winged or unwinged fruit is artificial (Anderson 1978). It is difficult to study this family primarily because of its large number of species, nomenclatural problems, and difficulties in identification by taxonomists. For example, glandular calyces are common among the neotropical Malpighiaceae, but it is possible to find eglandular calyces in species within the genera *Banisteriopsis*, *Byrsonima*, *Galphimia*, and *Pterandra* (Anderson 1990), making it difficult to distinguish these genera by using this morphological character. Such issues arise, in particular, because of the morphological variability and species synonymies (Gates 1982; Makino-Watanabe et al. 1993; Anderson 2001).

The floral architecture of all species in Malpighiaceae is very similar; therefore, characters such as leaf shape, length, width, and leaf pubescence as well as the extremely diverse fruit types are commonly used to sort out species within several genera (Anderson 1979; Mamede 1981; Davis et al. 2001). The phylogeny of Malpighiaceae is based on molecular analyses, which include fruit morphology, pollen grain morphology chromosome number, and distribution of species (Davis et al. 2001; Cameron et al. 2001); however we could not find evaluations that included anatomical characters.

Although the leaf is the organ that anatomically varies the most based on the hierarchic level (species, genus, or even the family) and despite the influence of environmental factors, many anatomical characters are useful for systematics, particularly the leaf epidermis (Metcalfe and Chalk 1979; Dickison 2000). Therefore, since the 19th century, taxonomists have investigated anatomical characters that may support species identification (Solereder 1908; Metcalfe and Chalk 1950, 1979).

Among the most recent works on anatomy applied to taxonomy, the research carried out on species belonging to

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the Brazilian flora stand out the most (Gomes et al. 2005; Rio et al. 2005; Fonsêca et al. 2007). Research on the anatomy of Malpighiaceae has been carried out on a few species without emphasis on the taxonomy (Arambarri et al. 2006; Ferreira 1981; Gavilanes and Ferreira 1974–1976; Guimaraes et al. 1985). However, in terms of anatomy as it applies to taxonomy, few references were found for this family (Mamede 1993; Attala 1997), which reveals how scarce these studies are.

It is important to highlight that the use of anatomical characters as a source of information for systematics necessitates an understanding of the character variation that a single individual can represent among the specimens of a particular species, group, or related taxa. The structural variation may be documented quantitatively or qualitatively (Dickison 2000). Thus, it is necessary to evaluate a higher number of specimens and, if possible, include specimens from collections in different locations.

In the Brazilian Cerrado, the Malpighiaceae stand out both in the number of woody species (Ratter et al. 1997) and other phytosociological parameters such as density, frequency, and absolute and relative dominance (Saporetti et al. 2003; Borges and Shepherd 2005). The importance of *Banisteriopsis, Byrsonima*, and *Heteropterys* must be emphasized since these genera have a high number of species that are difficult to identify when they are not fertile. In a recent review, 10 species of *Byrsonima*, 30 of *Banisteriopsis*, and 12 of *Heteropterys* were listed in the Cerrado vegetation (Mendonça et al. 2008). Therefore, anatomical characteristics from vegetative organs are useful to distinguish species.

This work describes the leaf anatomy of 16 species belonging to three genera of the Malpighiaceae family found in the Cerrado (Minas Gerais State, Brazil). The scope of this study is to improve the generic delimitation by contributing to the identification of species and constructing a dichotomous identification key that includes anatomical characters.

Materials and methods

Leaves of all Malpighiaceae species collected in Paraopeba National Reserve (FLONA; 19°16′S and 44°23′W; Minas Gerais State) were analyzed. This is a conservation unit managed by IBAMA (Brazilian Institute of Environment and Renewable Natural Resources) that has nearly 200 ha, of which 150 ha are the Cerrado (Neri et al. 2005).

The samples were kept in the herbarium at the Federal University of Viçosa (VIC), Minas Gerais, and included 16 species belonging to three genera: *Banisteriopsis* (seven species), *Byrsonima* (five species), and *Heteropterys* (four species). Samplings of the same species from voucher material kept in the Herbaria of the University of Brasilia (UB), University of São Paulo (SPF), and Rio de Janeiro Botanical Garden (RB) were also taken and used as replications (Table 1).

The criteria used for selecting the leaves were as follows: (1) herborized material quality, (2) fully expanded leaves, and (3) identification by experts in the Malpighiaceae family.

Leaves were boiled in distilled water until their submersion, treated with 2% potassium hydroxide for 2 h at room temperature (Smith and Smith 1942), dehydrated in ethylic series, and then stored in 70% ethanol.

The leaf blade (including basal, middle, and distal parts) and petiole were hand sectioned. Cross and longitudinal sections were cleared by means of a 50% sodium hypochlorite and stained with basic fuchsin-Astra blue. Slides were mounted in glycerogelatin and sealed with transparent nail polish (Kraus and Arduin 1997).

To analyze the venation pattern and stomata type, samplings of 20 mm^2 were subjected to diaphanization and staining with alcoholic safranin (Shobe and Lersten 1967). Slides were then mounted with synthetic resin (Permount - SP15-500, Fisher Scientific, NJ, USA).

The analysis and photographic records were performed under a photomicroscope (Olympus AX70TRF, Olympus Optical, Tokyo, Japan) with attached image capture. The material was also recorded by means of hand drawings made with the aid of a camera lucida attached to a light microscope (Olympus CBA, Olympus Optical, Tokyo, Japan).

All 16 Malpighiaceae species were compared according to their analyzed structural characteristics by using a presence/absence matrix (Table 2). This matrix used Sorensen's index and an average group linkage technique (also known as unweighted pair-group method using arithmetic averages, UPGMA) with the MVSP 3.13 m program (Mueller-Dombois and Ellenberg 1974). Also, principal component analysis (PCA) was calculated by using the same matrix and performed using NTSys software (Rohlf 1988; Sokal and Rohlf 1995).

The vascular bundle arrangement in the petiole was classified according to Howard (1979). Venation pattern and stomata type of the leaf blade are classified according to Metcalfe and Chalk classification (1979). Trichomes were analyzed according to the classification proposed by Theobald et al. (1979).

Results

Anatomic description

The petiole outlines of *Banisteriopsis anisandra* (Fig. 1) and *B. pubipetala* are concave-convex in cross-section.

Table 1 List of the voucher material species. Information about the vegetation is included when it was described on the voucher material label

Species	Herbaria			
	Federal University of Viçosa	University of Sao Paulo	University of Brasilia	Rio de Janeiro Botanic Garden
Banisteriopsis anisandra (Juss.) B. Gates	VIC 9726 Cerrado	SPF 35657 – Rocky Outcrop/MG		RB 227241
B. argyrophylla (Juss.) B. Gates	VIC 10014, VIC 10251 Cerrado	SPF 15643 Porto Ferreira/SP	UB 3547 Perto Córrego Terra Branca/DF	
B. campestris (Juss.) Little	VIC 9934 Cerrado		UB 6738 Chapada dos Veadeiros/GO, UB 7980 Serra dos Cristais/GO	
B. gardneriana (A. Juss.) W. R. Anderson and B. Gates	VIC 9140 Cerrado	SPF 44593 Vale do Rio Itacambiruçu/MG	UB 34517 Serra dos Pireneus/GO	
B. laevifolia (Juss.) B. Gates	VIC 9931 Cerrado	SPF 48780	UB 31733 Serra Geral do Paraná/GO	
B. malifolia (Nees & Mart.) B. Gates	VIC 9982 Jaboticatubas, São José da Serra, Cipó Camping Club/MG		UB 13369 Cristalina/GO, UB 11588 Diamantina/MG	
B. pubipetala (A. Juss.) Cuatrec.	VIC 10244 Cerrado	SPF 18052 Andaraí/BA	UB 34795 Estado de Goiás	
Byrsonima basiloba A. Juss	VIC 9963 Cerrado		UB 24112 Chapada dos Veadeiros/GO, UB 7545 Serra Geral do Paraná/GO	
B. coccolobifolia (Spreng.) Kunth	VIC 9833, VIC 25995, VIC 9131 Cerrado			
B. crassifolia (L.) Kunth	VIC 9152, VIC 9153 Cerrado			RB 211132
B. lancifolia A. Juss.	VIC 9753 Cerrado	SPF 170071 Alto Paraíso	UB 6343 Chapada dos Veadeiros/GO	
B. verbascifolia Rich. ex A. Juss.	VIC 9731, VIC 9157, VIC 9158, VIC 9159 Cerrado			
Heteropterys anoptera A. Juss.	VIC 9734 Cerrado		UB 7359 Chapada dos Veadeiros/GO, UB 23771 Serra do Espinhaço/MG	
H. byrsonimifolia A. Juss.	VIC 8943, VIC 9763 Serra do Cipó/MG, VIC 24126 Cerrado Stricto Sensu – Parque Estadual do Lajeado – Palmas/TO			
H. campestris A. Juss.	VIC 10015, VIC 25775/, VIC 25653 Cerrado			
H. tomentosa A. Juss.	VIC 10318 Cerrado		UB 17164 Serra de Xavantina/MT, UB 39721 Xavantina/MT	

Banisteriopsis argyrophylla, *B. campestris* (Fig. 2), *B. gardneriana* (Fig. 3), *B. laevifolia*, *B. malifolia*, and all species of *Byrsonima* (Fig. 4–6) and *Heteropterys* (Fig. 7 and 8) have a plan-convex petiole outline. The surface is slightly sinuous in *B. gardneriana* (Fig. 3), *B. malifolia,* and all species of *Byrsonima* (except in *B. crassifolia*) as well as *Heteropterys byrsonimifolia* (Fig. 7) and *H. tomentosa* (Fig. 8).

Three types of petiole vascular patterns are identified. In *Banisteriopsis gardneriana* (Fig. 3), *Byrsonima basiloba* (Fig. 4), *B. coccolobifolia, B. lancifolia* (Fig. 5), and in all species of *Heteropterys* (Fig. 7 and 8), an arc invaginating at the ends is found. A curved arc is observed in *Banisteriopsis anisandra* (Fig. 1), *B. argyrophylla, B. campestris* (Fig. 2), *B. pubipetala*, *B. laevifolia*, and *B. malifolia*. A pattern constituted by free bundles forming an arc with

Table 2 Presence (1) and absence (0) matrix of 16 Malpighiaceae species found in the FLONA of Paraopeba, Minas Gerais, Brazil

Analyzed characteristics	BaA	BaAR	BaC	BaG	BaL	BaM	BaP	ByB	ByCO	ByCR	ByLA	ByV	HA	HB	HC	HT
Single-layered epidermis on both sides (1)	0	1	1	0	0	1	1	1	1	0	0	0	1	1	1	0
Bilayered epidermis on some regions of the adaxial side (2)	1	0	0	1	1	0	0	0	0	1	1	1	0	0	0	1
Guard cells forming ledge only on the outer side (3)	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0
Subsidiary cells projected over the guard cells (4)	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1
One pair of leaf glands (5)	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0
Glands present at the petiole base (6)	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
Glands present in the lower part of the leaf (7)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Glands present in the middle part of the leaf (8)	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
V-shaped nonglandular trichomes (9)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Y-shaped nonglandular trichomes (10)	0	1	1	1	1	1	0	1	0	1	0	0	0	0	0	0
T-shaped nonglandular trichomes (11)	1	1	1	1	1	1	0	1	0	1	1	1	0	0	1	1
Isobifacial leaves (12)	0	0	1	0	0	1	0	0	1	1	1	1	1	0	0	0
Dorsiventral leaves (13)	1	1	0	1	1	0	1	1	0	0	0	0	0	1	1	1
Vascular bundles arranged in open arc (14)	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1
Vascular bundles arranged in closed arc with phloem in the midrib (15)	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0
Vascular bundles arranged in closed arc without phloem in the medullary region of the midrib (16)	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Petiole on cross-section with concave-convex shape (17)	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Petiole on cross-section with plan-convex shape (18)	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
Petiolar vascular system arranged as a curved arc (19)	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0
Petiolar vascular system arranged in an arc and invaginate at ends (20)	0	0	0	1	0	0	0	1	1	0	1	0	1	1	1	1
Petiolar vascular system comprised of free bundles forming an arc with invaginate ends (21)	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Two conspicuous accessory bundles in the petiole (22)	1	1	1	0	0	0	0	1	0	1	0	0	0	0	0	0
Four conspicuous accessory bundles in the petiole (23)	0	0	0	1	1	1	1	0	1	0	0	1	0	1	1	0
Camptodromous-brochidodromous-pinnate-like venation (24)	0	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1
Camptodromous-eucamptodromous-pinnate-like venation (25)	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
Primary vein with branched linear orientation (26)	0	1	1	1	1	1	1	1	0	0	0	0	1	1	1	0
Primary vein with nonbranched linear orientation (27)	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Primary vein with curved orientation (28)	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1
Randomly reticulated tertiary veins (29)	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1
Transversally branched tertiary veins (30)	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0
Orthogonal areoles (31)	1	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0
Irregularly shaped areoles (32)	0	1	0	1	1	1	1	1	1	0	1	0	1	0	1	1
Aeroles lacking (33)	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0

Table 2 continued

Analyzed characteristics	BaA	BaAR	BaC	BaG	BaL	BaM	BaP	ByB	ByCO	ByCR	ByLA	ByV	HA	HB	HC	HT
Areoles arranged in an oriented pattern (34)	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Randomly arranged areoles (35)	1	1	0	1	1	1	1	1	1	1	1	0	1	0	1	1
Well-developed areoles (36)	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0
Incompletely closed anastomose areoles (37)	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Imperfectly developed areoles (38)	1	0	0	1	1	1	1	1	0	0	1	0	1	0	0	1

BaA Banisteriopsis anisandra, *BaAR B. argyrophylla*, *BaC B. campestris*, *BaG B. gardneriana*, *BaL B. laevifolia*, *BaM B. malifolia*, *BaP B. pubipetala*, *ByB Byrsonima basiloba*, *ByCO B. coccolobifolia*, *ByCR B. crassifolia*, *ByLA B. lancifolia*, *ByV B. verbascifolia*, *HA Heteropterys anoptera*, *HB H. byrsonimifolia*, *HC H. campestris*, *HT H. tomentosa*

invaginate ends was found in *Byrsonima verbascifolia* (Fig. 6) and *B. crassifolia.*

Accessory vascular bundles are observed in all species of *Banisteriopsis* (Figs. 1–3). They are absent in *Byrsonima lancifolia* (Fig. 5), *Heteropterys anoptera*, and *H. tomentosa* (Fig. 8). Variations in the number of accessory bundles are observed. Two accessory bundles are found in *Banisteriopsis anisandra* (Fig. 1)*, B. argyrophylla, B. campestris* (Fig. 2), *Byrsonima basiloba* (Fig. 4), and *B. crassifolia*, and four in *Banisteriopsis gardneriana* (Fig. 3), *B. laevifolia, B. malifolia, B. pubipetala, Byrsonima coccolobifolia, B. verbascifolia* (Fig. 6), *Heteropterys byrsonimifolia* (Fig. 7), and *H. campestris.*

The presence of sclereids scattered among the cortical parenchyma is common in *Byrsonima* (Figs. 4–6).

Leaf blades have thickened cuticle in 14 species (Figs. 10–12), except in *Heteropterys campestris* and *H. tomentosa*. Cuticular flanges are observed in all species that have thickened cuticle except for *Banisteriopsis argyrophylla* and *B. pubipetala.*

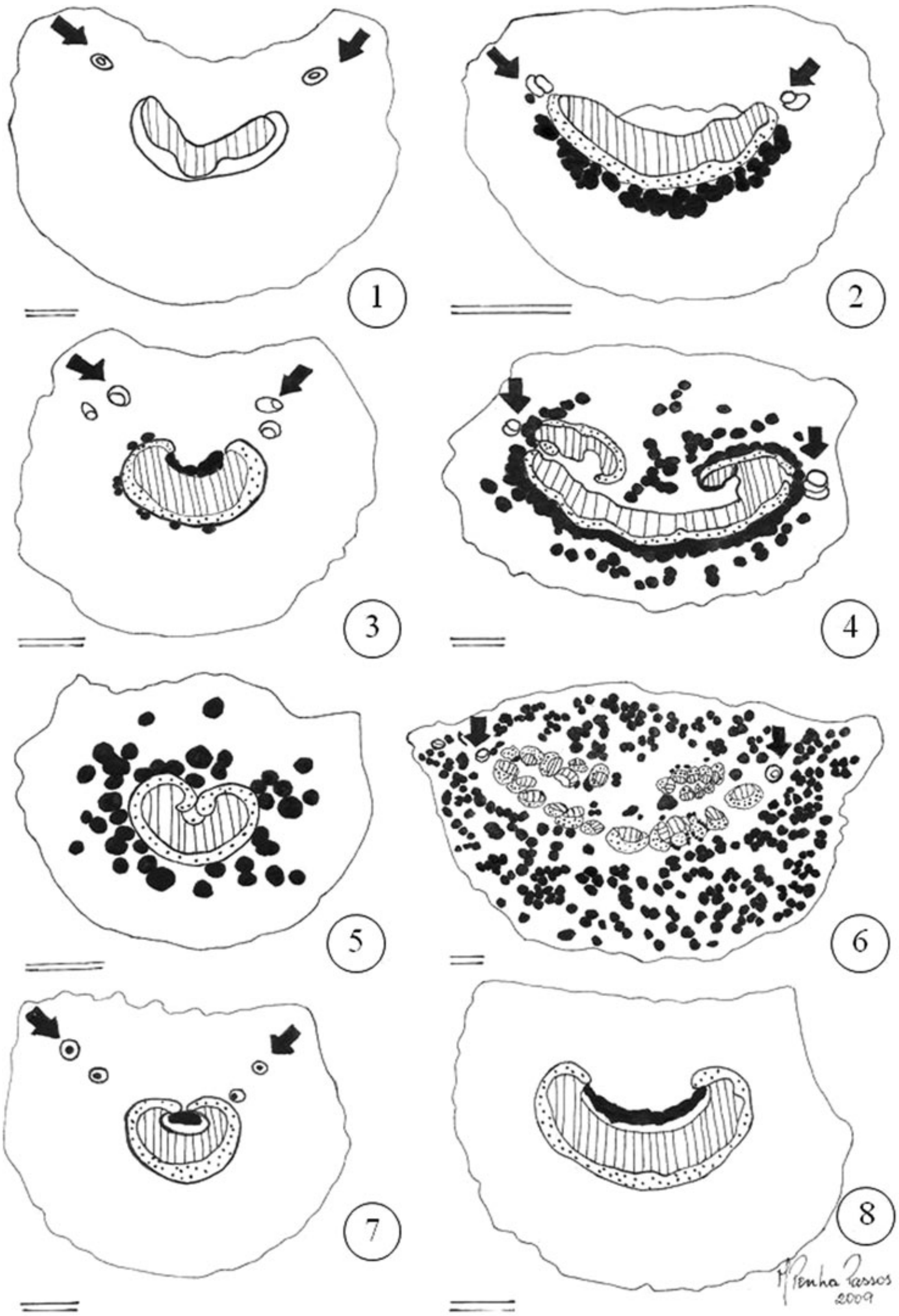
Leaf blades (Figs. 9–14) have a single-layered epidermis on the abaxial side of the leaf in all species. On the adaxial side of the leaf, the epidermis is single-layered in *Banisteriopsis campestris* (Fig. 10)*, B. argyrophylla, B. malifolia, B. pubipetala, Bysonima basiloba* (Fig. 12), *Heteropterys anoptera* (Fig. 14), *H. byrsonimifolia* (Fig. 13), and *H. campestris.* A noncontinuous bilayered epidermis is found in *Banisteriopsis anisandra* (Fig. 9)*, B. gardneriana, B. laevifolia*, *Byrsonima coccolobifolia* (Fig. 11)*, B. crassifolia, B. lancifolia*, *B. verbascifolia*, and *Heteropterys tomentosa.*

Stomata are paracytic (Fig. 15). In cross-section, subsidiary cells that are large in relation to the guard cells are observed. Cuticular ledges are poorly developed in *Banisteriopsis* and *Heteropterys* species (Figs. 16 and 17), while in *Byrsonima* species, the cuticular ledges are well developed in the guard cells (Figs. 18 and 19).

Three types of branched unicellular nonglandular trichomes are observed: T type (Fig. 20), Y type (Fig. 21), and V type (Fig. 22). The T type is absent only in *Banisteriopsis pubipetala*, *Byrsonima coccolobifolia*, *Heteropterys anoptera*, and *H. byrsonimifolia.* The Y type is found in *Banisteriopsis argyrophylla, B. campestris, B. laevifolia*, *B. malifolia*, *Byrsonima basiloba*, and *B. crassifolia*. The V type is observed only in *Byrsonima basiloba*. Trichomes are found on the abaxial side, and there are many of them in *Banisteriopsis* species, except *B. campestris*, and in *Byrsonima basiloba*. In *Banisteriopsis campestris*, the number of such structures is visibly lower. In *Byrsonima crassifolia, B. lancifolia*, *B. verbascifolia*, *Heteropterys campestris*, and *H. tomentosa*, the trichomes are observed on both sides, being higher in quantity in *B. verbascifolia.*

Mesophyll is dorsiventral in *Banisteriopsis anisandra* (Fig. 9), *B. argyrophylla*, *B. gardneriana*, *B. laevifolia*, *B. pubipetala*, *Byrsonima basiloba* (Fig. 12), *Heteropterys byrsonimifolia* (Fig. 13), *H. campestris*, and *H. tomentosa*. In *Banisteriopsis campestris* (Fig. 10), *B. malifolia*, *Byrsonima coccolobifolia* (Fig. 11), *B. crassifolia*, *B. lancifolia*, *B. verbascifolia*, and *Heteropterys anoptera* (Fig. 14), it is isobilateral.

The midrib (Figs. 26–31) is prominent and made up of collateral bundles arranged to form a single arc in all *Banisteriopsis* species (Figs. 26 and 27) and *Heteropterys* (Figs. 30 and 31). In *Byrsonima*, the midrib is prominent and made up of collateral bundles arranged in two arcs (Figs. 28 and 29), one curved towards the adaxial side and the other one towards the abaxial side, the latter one being smaller in most cases. In *Banisteriopsis anisandra* (Fig. 26) and *B. gardneriana*, the midrib shows a flat adaxial side. Near the vascular bundles, fibers are observed in *Banisteriopsis* (Fig. 26), *Byrsonima* (Figs. 28 and 29), and *Heteropterys* (Fig. 30), but not in *B. argyrophylla* (Fig. 27), *B. laevifolia*, and *H. campestris* (Fig. 31).



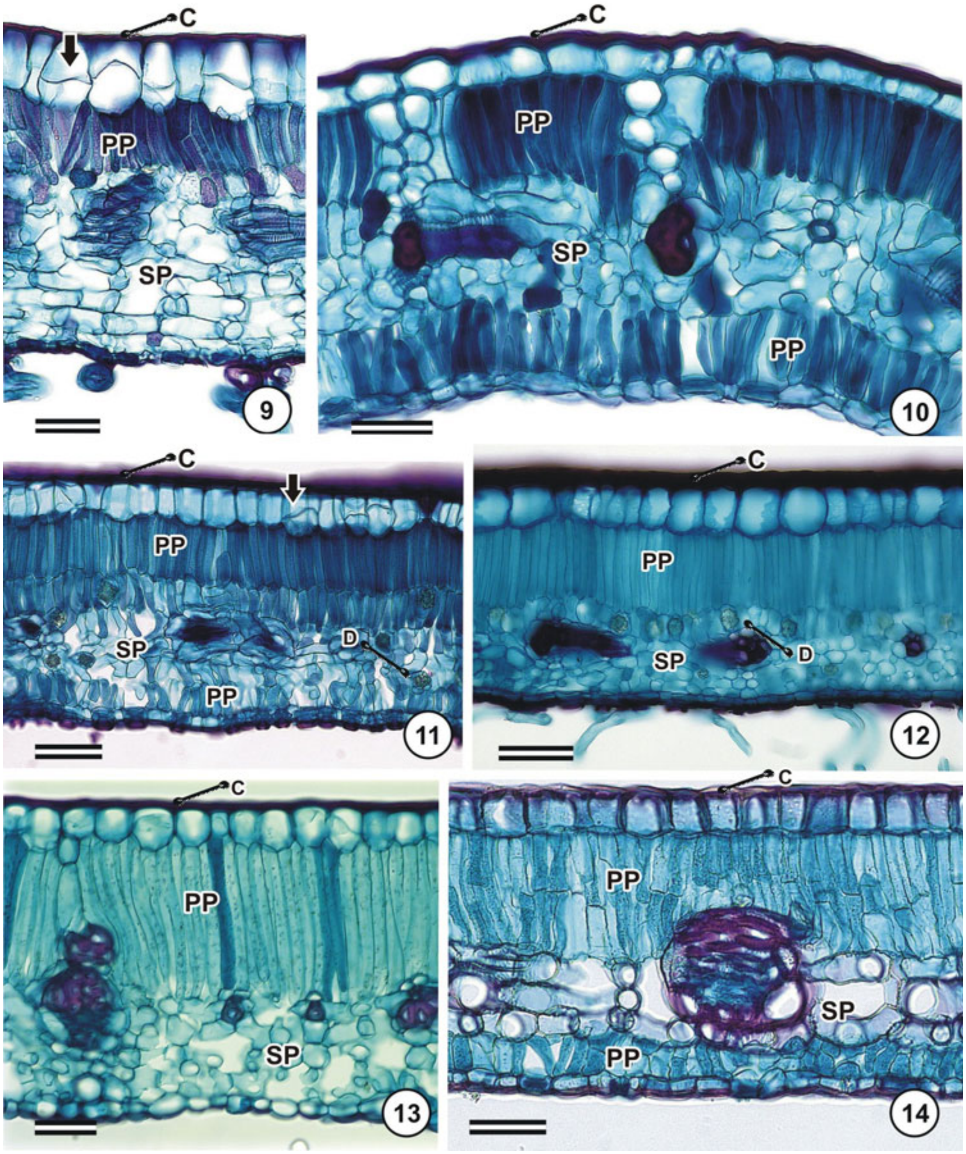
Figs. 1–8 Diagrammatic schemas of the petiole cross-section of Malpighiaceae species. **Fig. 1** *Banisteriopsis anisandra*. **Fig. 2** *Banisteriopsis campestris*. **Fig. 3** *Banisteriopsis gardneriana*. **Fig. 4** *Byrsonima basiloba*. **Fig. 5** *Byrsonima lancifolia*. **Fig. 6** *Byrsonima verbascifolia*. **Fig. 7** *Heteropterys byrsonimifolia*. **Fig. 8** *Heteropterys tomentosa*. *Arrow* Accessory bundles, *dot-filled areas* phloem, *striped areas* xylem, *dark areas* fibers. *Scale* = 400 μm

Phloem is found in the medullary region of the midrib in all *Byrsonima* species (Fig. 28), except in *B. coccolobifolia* (Fig. 29).

Druses and prismatic crystals occur in all *Banisteriopsis* and *Byrsonima* species, both in the mesophyll (Figs. 11 and 12) and the midrib, except in *Banisteriopsis pubipetala* where they are absent.

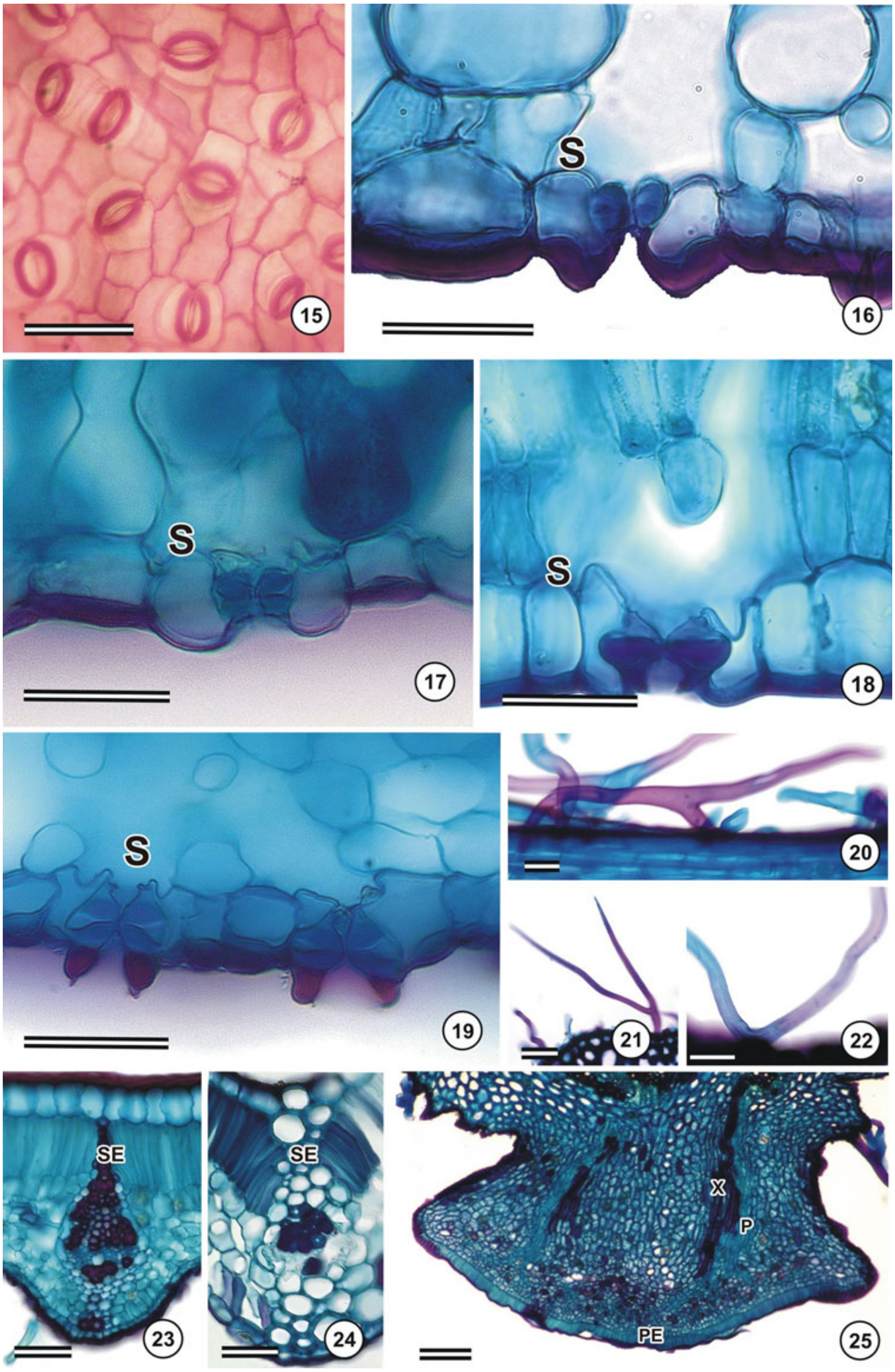
Sclerified sheath extensions are observed in larger-diameter vascular bundles in *Byrsonima basiloba* (Fig. 23), *Heteropterys anoptera*, and *H. byrsonimifolia*. In *Banisteriopsis argyrophylla*, *B. campestris* (Fig. 24), *B. laevifolia*, *B. malifolia*, *Byrsonima coccolobifolia*, *B. crassifolia*, *B. lancifolia*, *B. verbascifolia*, and *Heteropterys tomentosa*, they are parenchymatic.

Figs. 9–14 Leaf blade cross-sections of Malpighiaceae species. **Fig. 9** *Banisteriopsis anisandra*. **Fig. 10** *Banisteriopsis campestris*. **Fig. 11** *Byrsonima coccolobifolia*. **Fig. 12** *Byrsonima basiloba*. **Fig. 13** *Heteropterys byrsonimifolia*. **Fig. 14** *Heteropterys anoptera*. *C* Cuticle, *D* druses, *PP* palisade parenchyma, and *SP* spongy parenchyma, *arrow* bilayered epidermis. *Scale* **Fig. 9**, **10**, **13**, **14** = 50 µm; **Fig. 11** and **12** = 70 µm



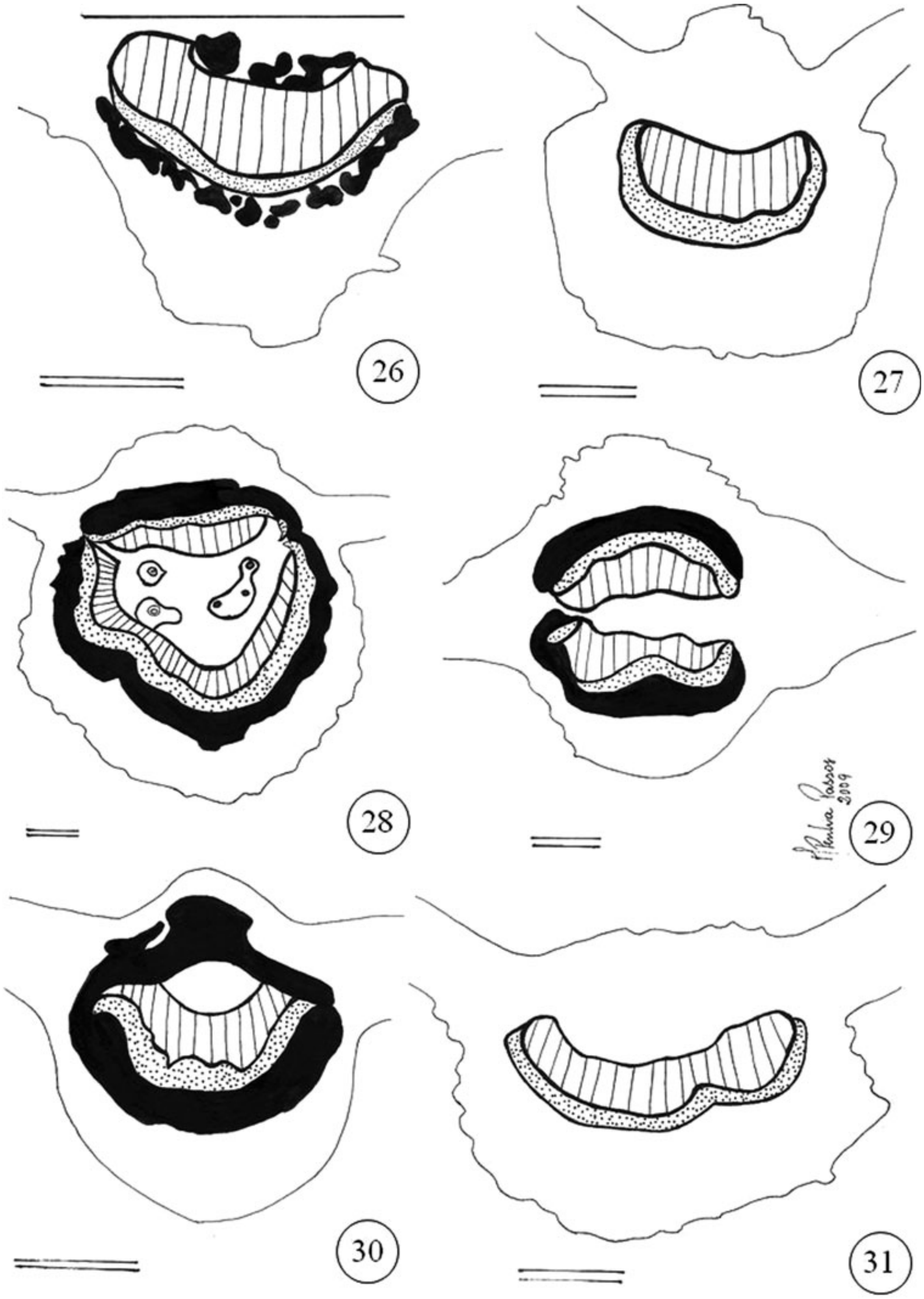
Glands on the lower leaf blade (Fig. 25) are only observed in *Banisteriopsis*, and only in five out of the seven analyzed species. Such structures are located at three different places: at the distal portion of the petiole (*B. anisandra, B. gardneriana, B. laevifolia*, and *B. malifolia*), on the lower abaxial side of the leaf blade (*B. campestris*), and on the third part of the leaf blade near the midrib (*B. laevifolia*). Anatomically, these structures are made up of a secretory palisade epidermis and a secretory subepidermic parenchyma (Fig. 25). Vascular endings of xylem and phloem converge to the subepidermic parenchyma (Fig. 25).

A camptodromous-brochidodromous pinnate venation pattern (Figs. 32–40) occurs in all species except *Banisteriopsis anisandra*, *B. laevifolia*, and *Byrsonima verbascifolia,* where a camptodromous-eucamptodromous pattern is found (not documented). The primary vein shows a straight pattern, except in *Banisteriopsis anisandra*, *Byrsonima crassifolia*, *B. verbascifolia*, *Heteropterys anoptera*, *H. byrsonimifolia*, *H. campestris*, and *H. tomentosa* where it is curved, and in *Byrsonima lancifolia* where it is nonbranched and linear. The tertiary veins are randomly reticulate in *Banisteriopsis*, *Byrsonima basiloba* (Fig. 35), *B. coccolobifolia* (Fig. 36), and *B. crassifolia* (Fig. 37), while in *Byrsonima lancifolia* (Fig. 38), *B. verbascifolia*, and *H. campestris* they are transversally branched. Terminal veins are branched in all species, except *Byrsonima verbascifolia,* where they are simple. Areoles in



Figs. 15–25 Diaphanized leaf blade (**15**). Leaf blade cross sections (**16**–**21**) of Malpighiaceae species. **Fig. 15** *Byrsonima crassifolia*: paracytic-like stomata. **Fig. 16** *Banisteriopsis pubipetala*. **Fig. 17** *Banisteriopsis laevifolia*. **Fig. 18** B*yrsonima coccolobifolia*. **Fig. 19** and **22** *Byrsonima basiloba*. **Fig. 20** T-shaped nonglandular trichome in *Byrsonima crassifolia*. **Fig. 21** Y-shaped nonglandular trichome in *Banisteriopsis argyrophylla*. **Figs. 22** and **23** *Byrsonima basiloba*. **Fig. 22** Detail of V-shaped nonglandular trichome in **Fig. 22**. **Figs. 24** and **25** *Banisteriopsis campestris*, detail of the glandular structure on the lower leaf blade shown in **Fig. 25** (*arrow*). *S* Stomata, *SE* sheath extension, *PE* palisade epidermis, *P* phloem, and *X* xylem. *Scale*: **Fig. 15**, **20**, **22–24** = 50 µm; **Fig. 16**–**19** = 30 µm; **Fig. 21** = 70 µm; **Fig. 24** = 150 µm

Banisteriopsis argyrophylla (Fig. 32), *Byrsonima coccolobifolia* (Fig. 36), and *H. campestris* show incompletely closed anastomoses; in *Banisteriopsis campestris* (Fig. 33), *Byrsonima crassifolia* (Fig. 37), and *H. byrsonimifolia* (Fig. 40), they are well developed; in *Banisteriopsis* species (Fig. 34), they are branched; and in *Byrsonima basiloba* (Fig. 35), *B. lancifolia* (Fig. 38), *B. verbascifolia*, *H. anoptera* (Fig. 39), and *H. tomentosa*, they are imperfect. The areole shape is irregular (Figs. 32, 34–36, 38, and 39), except for *Banisteriopsis campestris* (Fig. 33), *B. anisandra*, *Byrsonima crassifolia* (Fig. 37), and *H. byrsonimifolia* (Fig. 40), where they show an orthogonal pattern.

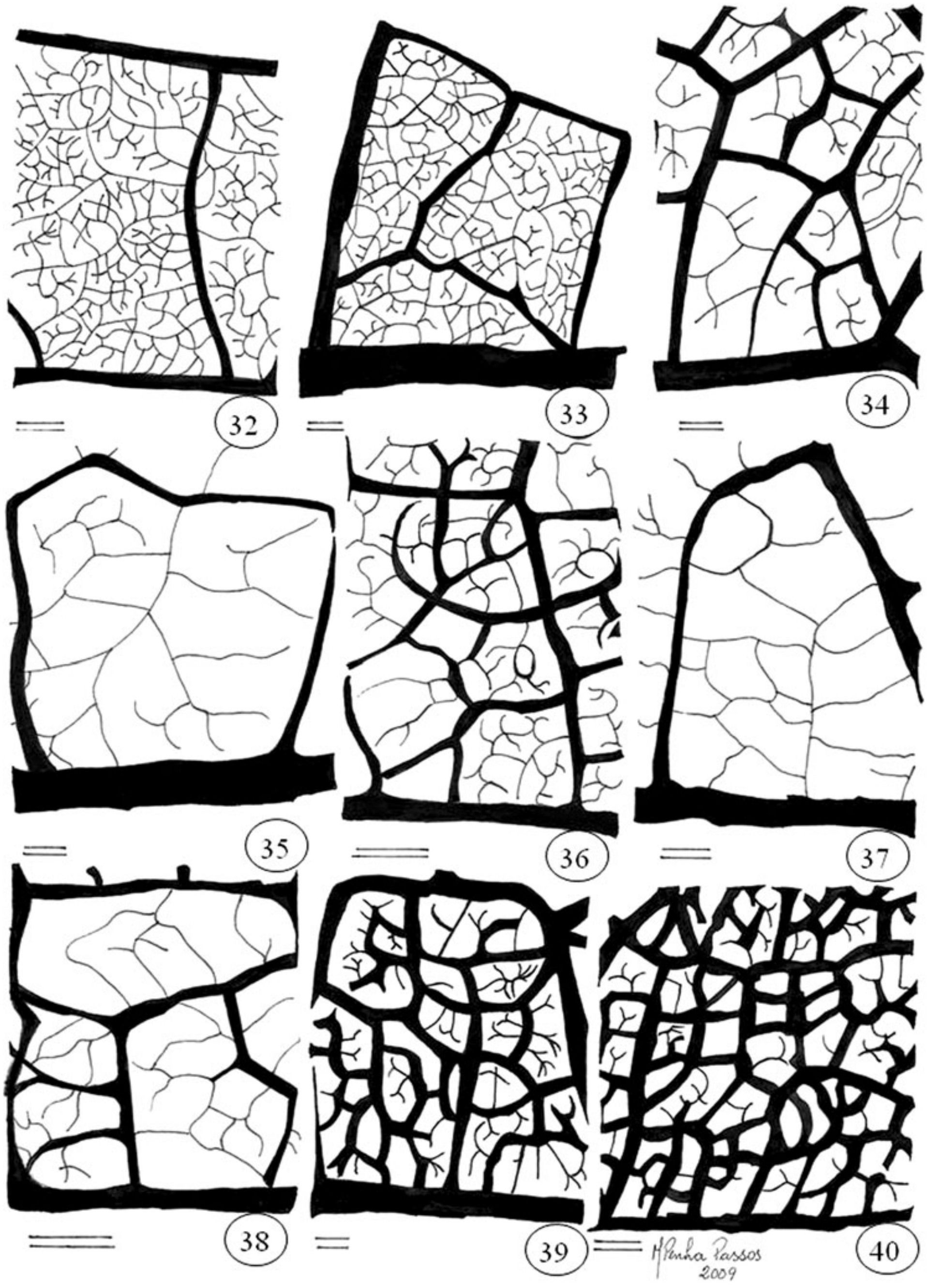


Figs. 26–31 Schemas of leaf blade cross-sections in Malpighiaceae species. **Fig. 26** *Banisteriopsis anisandra* with prominent midrib and flat adaxial side surface. **Fig. 27** *Banisteriopsis argyrophylla* with prominent midrib and absent fibers. **Fig. 28** *Byrsonima basiloba* with prominent midrib and phloem within the midrib. **Fig. 29** *Bysonima coccolobifolia* with prominent midrib and no phloem within the midrib. **Fig. 30** *Heteropterys anoptera* with prominent midrib and bundles arranged in open arc. **Fig. 31** *Heteropterys campestris* with prominent midrib and bundles arranged in open arc. *Dot-filled areas* Phloem, *striped areas* xylem, *dark areas* fibers. *Scale* = 300 μm

All species show looped venation endings on the leaf border, which are enclosed by secondary, tertiary, and quaternary arcs.

Similarity analysis

The similarity analysis (Fig. 41) was performed based on presence/absence (Table 2) to distinguish three groups: one includes *Byrsonima verbascifolia* and *B. crassifolia*; the second *Byrsonima coccolobifolia*, *Byrsonima lancifolia*, and *Byrsonima basiloba*; and the third, which groups all *Banisteriopsis* and *Heteropterys* species. The presence of phloem in the midrib rib and stomata with developed cuticular ledges in the guard cells is the characteristic that incorporated *Byrsonima* species in the first group. Regarding the second group, the major similarity among the species is the presence of a petiolar vascular bundle arranged in the shape of an arc, which invaginates at the ends and includes areoles of irregular shape in the blade. The third group, formed by *Banisteriopsis* and *Heteropterys* species,



Figs. 32–40 Diagrammatic schemas of the venation patterns in diaphanized leaf blades of Malpighiaceae species. **Fig. 32** *Banisteriopsis argyrophylla*: areole with incompletely closed anastomose and irregular shape. **Fig. 33** *Banisteriopsis campestris*: well-developed orthogonal areoles. **Fig. 34** *Banisteriopsis gardneriana*: branched areole with irregular shape. **Fig. 35** *Byrsonima basiloba*: randomly reticulated tertiary veins with imperfect areoles and irregular shape. **Fig. 36** *Byrsonima coccolobifolia*: randomly reticulated tertiary veins, areole with incompletely closed anastomose and irregular shape. **Fig. 37** *Byrsonima crassifolia*: randomly reticulated tertiary veins, well-developed orthogonal areoles. **Fig. 38** *Byrsonima lancifolia*: transversally branched tertiary veins, with imperfect areoles and irregular shape. **Fig. 39** *Heteropterys anoptera*: imperfect areoles with irregular shape. **Fig. 40**. *Heteropterys byrsonimifolia*: well-developed orthogonal areoles. *Scale* = 400 μm

contains well-developed subsidiary cells in relation to the guard cells and has a single vascular bundle arranged in an arc in the midrib.

In the PCA (Fig. 42), it was possible to distinguish two groups of characteristics, one which defines *Byrsonima* and the other *Banisteriopsis*. However, *Heteropterys* could not be distinguished (Fig. 42). The following characteristics are found exclusively in the *Byrsonima* genus: guard cells forming a ledge only on the outer side (3), V-shaped nonglandular trichomes (9), vascular bundles arranged in a closed arc with phloem in the midrib rib (15), vascular bundles arranged in a closed arc without phloem in the medullary region of the midrib (16), petiolar vascular system consisting of free bundles forming an arc with invaginate ends (21), primary vein with nonbranched linear orientation (27), primary vein with curved orientation (28), transversally branched tertiary veins (30), and no aerolation (33). The following were characteristic of the *Banisteriopsis* genus: one pair of leaf glands (5), glands present at the petiole base (6),

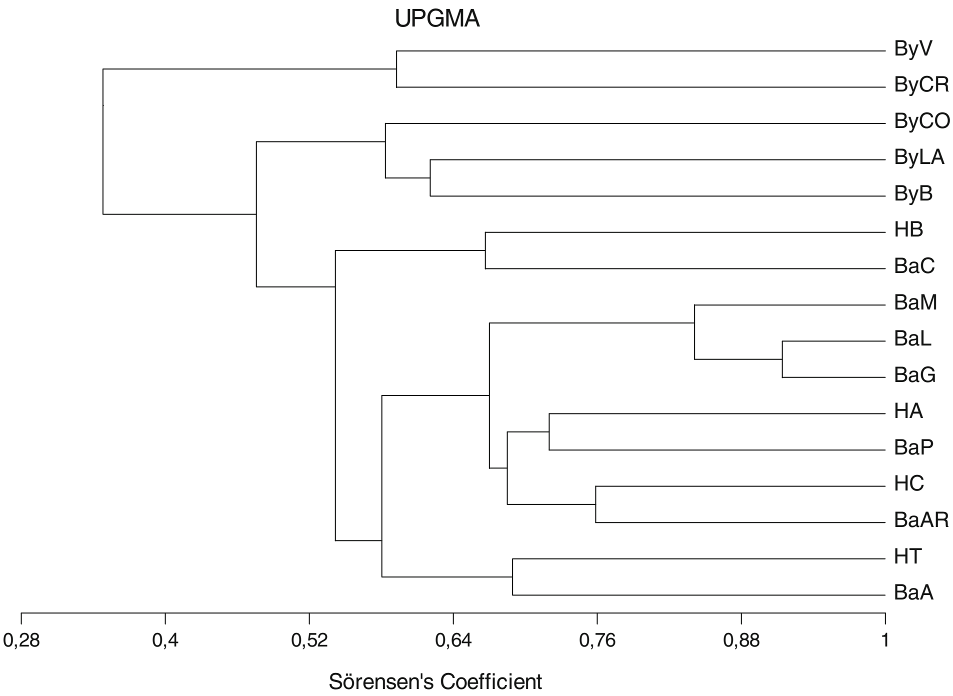


Fig. 41 Grouping analysis of *Banisteriopsis*, *Byrsonima*, and *Heteropterys* species. *BaA Banisteriopsis anisandra*. *BaAR B. argyrophylla*, *BaC B. campestris*, *BaG B. gardneriana*, *BaL B. laevifolia BaM B. malifolia*, *BaP B. pubipetala*, *ByB Byrsonima basiloba*, *ByCR B. crassifolia*, *ByCO B. coccolobifolia*, *ByLA B. lancifolia*, *ByV B. verbascifolia*, *HA Heteropterys anoptera*, *HB H. byrsonimifolia*, *HC H. campestris*, *HT H. tomentosa*

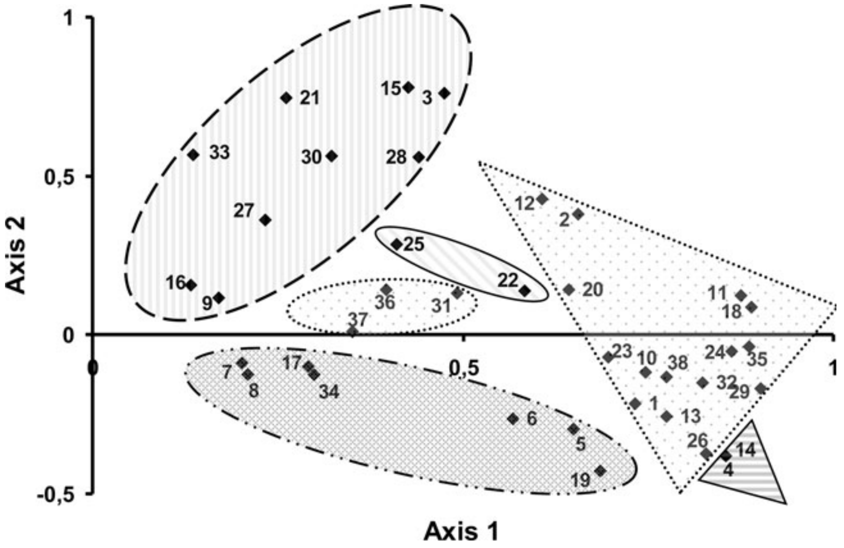


Fig. 42 Biplot principal component analysis (PCA) of *Banisteriopsis, Byrsonima*, and *Heteropterys* species. ▩ indicates characters for *Banisteriopsis* species; ▥ indicates characters for *Byrsonima* species; ⬚ indicates characters for *Banisteriopsis, Byrsonima*, and *Heteropterys* species; ▧ indicates characters for *Banisteriopsis* and *Byrsonima* species; and ▤ indicates characters for *Banisteriopsis* and *Heteropterys* species. The *triangles* indicate symplesiomorphies and *ellipses* synapomorphies

glands present in the lower part of the leaf (7), glands present in the middle part of the leaf (8), petiole with concave-convex shape on cross-section (17), petiolar vascular system arranged as a curved arc (19), and areoles arranged in an oriented pattern (34).

Both analyses pointed to *Byrsonima* as the most isolated group if compared to *Banisteriopsis* and *Heteropterys*. In a cluster analysis, *Byrsonima* formed two groups with its entire species placed before *Banisteriopsis* and *Heteropterys* species, which were grouped into clusters (Fig. 42).

In the PCA analysis (Fig. 42), *Byrsonima* showed nine exclusive traits and *Banisteriopsis* seven. The group of characters found near or at the end of axis 1 indicated by both triangles occurs in all three genera; however two of these characters (represented by the smaller triangle) are only present in *Banisteriopsis* and *Heteropterys*. Between the two major clusters (i.e., the two biggest ellipses, which have characters found exclusively in *Banisteriopsis* or *Byrsonima*), there are two smaller ellipses grouping characters for some species of the three genera or some species of *Banisteriopsis* and *Byrsonima*.

Identification key

1. Presence of a pair of glands on the leaf..2

2. One pair of glands on the lower abaxial side ....*Banisteriopsis campestris*

2'. One pair of glands near the third part of the midrib ....*Banisteriopsis laevifolia*

2". One pair of glands on the distal portion of the petiole......................................3

3. Absence of Y shape trichomes................................*Banisteriopsis anisandra*

3´. Presence of Y shape trichomes 4

4. Isobifacial leaves...*Banisteriopsis malifolia*

4". Dorsiventral leaves......................................*Banisteriopsis gardneriana*

1'. Absence of glands...5

5. Presence of phloem in the midrib rib..6

6. Dorsiventral leaves......................................*Byrsonima basiloba*

6'. Isobifacial leaves 7

7. Petiolar vascular system consists of a single bundle arranged in the form of an invaginated arc....................*Byrsonima lancifolia*

7'. Petiolar vascular constituted by free bundles forming an arc with invaginate ends..8

8. Two accessory bundles in the petiole..*Byrsonima crassifolia*

8'. Four conspicuous accessory bundles in the petiole..*Byrsonima verbascifolia*

5'. Absence of phloem in the midrib rib..9

9. Presence of trichomes..10

10. Absence of accessory bundles in the petiole...............................*Heteropterys tomentosa*

10'. Presence of two accessory bundles in the petiole.........................*Banisteriopsis argyrophylla*

10". Presence of four accessory bundles in the petiole ....*H. campestris*

9'. Absence of trichomes..11

11. Isobifacial leaves....................................12

12. Absence of accessory bundles in the petiole......................................*H. anoptera*

12'. Presence of four accessory bundles in the petiole...........*Byrsonima coccolobifolia*

11'. Dorsiventral leaves.................................13

13. Petiolar vascular system arranged in the form of a curved arc...........*Banisteriopsis pubipetala*

13`. Petiolar vascular system arranged in the form of an arc invaginated at ends...........................*H. byrsonimifolia*

Discussion

It was possible to identify meaningful characters for the three genera studied. These characters may be applied to the taxonomy of Malpighiaceae. Anatomy has been a useful tool for taxonomy and has been successfully used since the 19th century (Solereder 1908; Metcalfe and Chalk 1979). The importance of the anatomy as an additional tool for taxonomy is evident when fragmented material needs to be identified, when reproductive structures are not available, or even to indicate evolutionary trends and phylogenetic relationships among taxa (Metcalfe and Chalk 1979). Anatomy studies in the Malpighiaceae family may be very important once the fruit morphology is the main character used in taxonomy and phylogeny. It has been emphasized that molecular data combined with morphological characters may enhance the understanding of the evolution of Malpighiaceae (Cameron et al. 2001; Davis et al. 2001).

Regarding the vascular bundle in the petiole, the form of an arc with invaginated ends was considered the most common type among the studied species, observed in nine species among three genera. A pattern with a high number of free bundles forming an invaginated arc was restricted to the genus *Byrsonima*. The form of a curved arc was exclusive and predominant in *Banisteriopsis*. The petiole vascularization is a promising character and must be considered in future taxonomic reviews, especially at the genus level. Although references regarding the use of such a character in the Malpighiaceae taxonomy were not found, few studies have described this character for few species. In *Peixotoa hispidula* (Ferreira 1981), *Heteropterys angustifolia* (Arambarri et al. 2006), and *Mascagnia pubiflora* (Guimaraes et al. 1985), the petiole vascular bundle forms an arc, similar to the most common type of petiole vascularization among the species studied. The importance of the petiole vascularization for taxonomy has been reported for other groups, for instance in Melastomataceae, where the character helped distinguish five genera, or in the genus *Chamaecrista* (Leguminosae/Caesalpinioideae), where this characteristic was considered to be diagnostic for the studied species (Reis et al. 2004; Francino 2006).

The presence and number of accessory bundles were considered meaningful diagnostic characters for the analyzed species. Most of the species showed four or two accessory bundles. The absence of this structure was not very common. This character is useful when distinguishing species, being similar to what was described for the genus *Erythroxylum* of Erythroxylaceae (Bieras and Sajo 2004).

Three types of nonglandular trichomes (V, Y, and T types) were observed in 12 of the 16 analyzed species. Such types of trichomes are characteristic of Malpighiaceae and are considered to be diagnostic for this family (Metcalfe and Chalk 1979; Gates 1982; Judd et al. 2007). *Byrsonima basiloba* was the only species that showed the three types of trichomes; for this reason, they are considered to be diagnostically meaningful. Although the T type has been observed in individuals of the three analyzed genera, it was the only one found in *Heteropterys*. Nonglandular types of trichomes have been used in taxonomic reviews, as for *Banisteriopsis* where the Y or T, or Y and T types were found in 67 species. This characteristic was considered important and was used in the identification key presented for this genus (Gates 1982). It is important to emphasize that the lack of nonglandular trichomes on expanded leaves must be considered with care, as it is very common for these trichomes to be deciduous, as recorded for *Byrsonima coccolobifolia* (Beiguelman 1962a).

Paracytic type stomata were seen in all species, while a bilayered epidermis was found only in 7 of the 16 studied species (*Banisteriopsis anisandra, B. gardneriana, B. laevifolia, Byrsonima crassifolia, B. lancifolia, B. verbascifolia*, and *Heteropterys tomentosa*). These results confirm literature records, increasing the number of studied species and genera of the Brazilian flora (Beiguelman 1962a; Metcalfe and Chalk 1979; Attala 1997). *Mascagnia pubiflora* has an anomocytic stomata type (Guimaraes et al. 1985), which may be additional evidence of the polyphyletic hiraeoid clade that was demonstrated by molecular phylogenetic analysis (Cameron et al. 2001).

The mesophyll types found in many studied species are in accordance with the literature for individuals of the Malpighiaceae family (Beiguelman 1962b; Attala 1997; Pereira 2002). However, it is important to note that even though a dorsiventral mesophyll was recorded for *Byrsonima coccolobifolia* (Beiguelman 1962b), it is believed that such an interpretation is misleading. As the substomatal chambers in this species are large, and there are many stomata, the palisade parenchyma facing the abaxial side is constantly interrupted, which could make it difficult to recognize the isobifacial mesophyll.

The presence of phloem in the midrib was restricted to the *Byrsonima* genus, which makes this an important diagnostic character at the genus level.

One pair of glands, found in different places, was observed in five of the seven species of *Banisteriopsis*. Glands were absent in the other genera analyzed. Metcalfe and Chalk (1979), Anderson (1990), and Judd et al. (2007) have reported the presence of glands on vegetative organs in most genera and species of Malpighiaceae in both the New and Old World. They are commonly found on the petiole, on the leaf abaxial side, and on bracts and bracteoles. These structures are morphologically and anatomically similar to the analyzed species. Structures that resemble such glands were reported in *Banisteriopsis anisandra* and *B. gardneriana* (Attala 2004) and were characterized as extrafloral nectaries (Anderson 1990; Attala

2004). These glands are quite similar in their anatomical structure and occur in a position analogous to the position of elaiophores found on the sepals of most neotropical Malpighiaceae (Anderson 1990). Since herbarium material was used in the present work, tests to verify the presence of sugars in the glandular secretion of the *Banisteriopsis* were not performed.

The venation pattern for most species was brochidodromous. Only *Banisteriopsis anisandra, B. laevifolia*, and *Byrsonima verbascifolia* showed eucamptodromous type. According to Judd et al. (2007), one of the characteristics for most species of the Malpighiaceae family is the brochidodromous-like venation, which was confirmed by our observations and was related to *Peixotoa hispidula* (Ferreira 1981), further indicating the eucamptodromous pattern as a distinctive characteristic.

The morphology of the winged fruit (Gates 1982; Amorim 2002) allows differentiation between *Heteropterys* (dorsal wing with thickened abaxial border) and *Banisteriopsis* (dorsal wing with thickened adaxial border). However, the results of leaf anatomy show that at the species level, the clades are well defined for the species analyzed. It is so reliable that it was possible to build an identification key for those species.

The analysis of similarity showed that for the Malpighiaceae species found in the FLONA of Paraopeba, the genus *Byrsonima* is well delimited, since the analyzed species formed a consistent group and were isolated from *Banisteriopsis* and *Heteropterys*. These results are corroborated by the molecular phylogenetic analysis in which *Byrsonima* is allocated in the diverse clade of *Heteropterys* and *Banisteriopsis* (Cameron et al. 2001; Davis et al. 2001).

Intrafamilial classification and intergeneric relationships have not been agreed upon (Anderson 1978; Davis et al. 2001; Cameron et al. 2001). For the time being, according to molecular phylogeny analysis the only monophyletic group is the banisterioid (Cameron et al. 2001; Davis et al. 2001). The anatomical multivariate analyses showed that *Byrsonima* was derived before *Banisteriopsis* and *Heteropterys*, in keeping with the molecular phylogenetic analyses (Cameron et al. 2001; Davis et al. 2001).

In the PCA, single characters for *Byrsonima* are accumulated in the positive direction for axis 2, while the ones for *Banisteriopsis* are found on the negative side. These differences may be considered as another argument for *Banisteriopsis* being one of the several most derived genera of the banisterioid clade, which concurs with what Cameron et al. (2001) had earlier hypothesized while studying the molecular systematics of Malpighiaceae. The group of characters found near or at the end of axis 1 outlined by the two triangles (Fig. 42) suggests symplesiomorphies for the three genera; however two of these characters included in the smaller triangle (Fig. 42) are only present in *Banisteriopsis* and *Heteropterys*. *Byrsonima* seems to have derived first from the main branch of Malpighiaceae while *Banisteriopsis* and *Heteropterys* are thought to have derived later. These results corroborate the molecular phylogeny of Malpighiaceae (Cameron et al. 2001; Davis et al. 2001). Apparently, two smaller ellipses found in the PCA correspond to parallel apomorphies that occurred in some species of the three genera or in some species of *Banisteriopsis* and *Byrsonima*.

Anatomically, the analyzed 16 species showed differences that indicate the importance of anatomy for taxonomic evaluation. It was possible to indicate promising characters for future taxonomic and phylogenetic studies on the Malpighiaceae family, regarding not only the delimitation of *Banisteriopsis*, *Byrsonima*, and *Heteropterys* but also the other genera and species.

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