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Taxonomic relevance of leaf anatomy in *Banisteriopsis* C.B. Rob. (Malpighiaceae)

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ABSTRACT

We describe the leaf anatomy of 42 of the 60 species of the genus *Banisteriopsis*, one of the largest and most diverse genera of Malpighiaceae in the Cerrado domain. Our aim was to identify anatomical leaf characters useful for the taxonomy and systematics of this morphological diverse genus. Expanded leaves from herbarium specimens were rehydrated and processed according to usual anatomical methods for analysis under light microscopy. All characters were compared using the Unweighted Pair Group Method with Arithmetic Mean algorithm and expressed in dendrograms. The most useful leaf anatomical characters were petiole contour, presence/absence of vascular bundles in the petiole, the position of the vascular bundles in the petiole, shape of the midrib, presence/absence of sheath extension, mesophyll type, and both the presence/absence and shape of leaf glands. Our study indicates that the morphological groups proposed in the literature for *Banisteriopsis* are natural groups.

Keywords: leaf glands, Malpighiales, multivariate analysis, plant anatomy, stigmaphylloid clade

Introduction

Banisteriopsis is one of the most diversified genera of Malpighiaceae in Neotropical savannas, comprising ca. 60 species of shrubs and vines confined to the Americas (Gates 1982; Davis & Anderson 2010). The genus is easily recognized by its leaves bearing extra-floral nectaries on the leaf blades (Fig. 1), and by its greatly expanded glandular connectives (Gates 1982). Even though *Banisteriopsis* was taxonomically revised in the early 1980's (Gates 1982), it was evidenced to be polyphyletic in the first phylogenetic studies for Malpighiaceae (Cameron *et al*. 2001; Davis *et al*. 2001; Davis & Anderson 2010), leading to the segregation of some species to *Diplopterys* or to the newly described *Bronwenia* (Anderson & Davis 2006). After rendering *Banisteriopsis* monophyletic, these authors failed to morphologically re-circumscribe *Banisteriopsis sensu stricto*. The morphological characters currently used to distinguish the species of *Banisteriopsis* are mostly restricted to inflorescence architecture, flower, and fruit morphology (Gates 1982; Anderson & Davis 2006). As several floral characters are not retained in fruiting specimens (*e.g.*, color and floral display), correctly assigning newly collected specimens of Malpighiaceae to *Banisteriopsis* became one of the main challenges for general botanists.

Leaf anatomy applied to taxonomic purposes has historically been used as a useful tool for exploring additional morphological characters in taxonomically convoluted groups (Metcalfe & Chalk 1950; 1979). This approach makes it possible to correctly identify fragmented or sterile plant specimens, besides pointing out evolutionary trends in groups (Metcalfe & Chalk 1950; 1979). Nonetheless, few studies exploring the anatomy of specific genera in

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Malpighiaceae are available in the literature, such as Mamede (1993), Araújo *et al.* (2010), Possobom *et al.* (2015), Araújo & Meira (2016), and Mello *et al.* (2019).

In order to search for additional leaf characters that might contribute to specific and generic delimitation in *Banisteriopsis*, we present a thorough survey of the leaf anatomy of 42 species of this genus. Additionally, we provide a multivariate analysis of all the analyzed species to compare the dendrograms obtained with the most recent phylogenetic hypothesis and current infrageneric classification for *Banisteriopsis*.

Materials and methods

We analyzed leaves of 42 species of *Banisteriopsis* C.R. Rob , as well as leaves of closely related genera based on Davis & Anderson (2010) (Tab. 1, Fig. 1A-M). All leaves were obtained from exsiccates deposited in Brazilian herbaria (CEPEC, HUEFS, INPA, MBM, RB, SP, SPF, UB, UPCB, and VIC; acronyms according to Thiers 2019). Fully expanded and well-preserved leaves were sampled from three specimens for each taxon, except for rare species (*i.e.*, *Banisteriopsis martiniana* (A. Juss.) Cuatrec, and *B. quadriglandula* B. Gates). The leaves were rehydrated in boiling distilled water until complete submersion (within five to fifteen minutes, in general), embedded in KOH 2 % for full distention (two hours in general), washed and dehydrated in ethyl alcohol series and stored in 70 % ethanol. All specimens were hand free sectioned using a shaving blade to obtain transversal and longitudinal sections from the leaf blade (base, middle, margin, and apex) and petiole. We adopted the median region for anatomical descriptions since there were not significative differences among all sectioned portions of the leaf blade. All sections were clarified in 20 % sodium hypochlorite and stained in basic fuchsine – Astra blue, and the slides were mounted in glycerin jelly and sealed with colorless varnish (Kraus & Arduin 1997).

Part of the samples was diaphanized according to a modified Foster (1950) protocol. Samples measuring ca. 20 mm^2 were kept in a solution of sodium hydroxide for two hours, washed in distilled water several times, and then kept in 20 % sodium hypochlorite for 18 hours or until the tissue became translucid. After an additional wash, samples were dehydrated in a 50 % ethyl alcohol series overnight, and the slides were mounted in glycerin jelly and sealed with colorless varnish.

Vascular bundles and vein patterns were classified according to Howard (1979) and to Ellis *et al.* (2009), respectively. Hair types were classified according to Theobald *et al.* (1979). Macromorphological data were obtained from Gates (1982) for elaborating a binary matrix (Tab. 2). All analyses and picture records were performed using a photomicroscope (Olympus AX70TRF, Olympus Optical, Tokyo, Japan) with a U-Photo system with a digital camera coupled (AxioCam HRc; Zeiss, Göttingen, Germany).

The identification key was elaborated using the data from the matrix (Tab. 2) for all analyzed species, and a reduced matrix comprising all 18 species sampled by Davis & Anderson (2010) in the most recent molecular phylogeny for Malpighiaceae. Leaf and floral characters for all species were compared among each other by converting the binary matrix into a symmetric matrix for Sorensen's similarity. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was used in NTSYS-pc 2.2 software (Rohlf 2000; Sokal & Rohlf 1995) to generate dendrograms based on the Jaccard index, and using the species of *Diplopterys* A.Juss. , *Peixotoa* and *Stigmaphyllon* as outgroups. Results obtained from this analysis were also compared to the most recent molecular phylogeny study for Malpighiaceae (Davis & Anderson 2010).

Results

Leaf anatomy of ***Banisteriopsis***

Petiole contour in transversal section displayed significant morphological variance (Fig. 2). In most species (29 species), the contour is plane-convex (Fig. 2A), while in 11 species (*B. adenopoda*, *B. goiana*, *B. megaphylla*, *B. membranifolia*, *B. muricata*, *B. nummifera*, *B. oxyclada*, *B. prancei*, *B. paraguariensis*, *B. parviflora* and *B. quadriglandula*) it is concave-convex (Fig. 2C). In the remaining two species (*B. acerosa* and *B. scutellata*), the contour is circular (Fig. 2B). Two types of configurations of vascular bundles were observed in petioles: an open arch with convolute extremities, found in *B. gardneriana* and *B. martiniana* (Fig. 2E), and an open arch in the remaining species (Fig. 2A-D, F). The occurrence of accessory bundles (Fig. 2A, C-F) was observed in most analyzed species, except in *B. acerosa* (Fig. 2B) and *B. stellaris*, but the number of vascular bundles has also varied within the same species.

Sclerenchyma sheaths were recorded surrounding the vascular bundles in petioles of eight species [*B. anisandra*, *B. campestris*, *B. hatschbachii* (Fig. 2F), *B. irwinii*, *B. latifolia*, *B. megaphylla*, *B. pulchra* and *B. schizoptera*]. Disperse sclereids were observed in petioles of nine species (Fig. 2A), *B. arborea*, *B. cipoensis*, *B. confusa*, *B. gardneriana*, *B. goiana*, *B. hirsuta*, *B. muricata*, *B. sellowiana*, *B. malifolia* var. *apressa*, *B. malifolia* var. *malifolia*), and were absent in the remaining species (Fig. 2B-E).

Midribs presented a convex contour in both surfaces of leaf blades in most analyzed species (Fig. 3A-C, Tab. 2). Plane midribs were observed in the adaxial surface of leaf blades of eight species [*B. anisandra* (Fig. 3B), *B. calcicola*, *B. gardneriana*, *B. irwinii*, *B. paraguariensis*, *B. prancei*, and *B. sellowiana*]. Concave midribs were observed in the adaxial surface of leaf blades of five species [*B. andersonii*, *B. angustifolia*, *B. arborea*, *B. martiniana* var. *martiniana* (Fig. 3C) and *B. muricata*]. The epidermis is single-layered in most species (Fig. 3D), except

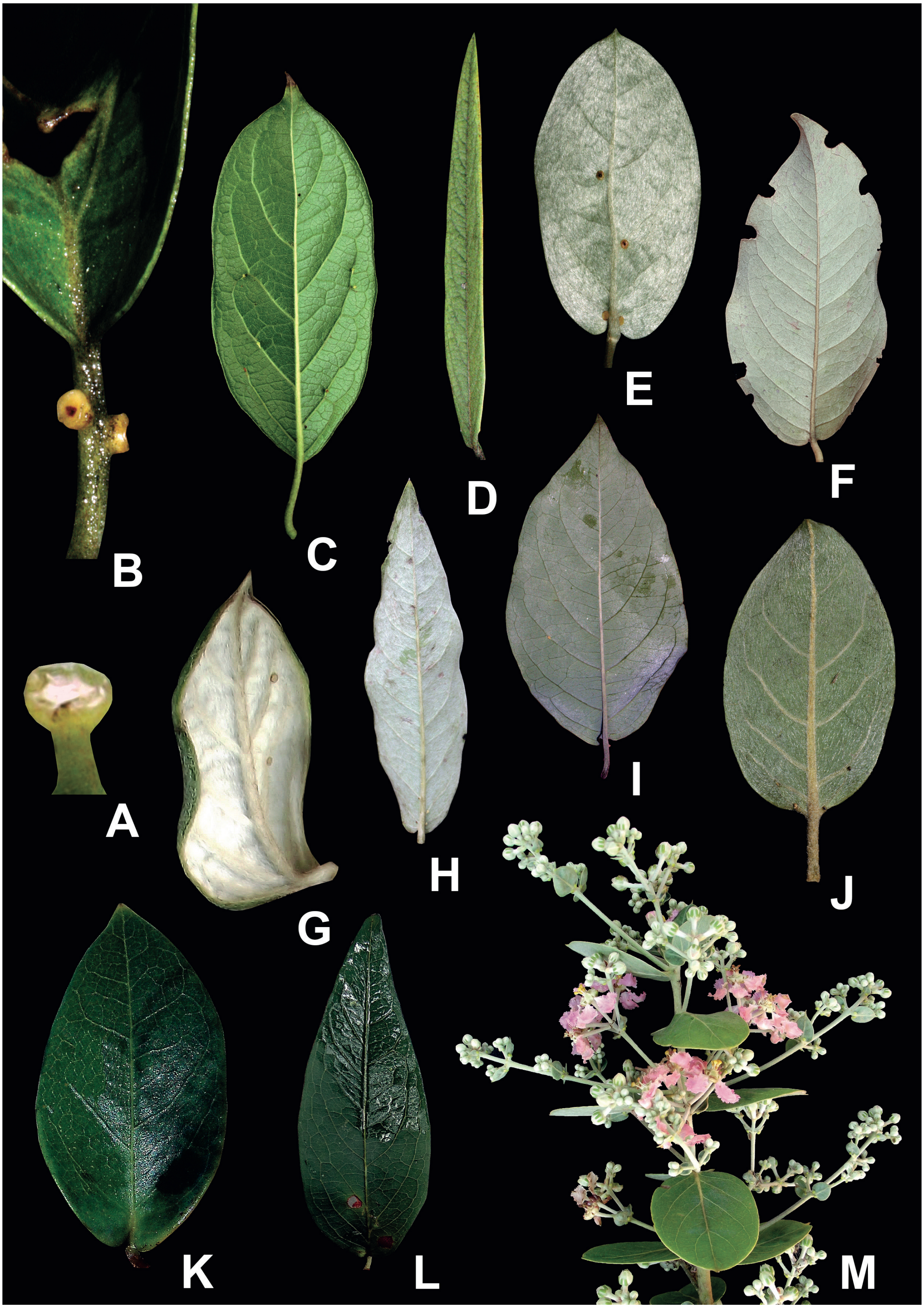

Figure 1. Leaves of some analyzed species of *Banisteriopsis*: **A.** stipitate leaf gland of *B. adenopoda*, **B.** sessile petiole glands of *B. membranifolia*, **C.** leaf of *B. adenopoda* in abaxial view, **D.** leaf of *B. angustifolia* in adaxial view, **E.** leaf of *B. argyrophylla* in abaxial view, **F.** leaf of *B. anisandra* in abaxial view, **G.** leaf of *B. harleyi* in abaxial view, **H.** leaf of *B. laevifolia* in abaxial view, **I.** leaf of *B. membranifolia* in abaxial view, **J.** leaf of *B. parviglandula* in abaxial view, **K.** leaf of *B. schizoptera* in adaxial view, **L.** leaf of *B. stellaris* in abaxial view, **M.** flowering branch of *B. variabilis* showing leaves, inflorescences and flowers in anthesis (photographs by R.F.Almeida).

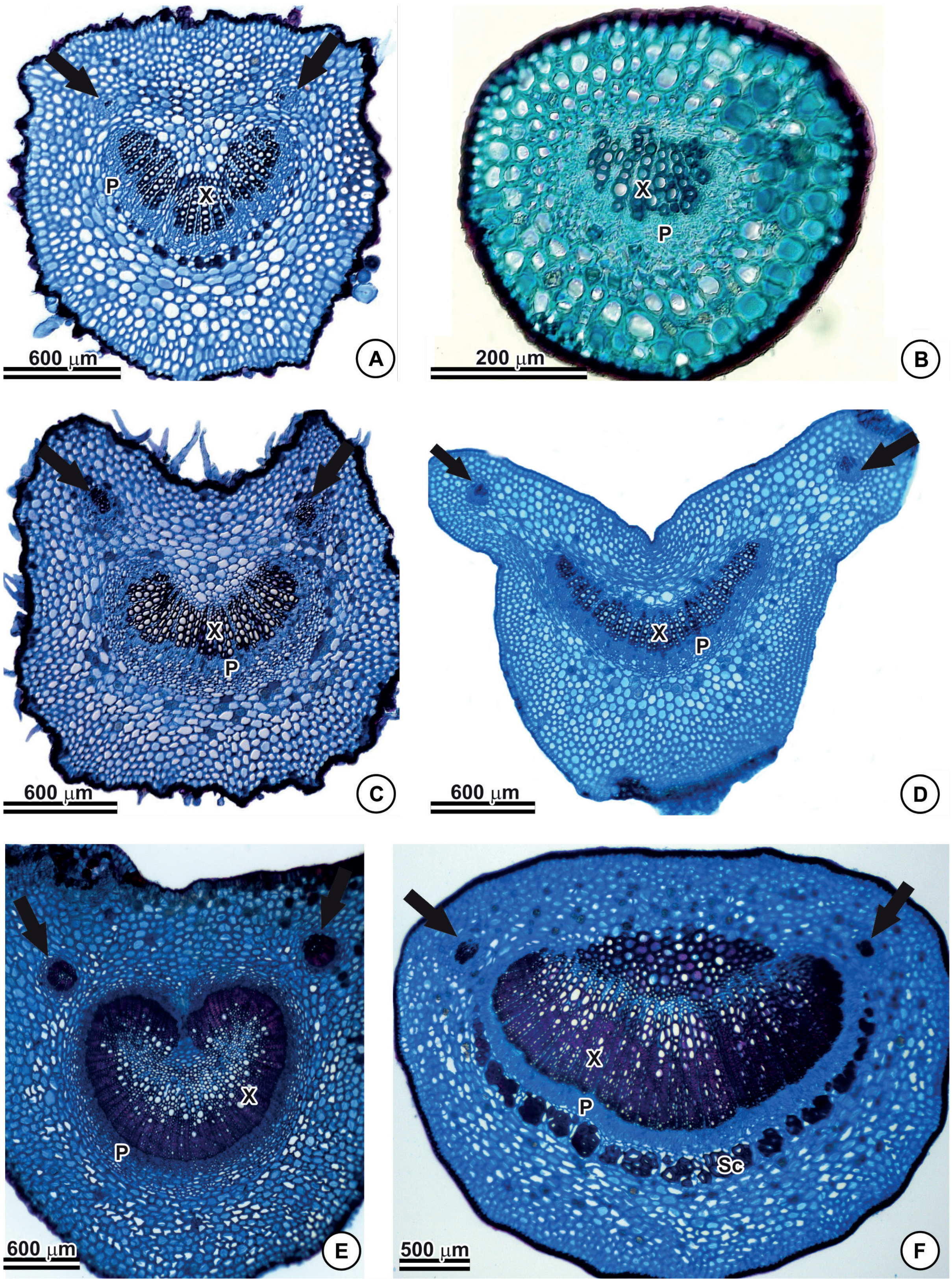

Figure 2. Shape and disposition of vascular bundles in petioles of *Banisteriopsis* species: A. plane-convex in *B. malifolia*, with vascular bundles in open arch, **B.** circular in *B. acerosa*, with vascular bundles in open arch, **C.** concave-convex in *B. membranifolia*, with vascular bundles in open arch, **D.** concave-convex in *B. parviflora*, with vascular bundles in open arch, **E.** circular in *B. martiniana*, with vascular bundles in open arch with convolute extremities, **F.** plane convex in *B. hatschbachii*, with vascular bundles in open arch. Abbreviations: arrow = accessory bundles; P = phloem; X = xylem; Sc = sclerenchymatic sheath.

in the abaxial surface of leaf blades in *B. martiniana* (Fig. 3E). This species presented two layers of cells and different layers of non-chlorophyll cells in some regions below the epidermis. Most species also showed unistratified adaxial epidermis (Fig. 3D, Tab. 2), and 16 species presented a bistratification (Fig. 3E-F, Tab. 2) in some regions of the adaxial epidermis. Also, several layers of non-chlorophyll cells were observed in the varieties of *B. martiniana* (Fig. 3E).

The mesophyll is isobilateral (Fig. 3D, F) in most species (Tab. 2), except in *B. adenopoda*, *B. anisandra*, *B. argyrophylla*, *B. calcicola*, *B. gardneriana*, *B. parviflora*, *B. sellowiana*, *B. martiniana* varieties, *B. megaphylla*, *B. multifoliolata* and *B. nummifera* in which the mesophyll is dorsiventral (Fig. 3E). All species showed collateral vascular bundles (Fig. 3A-C).

Paracytic stomata were observed in all species, with large subsidiary cells covering guard cells (Fig. 4A). Stomatal

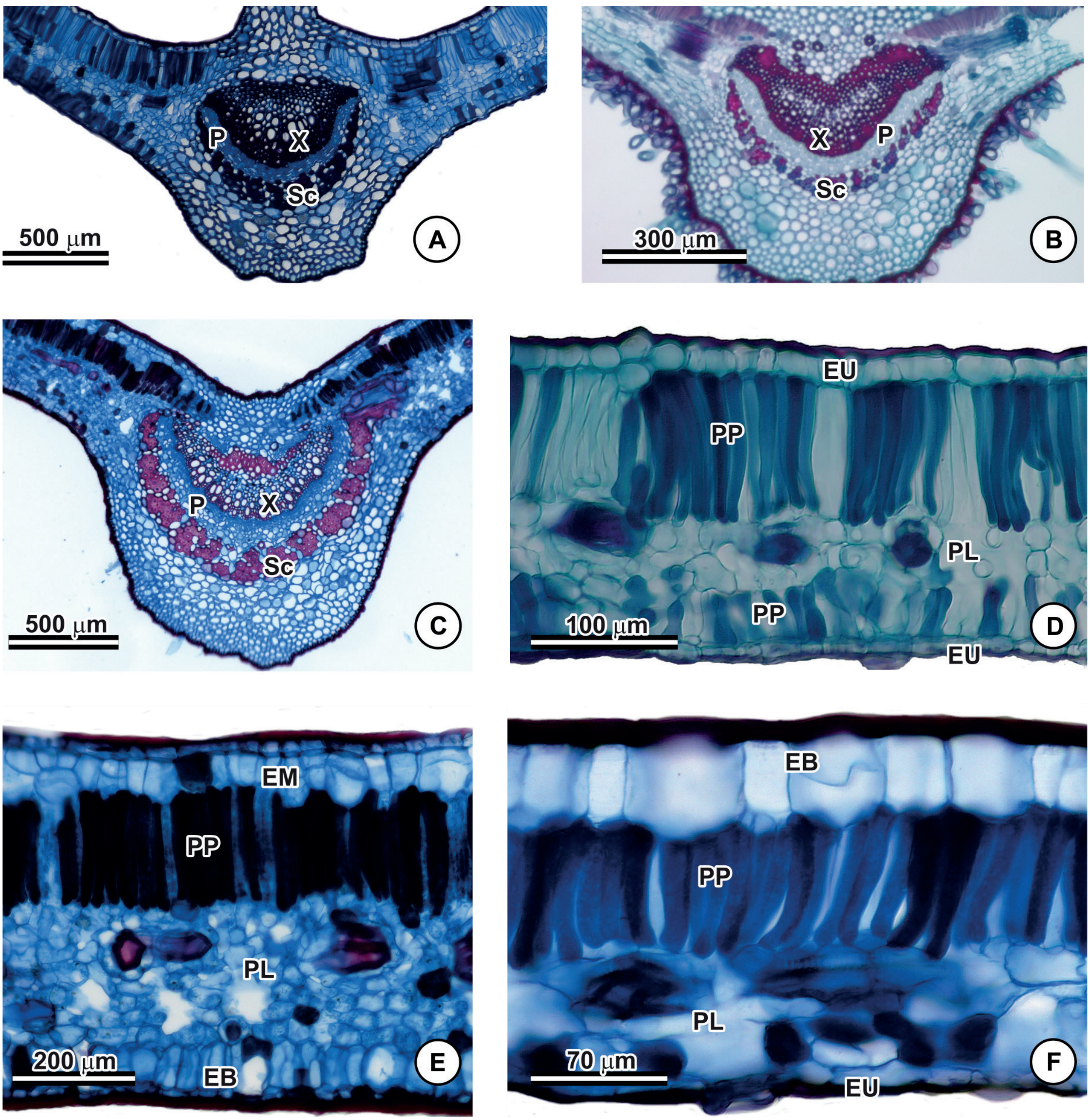

Figure 3. Anatomical characterization of leaf blades of *Banisteriopsis*: **A.** midrib transversal section of *B. hatschbachii*, **B.** midrib transversal section of *B. anisandra*, **C.** midrib transversal section of *B. martiniana* var. *martiniana*, **D.** mesophyll transversal section of *B. arborea*, **E.** mesophyll transversal section of *B. martiniana* var. *martiniana*, **F.** mesophyll transversal section of *B. acerosa*. Abbreviations: P = phloem; X = xylem; Sc = sclerenchymatous fibers; EU = unistratified epiderm; EM = multistratified epiderm; EB = bistratified epiderm; PP = palisade parenchyma; SP – spongy parenchyma.

Table 1. List of *Banisteriopsis* species and outgroups analyzed, and specimens examined from Brazilian herbaria (acronyms according to Thiers 2019).

Species	Specimens Examined
1. *Banisteriopsis acerosa* (Nied.) B.Gates	RB (331201, 244401, 201162); MBM (174055); UB (65222); SP (252951, 384090, 386918)
2. *B. adenopoda* (A.Juss.) B.Gates	RB (69270, 120913, 411546, 45432); SP (306339, 346371, 306335, 354763)
3. *B. andersonii* B.Gates	RB (193800, 244368, 90852); SP (142693, 153995, 143432)
4. *B. angustifolia* (A.Juss.) B.Gates	RB (57956, 195038, 326698); SP (409661, 390564, 253629)
5. *B. anisandra* (A.Juss.) B.Gates	RB (133639, 494729, 150011); UB (26924); SP (296650, 363454, 216329, 394821)
6. *B. arborea* B.Gates	RB (244371, 59841, 448021); MBM (35602); UB (58303, 58886)
7. *B. argyrophylla* (A.Juss.) B.Gates	RB (57894, 67619, 167016); UB (26972, 29428); SP (167185, 257888, 272633, 161943)
8. *B. basifixa* B.Gates	CEPEC (241574); RB (309636, 138858)
9. *B. byssacea* B.Gates	SP (286494, 287603, 286131, 286466)
10. *B. caapi* (Spruce *ex* Griseb.) Morton	INPA (98255, 124466, 192409); SP (113821, 270884, 45735)
11. *B. calcicola* B.Gates	MBM (204953); UB (58306); SP (320281, 319088, 386474)
12. *B. campestris* (A.Juss.) Little	RB (28103, 191338, 223130); UB (19522, 25538); SP (255661, 64956, 333747)
13. *B. cipoensis* B.Gates	RB (169904, 68846, 196228); UB (59361); SP (142685, 185216, 142666)
14. *B. confusa* B.Gates	RB (478486, 351217, 268035); INPA (104786); UB (58870, 65226); SP (267358, 369759)
15. *B. gardneriana* (A.Juss.) W.R.Anderson & B.Gates	RB (95614, 153189, 340322); INPA (120842); UB (59364, 43780); SP (411993, 405433, 296769)
16. *B. goiana* B.Gates	RB (167725, 141454, 141456); SP (247090, 314595, 409337)
17. *B. harleyi* B.Gates	RB (252166, 199408, 200020); UB (52128); SP (320286, 390563, 328052)
18. *B. hatschbachii* B.Gates	RB (141452); MBM (62215, 35681, 138625); UB (65218, 44865); SP (384087)
19. *B. hirsuta* B.Gates	MBM (159994); UB (62311, 65225); SP (272762)
20. *B. irwinii* B.Gates	RB (253058); MBM (62209, 34977); UB (61968); SP (256267, 319498, 333823)
21. *B. laevifolia* (A.Juss.) B.Gates	RB (208257, 208256, 141453, 345479); INPA (121113); UB (27610); SP (185291, 378737, 276298)
22. *B. latifolia* (A.Juss.) B.Gates	RB (244357, 78817, 451533); INPA (215076); SP (328863, 319491, 161946)
23. *B. malifolia* var. *apressa* B. Gates	RB (197481, 203185, 11692, 245137); UB (4534, 58889, 58840); SP (266351, 269834, 330459)
24. *B. malifolia* (Nees & Mart.) B. Gates var. *malifolia*	RB (227201, 167017, 318522); UB (2902, 29446); SP (247501, 352031, 247486)
25. *B. martiniana* (A. Juss.) Cuatrec. var. *martiniana*	RB (1012, 325635), SP (79198)
26. *B. martiniana* var. *subnervia* Cuatrec	SP (292853)
27. *B. megaphylla* (A.Juss.) B.Gates	RB (318523, 57964, 234958, 153191, 70740, 223124); UB (24276, 276680); SP (275693, 256227, 161240)
28. *B. membranifolia* (A.Juss.) B.Gates	RB (139047, 465297, 88808, 242941, 324061); UB (58359); SP (363619, 251636, 267592)
29. *B. multifoliolata* (A.Juss.) B.Gates	HUEFS (59557, 65929)
30. *B. muricata* (Cav.) Cuatrec.	RB (403165, 331387, 331331); INPA (104063, 92622, 112527, 117519); UB (58356); SP (345233, 354770, 292254)
31. *B. nummifera* (A.Juss.) B.Gates	RB (26312, 11703); HUEFS (72971); SP (311759, 258060, 292273)
32. *B. oxyclada* (A.Juss.) B.Gates	RB (462436, 256772, 380221); UB (58357); SP (321252, 338414, 306489)
33. *B. paraguariensis* B.Gates	RB (94220, 478482); MBM (323142, 45582, 79507)
34. *B. parviflora* (A.Juss.) B.Gates	MBM (350504, 57095); UB (65209); UPCB (16289); SP (153115, 26500)
35. *B. parviglandula* B.Gates	SP (329049, 276262, 301302); RB (1315)
36. *B. prancei* B.Gates	RB (11689); INPA (120862, 104884, 105558, 120766, 121147); UB (58933); SP (30263)
37. *B. pulchra* B.Gates	RB (360628); MBM (269100, 269096, 45572); UB (58358)
38. *B. quadriglandula* B.Gates	RB (247947); SP (153583)
39. *B. schizoptera* (A.Juss.) B.Gates	MBM (65895, 248945, 244219); UB (58865, 34428); SP (386630, 363464)
40. *B. scutellata* (Griseb.) B.Gates	SP (412014); CEPEC (67045, 102582, 124994)
41. *B. sellowiana* (A.Juss.) B.Gates	RB (280276, 109930)
42. *B. stellaris* (Griseb.) B.Gates	RB (72601, 238905, 214173); INPA (120693, 105103); UB (2160, 219); SP (386633, 251615)
43. *B. variabilis* B.Gates	RB (107054, 130807, 37877); UB (59350, 5211); SP (412422, 257872)
44. *B. vernoniifolia* (A.Juss.) B.Gates	MBM (64120, 249229, 65887); UB (58850); SP (402047, 275889, 167204)
45. *Diplopterys lutea* (Griseb.) W.R.Anderson & C.C.Davis	HUEFS (112131, 183169); SPF (37949)
46. *D. valvata* (W.R.Anderson & B.Gates) W.R.Anderson & C.C.Davis	HUEFS (43868, 39907, 45890)
47. *Stigmaphyllon cavernulosum* C.E.Anderson	VIC (2857, 7519)
48. *Peixotoa tomentosa* A.Juss.	VIC (35777, 35776, 35796)

Table 2. Morphological matrix of binary characters (absence/presence) including 42 species of *Banisteriopsis* and four outgroups: **1.** White petals from flowers in anthesis, **2.** yellow petals from flowers in anthesis, **3.** pink petals from flowers in anthesis, **4.** rounded, concave and sessile leaf glands, **5.** rounded, concave and stalked leaf glands, **6.** rounded, not concave, sessile leaf glands, **7.** concave-convex petiole in transversal section, **8.** plane-convex petiole in transversal section, **9.** cylindrical petiole in transversal section, **10.** vascular system of petioles disposed in open arch, **11.** vascular system of petiole arranged in open arc with convoluted extremities, **12.** accessory bundles, **13.** sclereid sheath surrounding petiole' bundle, **14.** concave midrib in adaxial view, **15.** plane midrib in adaxial view, **16.** convex midrib in adaxial view, **17.** unistratified epidermis on both surfaces, **18.** bistratified epidermis in some regions of the adaxial surface, **19.** bistratified epidermis in some areas of the abaxial surface, **20.** isobilateral mesophyll, **21.** dorsiventral mesophyll, **22.** sclereid groups throughout the mesophyll, **23.** sheath extension, **24.** quaternary veins, **25.** quintenary veins, **26.** Areoles, **27.** veinlets ending freely.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
B. acerosa	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0	1	1
B. adenopoda	1	0	0	0	1	0	1	0	0	1	0	1	0	0	0	1	1	0	0	0	1	0	1	1	1	1	1
B. andersonii	0	0	1	0	0	1	0	1	0	1	0	1	0	1	0	0	1	0	0	1	0	0	1	1	0	1	1
B. angustifolia	0	0	1	0	0	1	0	1	0	1	0	1	0	1	0	0	0	1	0	1	0	0	1	0	0	1	1
B. anisandra	0	1	0	0	0	1	0	1	0	1	0	1	1	0	1	0	0	1	0	0	1	0	0	0	0	1	1
B. arborea	0	0	1	0	1	0	0	1	0	1	0	1	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1
B. argyrophylla	1	0	0	0	1	0	0	1	0	1	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0
B. basifixa	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	1	0	1	0	1	0	0	1	1	1	1	0
B. byssacea	1	0	0	0	0	1	0	1	0	1	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0
B. caapi	0	0	1	0	0	1	0	1	0	1	0	1	0	0	0	1	1	0	0	1	0	0	0	1	1	1	1
B. calcicola	0	0	1	0	1	0	0	1	0	1	0	1	0	0	1	0	1	0	0	0	1	0	1	0	0	1	1
B. campestris	0	0	1	1	0	0	0	1	0	1	0	1	1	0	0	1	1	0	0	1	0	0	1	1	0	1	1
B. cipoensis	0	0	1	0	0	1	0	1	0	1	0	1	0	0	0	1	0	1	0	1	0	0	1	1	0	1	1
B. confusa	1	0	0	1	0	0	0	1	0	1	0	1	0	0	0	1	1	0	0	1	0	0	1	1	0	1	1
B. gardneriana	0	1	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	1
B. goiana	0	0	1	0	0	1	1	0	0	1	0	1	0	0	0	1	1	0	0	1	0	0	1	0	0	1	0
B. harleyi	1	0	0	1	0	0	0	1	0	1	0	1	0	0	0	1	0	1	0	1	0	1	0	0	0	1	1
B. hatschbachii	1	0	0	0	0	1	0	1	0	1	0	1	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1
B. hirsuta	1	0	0	0	0	1	0	1	0	1	0	1	0	0	0	1	1	0	0	1	0	1	1	1	0	1	1
B. irwinii	1	0	0	0	0	1	0	1	0	1	0	1	1	0	1	0	1	0	0	1	0	1	1	1	0	1	1
B. laevifolia	0	1	0	0	0	1	0	1	0	1	0	1	0	0	0	1	0	1	0	1	0	0	0	1	0	1	1
B. latifolia	1	0	0	0	0	1	0	1	0	1	0	1	1	0	0	1	1	0	0	1	0	0	1	1	0	1	1
B. malifolia	0	0	1	0	0	1	0	1	0	1	0	1	0	0	0	1	1	0	0	1	0	0	1	1	0	1	1
B. martiniana	0	1	0	1	0	0	0	1	0	0	1	1	0	1	0	0	0	1	1	0	1	0	0	1	0	1	1
B. megaphylla	0	0	1	0	0	1	1	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	1	1	0	1	1
B. membranifolia	0	0	1	1	0	0	1	0	0	1	0	1	0	0	0	1	1	0	0	1	0	0	0	1	0	1	1
B. multifoliolata	0	0	1	0	0	1	0	1	0	1	0	1	0	0	0	1	1	0	0	0	1	1	1	1	0	1	1
B. muricata	0	0	1	1	0	0	1	0	0	1	0	1	0	1	0	0	1	0	0	1	0	0	1	0	0	1	1
B. nummifera	0	1	0	0	0	1	1	0	0	1	0	1	0	0	0	1	0	1	0	0	1	1	0	1	0	1	1
B. oxyclada	0	0	1	1	0	0	1	0	0	1	0	1	0	0	0	1	1	0	0	1	0	0	1	0	0	1	1
B. paraguariensis	1	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	1	1
B. parviflora	0	1	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	1	0	0	1	1	0	1	0	1	1
B. parviglandula	0	1	0	0	0	1	0	1	0	1	0	1	0	0	0	1	1	0	0	1	0	1	1	1	0	1	1
B. prancei	0	0	1	0	0	1	1	0	0	1	0	1	0	0	1	0	1	0	0	1	0	0	1	1	0	1	1
B. pulchra	0	0	1	1	0	0	0	1	0	1	0	1	1	0	0	1	1	0	0	1	0	1	0	1	0	1	1
B. quadriglandula	0	0	1	0	0	0	1	0	0	1	0	1	0	0	1	0	1	0	0	1	0	1	0	0	0	1	1
B. schizoptera	1	0	0	1	0	0	0	1	0	1	0	1	1	0	0	1	0	1	0	1	0	1	1	1	0	1	1
B. scutellata	1	0	0	0	1	0	0	0	1	1	0	1	0	0	0	1	0	1	0	1	0	0	0	1	0	1	1
B. sellowiana	0	1	0	1	0	0	0	1	0	1	0	1	0	0	1	0	0	1	0	0	1	1	0	1	0	1	1
B. stellaris	1	0	0	1	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0	1	1
B. variabilis	1	0	0	1	0	0	0	1	0	1	0	1	0	0	0	1	1	0	0	1	0	0	1	1	0	1	1
B. vernoniifolia	1	0	0	0	0	1	0	1	0	1	0	1	0	0	0	1	1	0	0	1	0	1	0	0	0	1	1
Diplopterys lutea	0	1	0	1	0	0	0	1	0	1	0	1	1	0	0	1	1	0	0	0	1	0	0	0	0	1	0
Diplopterys valvata	0	1	0	0	0	1	0	1	0	0	1	1	1	0	0	1	1	0	0	0	1	0	0	0	0	1	0
Stigmaphyllon cavernulosum	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0
Peixotoa tomentosa	0	1	0	0	0	1	0	1	0	1	0	1	1	0	0	1	1	0	0	1	0	0	1	0	0	0	0

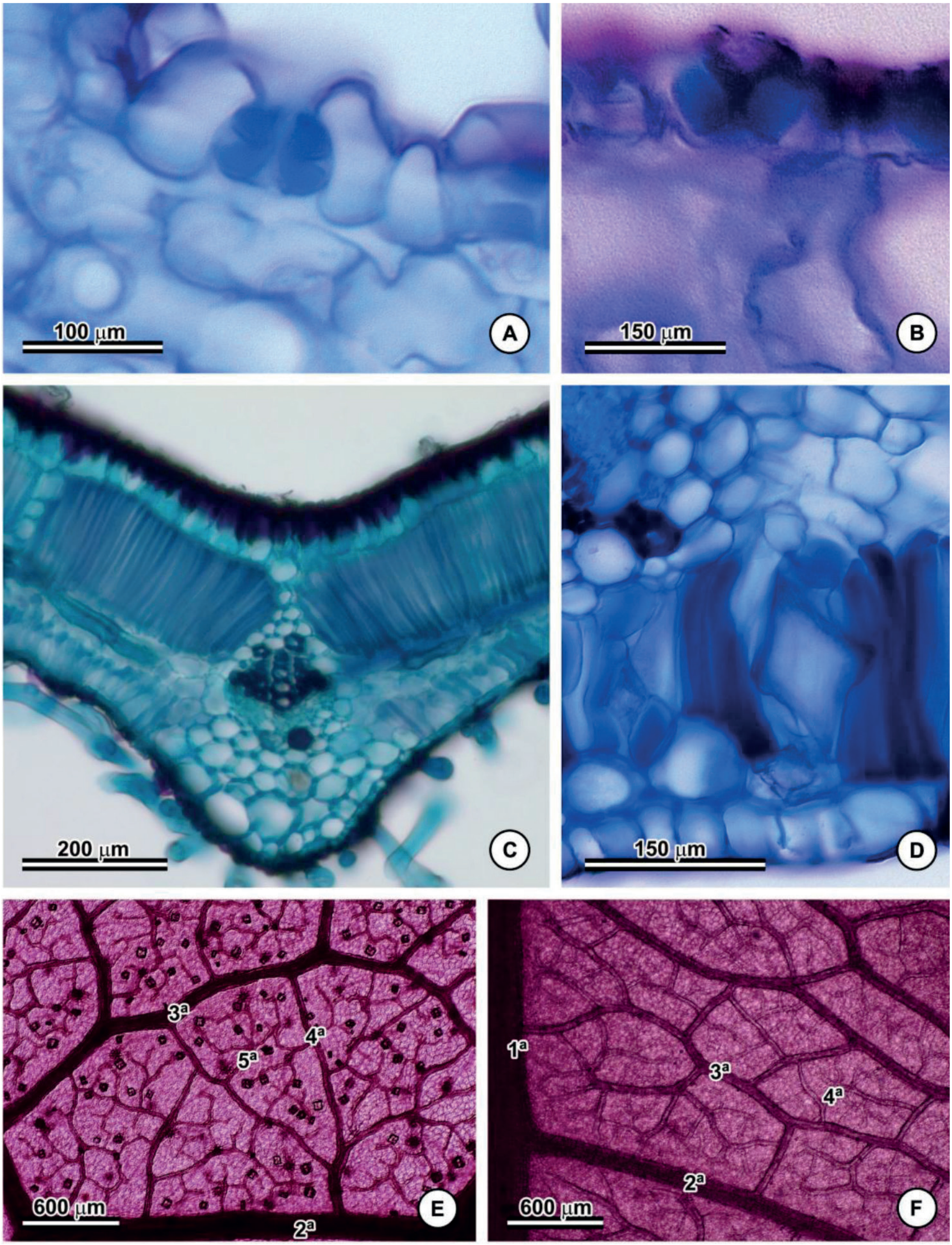

Figure 4. Mesophyll transversal sections and diaphanization of species of *Banisteriopsis*: **A.** detail of the stomata with subsidiary cells projected over guard cells in *B. byssacea*, **B.** details of the stomata with stomatal crests in *B. martiniana*, **C.** sheath extension in *B. andersonii*, **D.** prismatic crystals in *B. muricata*, **E.** the presence of quintenary veins in *B. adenopoda*, **F.** the absence of quintenary veins in *B. sellowiana*.

crests are absent (Fig. 4A), except in the varieties of *B. martiniana* (Fig. 4B), with subsidiary cells not covering guard cells. Malpighiaceous hairs were observed in most species (Fig. 4C), but it was not possible to distinguish their morphotypes (T, Y or V-shaped), due to these hairs being deciduous in most species, probably due to the re-hydratation method. In *Banisteriopsis acerosa* and *B. caapi*, we observed scars from deciduous hairs. On the other hand, *B. hatschbachii*, *B. latifolia*, *B. parviflora*, *B. sellowiana*, *B. stellaris* and in the varieties of *B. martiniana*, all leaves were always glabrous without hair scars (Fig.4E-F). The extension of the parenchymal (Fig. 4C) or sclerenchymatic sheath was observed in most species, except in *Banisteriopsis anisandra*, *B. argyrophylla*, *B. byssacea*, *B. caapi*, *B. gardneriana*, *B. harleyi*, *B. hatschbachii*, *B. laevifolia*, both varieties of *B. martiniana*, *B. membranifolia*, *B. nummifera*, *B. paraguariensis*, *B. parviflora*, *B. pulchra*, *B. quadriglandula*, *B. scutellata*, *B. sellowiana*, *B. stellaris* and *B. vernoniifolia*. The presence of prismatic crystals (Fig. 4D) was observed in most of the analyzed species.

All species showed pinnate vein pattern, absence of basal veins, with secondary veins ramified in loops, not reaching the margin of the leaf blade. Angle variation among midrib and secondary veins was uniform, with the secondary veins being decurrent and the last marginal vein looped. Quaternary veins were recorded in most species (Fig. 4E-F), except in *B. acerosa*, *B. angustifolia*, *B. anisandra*, *B. arborea*, *B. argyrophylla*, *B. byssacea*, *B. calcicola*, *B. goiana*, *B. harleyi*, *B. muricata*, *B. oxyclada*, *B. paraguariensis*, *B. quadriglandula* and *B. vernoniifolia*. Quintenary veins were recorded only in *B. adenopoda* (Fig. 4E) and *B caapi*. Areoles were observed in *B. argyrophylla* and *B. byssacea*, with veinlets ending freely in most species, except in *B. argyrophylla*, *B. byssacea* and *B. goiana*, in which it was absent.

A total of three morphological patterns for leaf glands were observed in almost all species, except in *B. quadriglandula*, in which glands were absent. Rounded, concave and stalked glands (Fig. 5A) were recorded in *B. adenopoda*, *B.arborea*, *B. argyrophylla*, *B. basifixa*, *B. calcicola* and *B. scutellata*. On the other hand, *B. acerosa*, *B. andersonii*, *B. angustifolia*, *B. anisandra*, *B. byssacea*, *B. caapi*, *B. cipoensis*, *B. gardneriana*, *B. goiana*, *B. hatschbachii*, *B. hirsuta*, *B. irwinii*, *B. laevifolia*, *B. latifolia*, *B. malifolia*, *B. megaphylla*, *B. multifoliolata*, *B. nummifera*, *B. parviglandula*, *B. prancei*, *B. quadriglandula*, *B. scutellata* and *B. vernoniifolia* all glands were rounded and sessile, without concavity (Fig. 5B). In the remaining species, glands were rounded concave and sessile (Fig. 5C) .

An identification key based on morphoanatomical characters for all studied species of *Banisteriopsis* is presented below:

1. Mesophyll dorsiventral (except for *B. laevifolia*), petals yellow in anthesis............ 2
1'. Mesophyll isobilateral (except for *B. argyrophylla*), petals pink to white in anthesis (Fig. 1M)............10
2. Unistratified epidermis .... *B. parviglandula*
2'. Bistratified epidermis at some portions 3
3. Guard cells with crests .... *B. martiniana*
3'. Guard cells with projections of subsidiary cells............4
4. Leaf glands rounded and stalked .... *B. basifixa*
4'. Leaf glands rounded and sessile 5
5. Leaf glands concave 6
5'. Leaf glands flat 7
6. Petioles transversally concave-convex............ *B. parviflora*
6'. Petioles transversally plane-convex............ *B. sellowiana*
7. Mesophyll isobilateral............ *B. laevifolia*
7'. Mesophyll dorsiventral 8
8. Vascular system of petioles in open arch with convoluted extremities .... *B. gardneriana*
8'. Vascular system of petioles in open arch without convoluted extremities............9
9. Midrib adaxially plane............ *B. anisandra*
9'. Midrib adaxially convex............ *B. nummifera*
10. Flowers with white petals in anthesis............11
10'. Flowers with pink petals in anthesis............25
11. Epidermis bistratified in some regions............12
11'.Epidermis unistratified 16
12. Leaf glands rounded and stalked............ *B. scutellata*
12'. Leaf glands rounded and sessile............13
13. Glands with concavity............14
13'. Glands without concavity............ *B. acerosa*
14. Accessory bundles present............15
14'. Accessory bundles absent............ *B. stellaris*

15. Presence of a sclereid sheath around vascular bundles in the petiole……*B. schizoptera*
15'. Absence of a sclereid sheath around vascular bundles in the petiole……*B. harleyi*
16. Petiole contour concave-convex……17
16'. Petiole contour plane-convex……18
17. Presence of sheath extension in the mesophyll……*B. adenopoda*
17'. Absence of sheath extension in the mesophyll……*B. paraguariensis*
18. Mesophyll dorsiventral……*B. argyrophylla*
18'. Mesophyll isobilateral……19
19. Midrib contour adaxially plane……*B. irwinii*
19'. Midrib contour adaxially concave……20
20. Leaf glands rounded with concavity……*B. confusa*, *B. variabilis*
20'. Leaf glands rounded without concavity……21
21. Sheath extension present……22
21'. Sheath extension absent……23
22. Sclereid sheath extension present around petioles' vascular bundles……*B. latifolia*
22'. Sclereid sheath extension absent around petioles' vascular bundles……*B. hirsuta*
23. Mesophyll sclereids present……*B. vernoniifolia*
23'. Mesophyll sclereids absent……24
24. Sclereid sheath extension present around petioles' vascular bundles……*B. hatschbachii*
24'. Sclereid sheath extension absent around petioles' vascular bundles……*B. byssacea*
25. Epidemis unistratified……27
25'. Epidermis bistratified in some regions……26
26. Midrib adaxially concave……*B. angustifolia*
26'. Midrib adaxially convex……*B. cipoensis*
27. Leaf glands present……28
27'. Leaf glands absent……*B. quadriglandula*
28. Leaf glands rounded and stalked……29
28'. Leaf glands rounded and sessile……30
29. Mesophyll dorsiventral……*B. calcicola*
29'. Mesophyll isobilateral……*B. arborea*
30. Glands with concavity……31
30'.Glands without concavity……35
31. Petiole's contour plane-convex……32
31'. Petiole's contour concave-convex……33
32. Sheath extension present……*B. campestris*
32'. Sheath extension absent……*B. pulchra*
33. Midrib adaxially concave……*B. muricata*
33'. Midrib adaxially convex……34
34. Mesophyll sheath extension present……*B. oxyclada*
34'. Mesophyll sheath extension absent……*B. membranifolia*
35. Petiole transversally concave-convex……36
35. Petiole transversally plane-convex……38
36. Midrib adaxially plane……*B. prancei*
36'. Midrib adaxially convex……37
37. Mesophyll dorsiventral……*B. megaphylla*
37'. Mesophyll isobilateral……*B. goiana*
38. Midrib adaxially concave……*B. andersonii*
38'. Midrib vein adaxially convex……39
39. Sheath extension present……40
39'. Sheath extension absent……*B. caapi*
40. Mesophyll dorsiventral……*B. multifoliolata*
40'. Mesophyll isobilateral ……*B. malifolia var. malifolia*, *B. malifolia var. apressa*

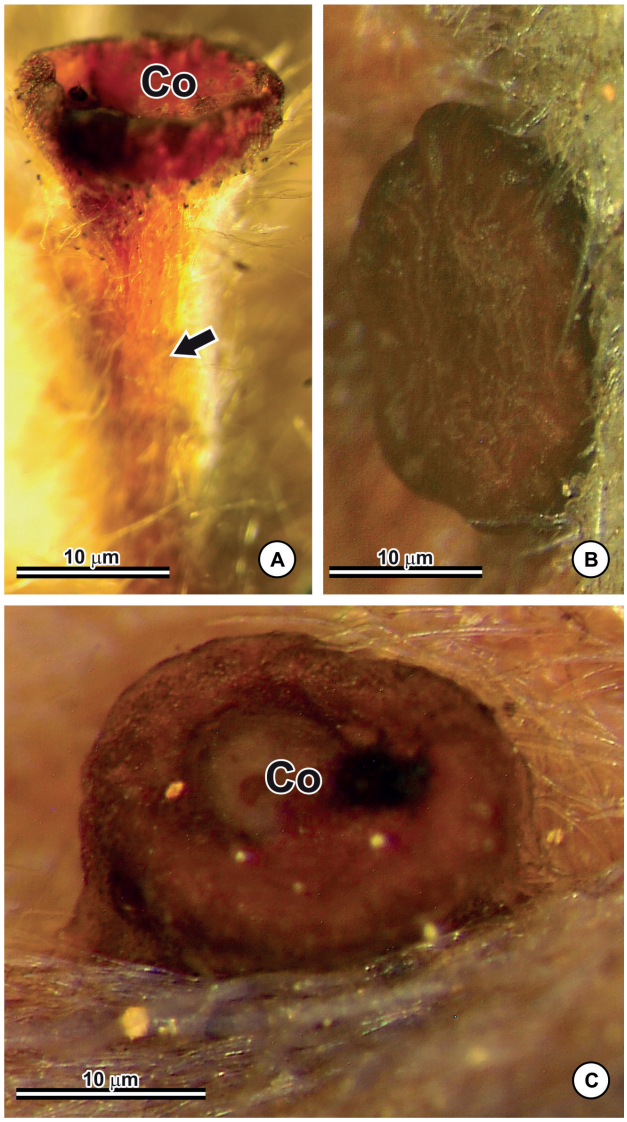

Figure 5. Shape of leaf glands in *Banisteriopsis*. **A.** *B. scutellata* – Rounded gland with concavity and stalked, **B.** *B. goiana* – Rounded gland without concavity and sessile, and **C.** *B. campestris* – Rounded gland with concavity and sessile. Co = concavity, arrow = stalk.

Leaf anatomy of outgroups species

The outgroup species show yellow petals, unistratified epidermis, presence of accessory bundles in petioles (Fig. 6A-B), midrib adaxially convex (Fig. 6C), and course of secondary veins brochidodromous. Rounded and sessile leaf glands were present in *D. lutea* (with concavity), *D. valvata*, and *P. tomentosa* (without concavity), and absent in *S. cavernulosum*. The mesophyll is isobilateral (Fig. 6D) only in *P. tomentosa* and dorsiventral (Fig. 6E) in the remaining species. Sheath extension (Fig. 6F) was present only in *P. tomentosa*. *S. cavernulosum* showed a concave-convex petiole contour (Fig. 6A), while the remaining species were plane-concave (Fig. 6B). Vascular bundles of petioles in *D. valvata* and *S. cavernulosum* are the open arch type with convoluted extremities (Fig. 6A), while in *D. lutea* and *P. tomentosa* it is only open arch type (Fig. 6B). Areoles were found only in *D. lutea* and *D. valvata*.

Multivariate analysis

The similarity analysis with UPGMA (Fig. 7) arranged all *Banisteriopsis* species in four groups, besides the outgroups. *Banisteriopsis* was grouped based on the concave shape of its leaf glands, and primary vein adaxially convex. The first group comprises *B. nummifera*, *B laevifolia*, *B. sellowiana*, *B. martiniana*, and *B. gardneriana*, which share the presence of yellow petals and quaternary veins. The second group comprises *B. adenopoda*, *B. argyrophylla* and *B. calcicola*, which share the presence of a dorsiventral mesophyll, unistratified epidermis on both surfaces, and rounded and stalked glands with a concavity. The third group comprises *B. paraguariensis*, *B. prancei*, *B. muricata*, *B. harleyi*, *B. confusa*, *B. latifolia*, *B. caapi* and *B. pulchra*, which do not share exclusive characters but show the presence of accessory vascular bundles, isobilateral mesophyll and vascular system of petioles in open arch. And a fourth group comprising *B. acerosa* and *B. angustifolia*, which share the presence of sheath extensions and rounded, and sessile leaf glands.

Discussion

Leaf anatomy

The anatomical and macromorphological characters used on the identification key allowed the distinction of 40 from the 43 sampled species. The most relevant characters used to differentiate the species: petal color, type of leaf glands, presence/absence of bistratified epidermis, shape of petioles, shape of the midrib, position of vascular bundles on petioles, presence/absence of sheath extension, presence/absence of sclereid sheath around petiole's bundles, and mesophyll type.

A total of three patterns of petiole contour were observed in the 42 analyzed species of *Banisteriopsis*. This difference was considered relevant to distinguish the species, corroborating the results of Araújo *et al.* (2010) that the petiole contour is a resourceful character to help identifying species of Malpighiaceae. Petiole anatomical characters are regarded as useful to distinguish taxa due to its stability in response to different environmental variables (Metcalfe & Chalk 1979). This character has successfully been applied to comparative anatomical studies in several plant families (Rio *et al.* 2005; Costa *et al.* 2010; Moraes *et al.* 2011; Ogundare & Saheed 2012; Almeida Jr. *et al.* 2013; Coutinho *et al.* 2013). Previously, only two types of petiole contour were reported in the literature for Malpighiaceae, concave-convex, and plane-convex (Araújo *et al.* 2010). The results obtained in this study corroborate those data and add a third new type of petiole contour to the family, the circular one.

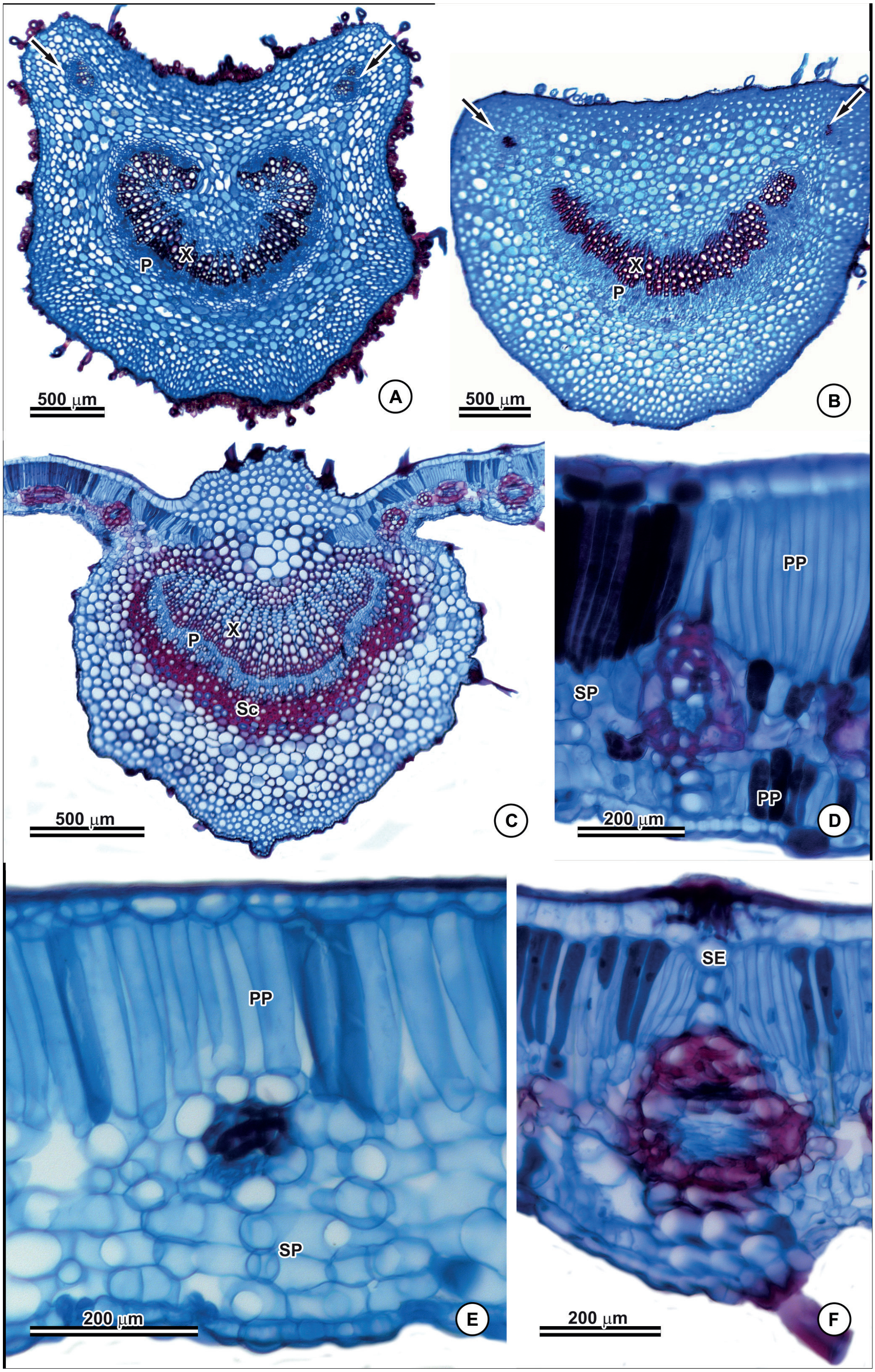

Figure 6. Anatomical characters of petioles and leaf blades of outgroups: **A.** petiole in transversal section of *S. cavernulosum*, **B.** petiole in transversal section of *D. lutea*, **C.** midrib in transversal section of *P. tomentosa*, **D.** mesophyll in transversal sections of *P. tomentosa*, **E.** mesophyll in transversal sections of *S. cavernulosum*, **F.** sheath extension in *P. tomentosa*. Abbreviations: P = phloem; PP = palisade parenchyma; Sc = sclerenchymatous fibers; SE = sheath extension; SP – spongy parenchyma; X = xylem.

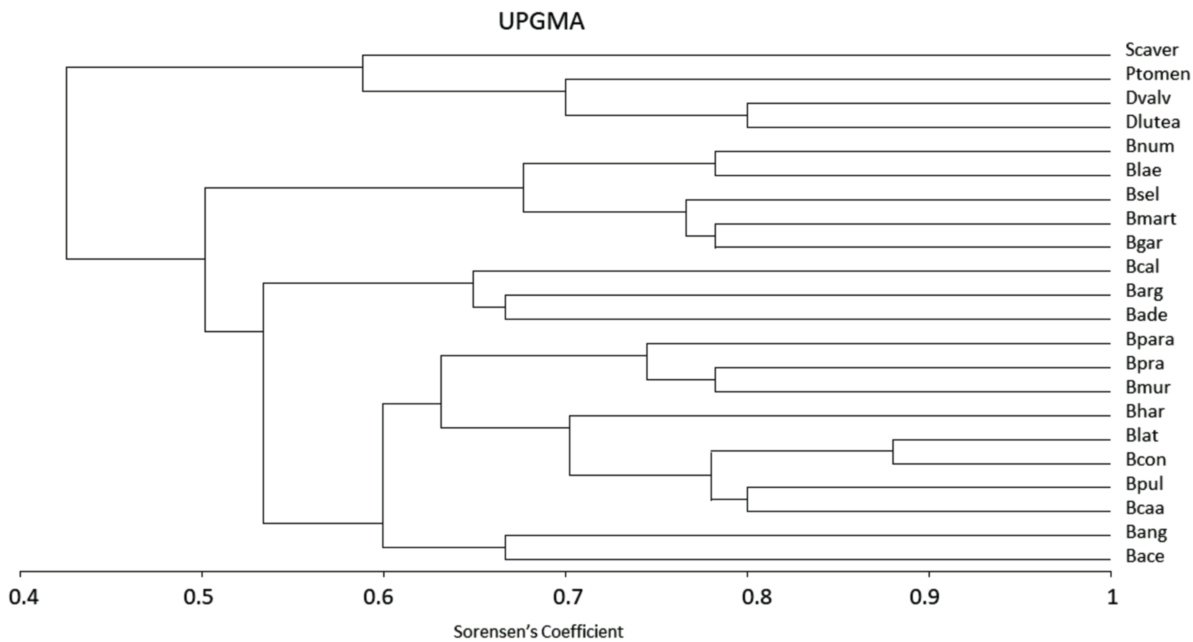

Figure 7. Cluster analysis (UPGMA) of species of *Banisteriopsis*. Abbreviations: Bace *B. acerosa*, Bade *B. adenopoda*, Bcon *B. confusa*, Blat *B. latifolia*, Bpul *B. pulcra*, Bhar *B. harleyi*, Bpara *B. paraguariensis*, Bmur *B. muricata*, Bang *B. angustifolia*, Bpra *B. prancei*, Bcaa *B. caapi*, Blae *B. laevifolia*, Bnum *B. nummifera*, Bgar *B. gardneriana*, Bsel *B. sellowiana*, Bmart *B. martiniana*, Barg *B. argyrophylla*, Bcal *B. calcicola*, Dlutea *Diplopterys lutea*, Dvalv *D. valvate*, Ptomen *Peixotoa tomentose*, Scaver *Stigmaphyllon cavernulosum.*

The petiole vascular bundles showed two different types of conformation in the studied species. Our results showed that the position of vascular bundles on petioles is useful to the group and distinguish species of *Banisteriopsis*, corroborating the data of Metcalfe & Chalk (1950) and Araújo *et al.* (2010) for Malpighiaceae. Characters related to vascularization patterns of petioles have been widely used to improve the taxonomy of different families of flowering plants, such as Melastomataceae (Reis *et al.* 2004), Myrtaceae (Oliveira *et al.* 2011), Rubiaceae (Moraes *et al.* 2011), Sapotaceae (Almeida Jr. *et al.* 2013) and Leguminosae (Francino 2006; Coutinho *et al.* 2013). Accessory bundles occurred in 40 of 42 species of *Banisteriopsis*, varying in the number of bundles, sometimes in specimes from the same species. Even though the occurrence of accessory bundles is an important character to help distinguishing species within Malpighiaceae (Araújo *et al.* 2010) and different families of flowering plants (Bieras & Sajo 2004; Moraes *et al.* 2011; Coutinho *et al.* 2013; Almeida Jr. *et al.* 2013), it was inconstant and not taxonomically significant (homoplastic) in our study. Only six from the 42 analyzed species did not present hairs, and due to the lack of studies on leaf ontogeny for *Banisteriopsis*, it is not possible to assert if those species are truly glabrous or just glabrescent at age.

The bistratified epidermis was found in 15 from the 42 analyzed species, indicating that this character is typical in the genus. The bistratified epidermis was mentioned by Metcalfe & Chalk (1950) for species of *Acridocarpus*, *Banisteriopsis*, *Blepharandra*, *Hiraea*, and *Tetrapterys*. Additionally, Araújo *et al.* (2010) mentioned this character for *Banisteriopsis*, *Byrsonima*, and *Heteropterys*. Although this character has limited applicability in a phylogenetic context, it has already been used to distinguish species of *Manilkara* Adans. (Sapotaceae) (Almeida Jr. *et al.* 2013). Stomata with subsidiary cells larger than guard cells and absence of stomatic crests occurred in 41 from 42 species. Those characters were previously useful to distinguish species in *Banisteriopsis* (Araújo *et al.* 2010) and *Byrsonima* (Beiguelman 1962), the latter also showing stomata crests, but further studies in the remaining genera of Malpighiaceae are needed in order to test its taxonomic relevance at the generic level.

Isobilateral mesophyll was predominant in relation to dorsiventral one, being found in 30 from 42 studied species. The occurrence of those types of mesophyll was previously reported for *Banisteriopsis* and other genera of Malpighiaceae such as *Acridocarpus*, *Banisteriopsis*, *Blepharandra*, *Byrsonima*, *Heteropterys*, *Hiraea*, and *Tetrapterys* (Metcalfe & Chalk 1950; Pereira 1953; Araújo *et al.* 2010). However, this is the first time that it is pointed out as a taxonomically relevant character at the generic level for *Banisteriopsis*. The type of mesophyll has been used in different anatomical studies applied to taxonomy, and allied to additional characters, was useful in distinguishing species of Apocynaceae (Rio *et al.* 2005), Celastraceae (Gomes *et al.* 2005), Leguminosae (Coutinho *et al.* 2013), Myrtaceae (Gomes *et al.* 2009), and Piperaceae (Gogosz *et al.* 2012).

The variation in the shape of the midrib observed in the studied species was useful to elaborate an identification key, corroborating its taxonomic relevance for Malpighiaceae

(Araújo *et al.* 2010). In the study of Bieras & Sajo (2004), this character was also useful to distinguish species of Erythroxylaceae. Data on the venation patterns from the 42 species of *Banisteriopsis* studied showed limited variation, with a conserved venation pattern that might be taxonomic significant at the generic level. Future studies on character evolution in the Stigmaphylloid clade might shed some light on this matter.

Araújo & Meira (2016) have already studied the anatomy and macromorphology of leaf glands in *Banisteriopsis*. These authors observed a variation pattern in relation to the shape of theses structures, but have not observed any significant difference in the anatomy of these glands. The same patterns were observed in this study, with the macromorphological differences being important in differentiating species in *Banisteriopsis*.

Multivariate analysis

Banisteriopsis was characterized in the UPGMA analysis and distinguished from *Diplopterys*, *Peixotoa* and *Stigmaphyllon* by leaf glands concave, primary vein adaxially convex and veinlets ending freely. Since Davis & Anderson (2010) recovered *Banisteriopsis* as monophyletic in the last molecular phylogeny for the family, those anatomical characters might be recovered as morphological synapomorphies for the genus in future character-mapping studies. It seems that only the type of mesophyll characterized the two main groups recovered by the UPGMA analysis, as described in the first step of the abovementioned identification key. These two major groups roughly represent the two subgenera currently recognized in *Banisteriopsis* (subg. *Banisteriopsis* and *Hemiramma*) (Gates 1982; Anderson & Davis 2006), and were recovered as monophyletic by Davis & Anderson (2010). Thus, future studies of character mapping might recover these anatomical characters as synapomorphies for the subgenera of *Banisteriopsis*.

Our results also corroborate some of the informal groups of *Banisteriopsis* proposed by Gates (1982) in the last taxonomic revision for this genus. Those informal groups are partially recovered as monophyletic by Davis & Anderson (2010), with four major lineages in the genus. Group 1 from Davis & Anderson (2010) comprises *B. prancei*, *B. martiniana*, *B. sellowiana*, *B. nummifera*, and *B. gardneriana*. It coincides with the group formed in the similarity analysis, except by the presence of *B. laevifolia*. This group is almost entirely represented by species from the informal group *B. martiniana* and *B. nummifera* from Gates (1982). Group II from Davis & Anderson (2010) comprises *B. latifolia*, *B. confusa*, *B. adenopoda*, *B. pulchra*, and *B. caapi*, which partially corresponds to group III of the similarity analysis. This group contains species from four different informal groups proposed by Gates (1982) and was only grouped by sharing homoplastic characters. On the other hand, group II obtained in the similarity analysis comprises *B. calcicola*, *B. argyrophylla*, and *B. adenopoda*. Those species are assigned to three different informal groups by Gates (1982) and three clades by Davis & Anderson (2010). The same pattern was found in group IV from the similarity analysis, which comprises *B. angustifolia* and *B. acerosa*, both assigned to two different informal groups by Gates (1982), and two clades by Davis & Anderson (2010).

Conclusions

The present study included 75 % of all currently accepted species of *Banisteriopsis* and 90 % of all species occurring in Brazil. Our results evidenced that characters related to the shape of leaf glands, the shape of the midrib in the adaxial surface of leaves and veinlets ending freely might be important in characterizing genera in Malpighiaceae. Additionally, we corroborated part of the informal groups currently recognized in *Banisteriopsis*, which are already confirmed as monophyletic, opening the possibility to recognize them as infrageneric ranks in future studies of character evolution in *Banisteriopsis*.

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