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Original Article

From the present to the past: a broad study of anatomical diversification in the leaves of the large Byrsonimoid lineage (Malpighiaceae)

João Victor Da Costa Santos[1,*], Josiane Silva Araújo[2], Miller Melo Sanches[1], Marcelo Rodrigo Pace[3], Brenda Hernández-Hernández[3], Renata Maria Strozi Alves Meira[1]

[1]Departamento de Biologia Vegetal, Programa de Pós-Graduação em Botânica, Universidade Federal de Viçosa, Viçosa, Minas Gerais, 36570-900, Brazil
[2]Campus Heróis do Jenipapo, Universidade Estadual do Piauí, Campo Maior, Piauí, 64280-000, Brazil
[3]Instituto de Biología, Departamento de Botánica, Universidad Nacional Autónoma de México, Ciudad de México, 04510, Mexico

[*]Corresponding author. Departamento de Biologia Vegetal, Programa de Pós-Graduação em Botânica, Universidade Federal de Viçosa, Avenida Peter Henry Rolfs, Viçosa, Minas Gerais, 36570-900, Brazil. E-mail: victorbarrosov@gmail.com

ABSTRACT

Recent phylogenetic studies and taxonomic revisions in the Malpighiaceae family have sparked renewed interest in leaf anatomy. Byrsonimoid *s.l.* is a large lineage of trees that includes three main clades: Acmantheroid, Byrsonimoid *s.s.*, and Galphimioid, which are sister to all other Malpighiaceae. This lineage has a long history of taxonomic changes, and while it is currently well-supported by molecular data, many clades still lack morpho-anatomical synapomorphies. In this study, we outline and map anatomical traits to document the diversity, potential synapomorphies, and evolution of characters in Byrsonimoid *s.l.*, enhancing our understanding of their systematics. Samples were collected from herbarium vouchers or in the field and processed using standard anatomical methods for light microscopy. Twenty-nine characters were mapped onto a well-supported phylogeny for Byrsonimoids *s.l.* Seventeen potential synapomorphies and one autapomorphy were identified. The presence of hypodermis, leaf secretory structures, the configuration of the vascular system of the petiole and midrib, cortical sclereids in the petiole, and the fibre cap at the margin are considered evolutionary novelties within the various clades of Byrsonimoid *s.l.* The probabilistic and maximum likelihood analyses allow us to identify at least 12 ancestral traits in Byrsonimoid *s.l.* that include: T-shaped trichomes, mucilaginous and non-papilous epidermis, stomata with ridges and that are level with the epidermis, absence of both nectaries and hypodermis, open arch without convoluted ends in petiole vascular system conformation and medullary bundles, midrib biconvex contour, and dorsiventral mesophyll with sclerified sheath surrounding the bundles. Our findings help clarify the anatomical diversity within the Byrsonimoid lineage and its distribution across the phylogeny.

Keywords: extrafloral nectaries, laticifers, leaf structure, mucilaginous idioblasts, malpighiales, character evolution

INTRODUCTION

Malpighiaceae Juss. is a pantropical plant family and comprises monophyletic group, which consists of 14 mostly well-supported clade, of which the Byrsonimoid *s.l.* is the sister group to all other Malpighiaceae (Cameron *et al.* 2001, Davis *et al.* 2001, Davis and Anderson 2010). The Byrsonimoid clade includes three of these 19 clades, with Byrsonimoid *s.s.* being sister to the Acmantheroid clade, with both of these clades are sister to the Galphimioid clade, all of which are strictly Neotropical (Davis and Anderson 2010).

Traditionally, fruit morphology was the main characteristic to categorize the infrafamilaire groups of Malpighiaceae (Niedenzu 1901, 1928). However, even before the advent of molecular phylogenies (Anderson 1977), it became evident that fruit evolution within the family experienced multiple events of homoplasy (Davis *et al.* 2001). This highlighted the necessity of examining other characters, particularly those related to flowers and the vegetative organs (Anderson 1977, 1981, Davis and Anderson 2010).

The Byrsonimoid *s.s.* comprises the genera *Byrsonima* Rich ex. kunth (135 spp.), *Blepharandra* Griseb. (six spp.), and *Diacidia* Griseb. (11 spp.); the Acmantheroid clade comprises the genera *Acmanthera* Adr. Juss. Griseb. (seven spp.), *Coleostachys* A. Juss. (one sp.), and *Pterandra* A. Juss. (15 spp.); and the Galphimiod clade comprises *Andersonioxa* C. Davis & Amorim (three spp.) (Davis *et al.* 2020a, 2020b), *Galphimia* Cav. (26 spp.), *Lophanthera* A. Juss. (three spp.), *Spachea* A. Juss. (six spp.),and *Verrucularia*

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A. Juss. (two spp.) (Davis and Anderson 2010). These relationships have been strongly supported by the most recent molecular phylogenies (Davis and Anderson 2010, Davis *et al.* 2014), but still lack a taxonomic treatment for a more natural classification. Furthermore, the relationships between groups are still the subject of taxonomic investigations, resulting in substantial changes and taxa being recircumscribed (Davis *et al.* 2020a, 2020b, Almeida and Van den Berg 2021, Almeida *et al.* 2024).

On the basis of morphological data, Anderson (1977) proposed a group that unites most of the previously mentioned taxa. He established the subfamily Byrsonimoideae, which would be considered monophyletic only if the genera *Burdachia* and *Glandonia* are excluded (Davis and Anderson 2010). However, *Burdachia* and *Glandonia* currently comprise the Mcvaughia clade, distantly related to the Byrsonimoid *s.l.* in the family's phylogenetic hypothesis (Davis and Anderson 2010). This means that purely morphological characters do not represent a basis for the natural classification of these groups. Given this gap, other approaches deserve to be considered.

Byrsonima is the most diverse genus of the Byrsonimoid *s.l.*, with ∼135 species (Anderson *et al.* 2006), and the second largest in the family, after *Heteropterys* A. Juss. (Anderson 1997a, 2011). Infrageneric categories within *Byrsonima* were delimited by Niedenzu (1901, 1928) in the beginning of the 20th century (1901, 1928) and have been used in classical taxonomical studies (Cuatrecasas 1958). However, these categories (both subgenera and sections) represent polyphyletic groups (Davis and Anderson 2010), highlighting the need for further studies on group diversity to propose and support new taxonomic categories.

From this perspective, leaf anatomy provides valuable characters for taxonomic delimitations, particularly in groups with hard-to-recognize species (Metcalfe and Chalk 1950, 1979, Solereder 1908). In Malpighiaceae, anatomical characters have been critical to distinguishing species in various genera, such as *Banisteriopsis* C.B.Rob. (Araújo *et al.* 2020), *Byrsonima* (Santos *et al.* 2020), *Glandonia* Griseb. (Guesdon *et al.* 2018), *Mcvaughia* W.R.Anderson (Almeida *et al.* 2019). In a phylogenetic study, the presence of fibres in the abaxial face of vascular system of the leaf blade were suggested as a synapomorphy for *Amorimia* W.R.Anderson (Mello *et al.* 2019).

Given this scenario of gaps, its long history of taxonomic changes in the group, and the power of leaf anatomy to delimit lineages in Malpighiaceae, our objective herein was to conduct a comprehensive comparative study of leaf anatomy in the Byrsonimoid *s.l.*, to evaluate the evolution of characteristics within the group, identifying which characters are conserved (potential synapomorphies), which are convergent (possibly adaptive to certain ecological conditions), and which appear to have evolved randomly.

MATERIAL AND METHODS

Members of Byrsonimoid *s.l.* lineage were anatomically analysed here (Table 1). Samples of mature leaves were collected in the field for *Byrsonima daelbata*, *B. verbascifolia*, *B. sericea*, and *Pterandra pyroidea*, being fixed in FNT (neutral buffered formalin) (Lillie 1965) and stored in the same fixative. Voucher materials were deposited at the Herbarium of Universidade Federal de Viçosa (Herbário da Universidade Federal de Viçosa- VIC) (Table 1). Samples of the remaining species were obtained from voucher specimens in good condition, deposited in national and foreign herbaria (Table 1; acronyms follow Thiers 2025, continuously updated). Leaves from the third to the fifth stem node obtained from fresh material, fixed samples, or herbarium vouchers were processed and analysed. However, due to the unavailability of dried material in good conditions, it was unfeasible to analyse the petiole traits of the following species: *Byrsonima affinis*, *B. arctostaphylloides*, *Diacidia cordata*, and *Spachea elegans*. For the same reason, the types of trichome in *Byrsonima cipoensis*, *B. poeppigiana*, *B. rigida*, *Galphimia angustifolia*, *G. glandulosa*, *G. glauca*, *G. gracilis*, *G. langlassei*, *G. mexiae*, *G. mirandae*, *G. multicaulis*, *G. oaxacana*, *G. speciosa*, and *Spachea membranacea* have not been scored. The leaf margin of *Galphimia mexiae* was also not evaluated.

Freehand sections were stained with Safrablau (Kraus and Arduin 1997) and histochemical tests for the detection of total polysaccharides, using the PAS reagent (O'Brien and McCully 1981), were performed on samples of the species collected and fixed in the field.

Character delimitation and anatomical description

We delimited 29 characters according to their diversity. Leaves were boiled in hot water for rehydration. Subsequently, they were left for ∼2 hours in 2% potassium hydroxide, washed four times in the water with an interval of 20–40 minutes between each wash (Smith and Smith 1942, modified). These leaves were dehydrated in ethanol and then stored in 70% ethanol.

The stored samples from both dried and fixed field-collected material were embedded in histological methacrylate resin (Historesin, LeicaInstruments, Heidelberg, Germany, prepared according to the manufacturer's instructions) and sectioned with 5 μm thicknesses, in a rotating automatic microtome (model RM2265, Leica Microsystems Inc., Deerfield, USA), using glass knives. Histological sections were stained in toluidine blue at pH 4.7 (O'Brien and McCully 1981) and the slides were mounted with synthetic resin (Permount-Fisher). Images were taken using an optical microscope (Model AX70TRF, Olympus Optical, Tokyo) equipped with a digital camera (AxioCam HRc; Zeiss, Gottingen, Germany) at the Laboratory of Plant Anatomy of Universidade Federal de Viçosa, Brazil.

Multistate matrix and identification key performed

We used selected anatomical characters to produce binary and multistate character matrices with their respective encoded characters (Table 2). The selection of characters was based on data traditionally used in applied anatomy studies in Malpighiaceae, which do not exhibit variation in response to environmental conditions (Araújo *et al.* 2010, 2020, Santos *et al.* 2020, 2023, Vilarinho *et al.* 2023, Silva *et al.* 2025).

The presence or absence of a given neomorphic feature was considered in the construction of the binary matrix. For the multistate matrix (Table 2), the transformational character states with mutual occurrence (more than one) or restricted in each taxon was considered. This character encoding, as well as the assignments to the concepts of neomorphic and transformational characters, are based on Assis (2019) revised criteria.

Table 1. Species analysed from the three clades of Byrsonimoid *s.l.*

Clade	Species	Voucher
Byrsonimoid *s.s.*	*Byrsonima correifolia* A. Juss.	Perillo 420 (BHCB 153316); L. Perillo 308 (BHCB 153204); Neto 692 (BHCB 24155)
	B. bumeliifolia A. Juss.	Harley 20779 (NYBG 472489)
	B. sericea DC.	Campos s.n. (BHCB 17230); Rezende 7149 (BHCB 200058); Souza, Machado e Araujo (BHCB 195154)
	B. umbellata Mart. ex A. Juss.	Souza, Souza, Scalon, Romão e Araújo 24738 (BHCB 162788); Costa e Horta s.n (BHCB 22523); s.n (BHCB 36142)
	B. guilleminiana A. Juss.	Lemos-Filho e Lovato s.n. (BHCB 174568); Stehmann s.n. (BHCB 18939); Heringer 5644 (IBGE)
	B. subterranea Brade&Markgr.	Barreto 7682 (BHCB 66710); s.n. (BHCB 49553); Fonseca s.n. (BHCB 11670).
	B. variabilis A. Juss.	Castro 8190 (BHCB); M.C.H. Mamede 5739 (SPF)
	B. cipoensis Mamede	s.n (BHCB 39742); Pereira 776 (BHCB 19808)
	B. vacciniifolia A. Juss.	Souza, Lorenzi e Romão 29594 (BHCB 162789); Mota e Vasconcelos 3937 (BHCB 184553); Azevedo, Oliveira Stehmann e Fernandes 560 (BHCB 194314)
	B. clausseniana A. Juss.	Mori 12930 (CEPEC); Lemos-Filho e Lovato s.n. (BHCB 169434); Lemos-Filho 169472 (BHCB)
	B. triopterifolia A. Juss.	Hyghadha 50215; Harley 54506 (HUEFS); Queiroz 3582 (BHCB 173277)
	B. rigida A. Juss.	Hatschbach 63620 (MBM); Tameirão Neto 2221 (BHCB 33697); s. n. (BHCB 25817)
	B. stannardii W.R. Anderson	Stehmann, Mota, Morais e França 3676 (BHCB 87036); s.n. (BHCB 61137)
	B. blanchetiana Miq.	Costa s.n. (BHCB 35079)
	B. arctostaphylloides Nied.	CFCR 2672; s.n. (BHCB 38868)
	B. affinis W.R.Anderson	Lemos-Filho e Lovato s.n. (BHCB 175233); Simon 581 (SPF)
	B. bahiana W.R.Anderson	HRB 55158
	B. gardneriana A. Juss.	Lemos-Filho e Lovato s.n. (BHCB 169462)
	B. laxiflora Griseb.	s.n. (BHCB 40914); Costa (BHCB 14399)
	B. brachybotrya Nied.	UPCB 33689; Silva 3837 (MBM)
	B. dealbata Griseb.	Martins s.n. (BHCB 8407); Barreto 7676 (BHCB 67897)
	B. intermedia A. Juss.	s.n. (BHCB 28247); Faria s.n. (BHCB 8556); Faria s.n. (BHCB 8558)
	B. morii W.R.Anderson	Almeida 889 b
	B. microphylla A. Juss.	Callejas 1742 (K)
	B. indorum S. Moore	Sasaki 2036 (K)
	B. linearifolia A.Juss.	Pirani 1558 (SPF)
	B. lanulosa W.R.Anderson	Francener 1354
	B. poeppigiana A. Juss.	Prata 396
	B. crispa A. Juss.	Nee 34362 (NYBG); Costa s.n. (BHCB 22372); Gomes 734 (BHCB 69526)
	B. aerugo Sagot	Evans 2736 (MG)
	B. verbascifolia (L.) DC.	s.n. (BHCB 28246); Faria s.n. (BHCB 4611); s.n. (BHCB 47487)
	Blepharandra hypoleuca (Benth.) Griseb.	Cardona 2099 U500685135
	D. aracaensis W.R. Anderson	Prance 29105 U501875182
	Diacidia cordata (Maguire) W.R.Anderson	Maguise 37320
	D. vestita (Benth.) Benth. & Hook. f. ex B.D. Jacks.	Tate 563

(Continued)

Phylogenetic reconstruction

To obtain the phylogeny we used the following markers: ITS, ETS, *trn*L-F, *ndh*F, *phy*C, *psb*A for 84 taxa, from which 53 corresponded to Byrsonimoid *s.l.* Sequences of ingroup and outgroup taxa were provided by Augusto F. N. Gonzaga (Gonzaga 2016). The phylogeny was adapted to contain the anatomically evaluated taxa, comprising a total of 53 species and 10 genera of Byrsonimoid *s.l.* For a complete list with voucher information and GenBank accession numbers see the supplementary S1 available in Bueno *et al.* (2025). We aligned each marker individually using MAFFT v.7.490 (Katoh and Standley 2013) with a post visualization and edition in Mesquite (Maddison and Maddison 2011). In the same software we assembled a concatenated matrix with a final length of 3926 nucleotides.

For each marker and for the concatenated matrix, a total evidence approach followed. We assessed evolutionary models using

Table 1. Continued.

Clade	Species	Voucher
Galphimioid	*Galphimia angustifolia* Benth.	Seigler 12063; Daniel 6818 (MEXU)
	G. brasiliensis (L.) A. Juss.	A. M. de Carvalho 544; CEPEC 15695 (CEPEC)
	G. elegans Baill.	Figueroa 264; Parada 721 (MEXU)
	G. glandulosa Cav.	Martinez 459; Hernandez 569321 (MEXU)
	G. glauca Cav.	Pantoya 485; Mendonza s/n (MEXU)
	G. gracilis Bartl.	Sanches 5 (VIC) G. Hatschbach 56354 (CEPEC)
	G. langlassei (SF Blake) C.E. Anderson	s/n; Sánchez 04 (VIC; MEXU)
	G. mexiae C.E.Anderson	Agala 621; Téllez 13708 (MEXU)
	G. mirandae C.E.Anderson	Teguco 7 (MEXU)
	G. multicaulis A. Juss.	Barriga 4760; Garcia-Mendonza 9899 (MEXU)
	G. oaxacana C.E.Anderson	Pascual 1145; 335
	G. speciosa C.E.Anderson	Pace 1249; Cardona 163; Sanches 7; Sanches 8 (MEXU)
	G. vestita S. Watson	Peina 899; Reina 2006 (MEXU)
	Spachea correae Cuatrec. & Croat	Moreno 23351
	S. elegans (G.Mey.) A.Juss	SRHILL 27352 U.S. 00618966
	S. herbert-smithii (Rusby) Cuatrec	SMITH 1898 (P02428735)
	S. membranacea Cuatrec.	Faulsom 3545; Folsom 3543 (MEXU)
	S. tricarpa A. Juss.	Rimachi 8955 (MBM 141935)
	Lophanthera lactescens Ducke	Faria 41 (BHCB 8557); Domingos s.n. (BHCB 8428); s.n. (BHCB 3123); Barreto 7479 (BHCB 67541)
	L. longifolia (Kunth) Griseb.	Pires 80 (NYGB)
	L. pendula Ducke	Leidon; Maas(?) PJM 6731 (INPA)
	Verrucularia glaucophylla A. Juss.	Roque 1182 (ALCB); Ganev 2499 (BHCB 84566)
Acmantheroid	*Acmanthera latifolia* (Adr. Juss.) Griseb.	Prance 11753 (NYBG); Lohmann 109 (NYBG)
	Coleostachys genipifolia A. Juss.	Vidal 712 (BHCB 145177); Giorni; Viana; Silva; Silva 82 (BHCB 130791); Arruda, Paula, Ranieri, Pivari e Pereira 1298 (BHCB 160500)
	Pterandra arborea Ducke	Prance 2122 (NYGB); Assunção 365 (NYBG)
	P. sericea W.R. Anderson	Maguire 32715 (NYBG); 3440B
	P. pyroidea A. Juss.	HUEFS 26685; Gontijo e Braga 754 (BHCB 178131); Rezende e Justo 6087 (BHCB 185427)
	P. hatschbachii W.R. Anderson	G. Hatschbach 35085 (BHCB 48232); s. n. (BHCB 48274)

jModelTest v.2.1.7 (Darriba *et al.* 2012). Each model was selected with the Akaike information criterion (Akaike 1974).

For the individual locus and concatenate matrix, we implement a maximum likelihood analysis using IQtree-web server v.1.6.12 (Trifinopoulos *et al.* 2016), with the Ultrafast bootstrap analysis (Thi Hoang *et al.* 2017) for 1000 iterations and the GTR + gamma model. For the concatenated matrix, we allowed a mixed model.

Ancestral character state reconstruction

From the analysis of petiole and leaf blade morpho-anatomical characters, we codified a matrix with 28 unordered, binary, or multistate characters (Table 2) that present anatomical variation and potential taxonomic value. The character encoding followed the recommendations of Sereno (2007) for morphological phylogenies, starting with the delimitation of primary homology hypotheses (sensu De Pinna 1991). Character states were reconstructed onto the previously mentioned molecular phylogeny. Anatomical character evolution was performed under the likelihood Ancestral States on Mesquite v.3.03 (Maddison and Maddison 2011) to match the same inference used in the phylogenetic reconstruction. Characters representing potential synapomorphies are highlighted in Figs 1–4. Some of the characters representing possible symplesiomorphies are available in the supplementary material. Furthermore, we constructed an infographic representing the anatomical diversification of leaves and probable synapomorphies in Byrsonimoid *s.l.* (Fig. S2 in the Supporting Information).

RESULTS

The leaf shape

Small, ericoid leaves were observed in *Byrsonima linearifolia* (Fig. 5A), *Galphimia angustifolia*, and *G. vestita*, while all other species had much larger leaves, and therefore are not illustrated. The petiole contour varied among species and genera (Fig. 5B–K), although there is no conclusive probability for the ancestral condition of the Byrsonimoid *s.l.* The petiole is biconvex in *Acmanthera latifolia* (Fig. 5B), *Byrsonima aerugo*, *B. bahiana*, *B. blanchetiana*, *B. brachybotrya* (Fig. 5C), *B. correifolia*, *B. bumellifolia*, *B. indorum*, *B. microphylla*, *B. morii*, *B. poeppigiana* (Fig. 5D), *B. stanardii*, and *Lophanthera longifolia* (Fig. 5E), bearing a central protuberance only in *Byrsonima crispa*, *B. laxiflora*, and *B. poeppigiana* (Fig. 5D); and plane-convex in *Diacidia aracaensis* (Fig. 5F), *Galphimia oaxacana*, *G. elegans*, *G. glauca*, *G. vestita*, *Pterandra* species, illustrated in *P. sericea* (Fig. 5G), *Spachea membranacea*, as well as in the remained evaluated species of *Byrsonima*, which was documented in *B. rigida* (Fig. 5H); concave–convex in *Blepharandra hypoleuca*, *Diacidia vestita*

Table 2. Binary and multistate matrix of Byrsonimoid *s.l.* **Character 1**: Trichomes, type: (-) Not applicable (0) T-shaped (1) Y-shaped. **Character 2**: Stomata on the leaves, position: (0) hypostomatic (1) amphistomatic. **Character 3**: Stomata, projected cells: (0) absent (1) present. **Character 4**: Stomata, ridges: (0) absent (1) present. **Character 5**: Stomata, level from epidermal cells: (0) below the epidermis level (1) same of epidermis level. **Character 6**: Papillose epidermis: (0) absent (1) present. **Character 7**: Epidermis, content: (0) mucilage (1) phenolic compounds (2) absent. **Character 8**: Hypodermis: (0) absent (1) present. **Character 9**: Subepidermal idioblasts: (0) absent (1) present. **Character 10**: Petiole, contour: (0) concave–convex (1) plane-convex (2) biconvex (3) biconvex with two protuberances (4) circular. **Character 11**: Petiole, vascular system conformation: (0) bundles arranged in an open arch (1) open arch with convoluted ends (2) concave–convex closed arch (3) plane-convex closed arch (4) open arch with one dorsal bundle (5) open arch without convoluted ends. **Character 12**: Petiole, medullary bundles: (0) absent (1) present. **Character 13**: Accessory bundles in the petiole, number: (0) absent (1) one pair of bundles (2) more than one pair of bundles. **Character 14**: Petiole: (0) parenchymal pericycle (1) pericycle fibres. **Character 15**: Petiole, cortical sclereids: (0) absent (1) present. **Character 16**: Petiole, nectaries: (0) absent (1) present. **Character 17**: Leaf blade, nectaries: (0) absent (1) present. **Character 18**: Midrib, contour: (0) concave–convex (1) plane-convex (2) biconvex. **Character 19**: Midrib, vascular system conformation: (0) open arch (1) open arch and 2 to 3 bundles subjacent the adaxial face (2) plane-convex (3) biconvex (4) circular (5) circular with one dorsal bundle. **Character 20**: Midrib: (0) parenchymal pericycle (1) pericycle fibres. **Character 21**: Midrib, medullary vascular bundles: (0) absent (1) present. **Character 22**: Mesophyll, type: (0) dorsiventral (1) isobilateral. **Character 23**: Mesophyll, sheath extension of bundles: (0) absent (1) present. **Character 24**: Mesophyll, sheath extension of bundles: (0) parenchymatic (1) sclerified. **Character 25**: Mesophyll, crystals in bundle sheath extension: (0) absents (1) prismatic crystals (2) druses. **Character 26**: Margin, curvature: (0) straight (1) slightly curved (2) revolute. **Character 27**: Marginal tooth with nectary at the base: (0) absent (1) present. **Character 28**: Margin, fibre cap: (0) absent (1) present. **Character 29**: Laticifers: (0) absents (1) presents.

Species/characters	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	28	29
B. umbellata	0	0	0	1	1	0	0/1	0	0	-	1	0	1	0	1	0	0	2	3	1	0	0	1	1	0	1	0	0	0
B. brachybotria	0	0	0	1	1	0	0	0	0	2	1/2	0	1	0	1	0	0	2	2	0	0	1	1	1	0	1	0	0	0
B. sericea	0	0	0	1	1	0	0	0	0	1	1/3	0	1	0	1	0	0	2	3	0	1	0	1	0	0	0	0	0	0
B. verbascifolia	0/1	0	0	1	1	0	0/1	0	0	1	1	0	1	0	1	0	0	2	3	0	1	0	1	1	0	0	0	0	0
B. intermedia	1	0	0	1	1	0	0	0	0	1	1	0	1	0	1	0	0	2	3	0	0	0	1	1	0	0	0	0	0
B. stannardii	0	0	0	1	1	0	0	0	0	2	1	0	1	0	1	0	0	2	3	0	1	0	1	0	0	0	0	0	0
B. variabilis	0	0	0	1	1	0	0	0	0	1	1	0	2	0	1	0	0	2	2	0	1	0	1	1	0	0	0	0	0
B. dealbata	0	0	0	1	1	0	0	0	0	1	1	0	1	0	1	0	0	2	3	0	0	0	0	-	0	0	0	0	0
B. subterranea	0/1	0	0	0	1	0	0	0	0	1	1	0	1	0	1	0	0	2	3	0	1	0	1	0	0	0	0	0	0
B. linearifolia	0	0	0	1	1	0	0	0	0	1	1	0	1	0	1	0	0	2	3	0	0	0	1	1	0	2	0	0	0
B. guilleminiana	0	0	0	1	1	0	0	0	0	1	1	0	1	0	1	0	0	2	3	0	1	0	1	1	0	0	0	0	0
B. crispa	0	0	0	1	1	0	0	0	0	3	1	0	1	0	1	0	0	2	3	0	1	0	1	1	0	1	0	0	0
B. lanulosa	0	0	0	1	1	0	0	0	0	1	1	0	2	0	1	0	0	2	3	0	0	0	1	1	0	2	0	0	0
B. affinis	0	0	0	1	1	0	0	0	0	-	-	0	1	0	1	0	0	2	3	0	1	0	1	1	0	2	0	0	0
B. poeppigiana	-	0	0	1	1	0	0	0	0	3	1/3	0	2	0	1	0	0	2	3	0	1	0	1	1	0	0	0	0	0
B. laxiflora	0	0	0	1	1	0	0	0	0	3	1	0	2	0	1	0	0	2	3	0	1	1	1	1	0	1	0	0	0
B. cipoensis	-	0	0	1	1	0	0	0	0	1	1	0	2	0	1	0	0	2	3	0	0	1	1	1	0	0	0	0	0
B. morii	0	0	0	1	1	0	0	0	0	2	1	0	2	0	1	0	0	2	3	0	0	1	1	1	0	1	0	0	0
B. correifolia	0/1	0	0	1	1	0	0	0	0	2	1	0	1	0	1	0	0	2	3	0	0	0	1	1	0	2	0	0	0
B. gardneriana	0	0	0	0	1	0	0	0	0	1	1	0	1	0	1	0	0	2	1	0	0	0	1	1	0	0	0	0	0
B. vacciniifolia	0	0	0	1	1	0	0	0	0	1	1	0	2	0	1	0	0	2	3	0	0	0	1	1	0	0	0	0	0
B. microphylla	0	0	0	1	1	0	0	0	0	2	1	0	2	?	1	0	0	2	3	0	0	1	1	1	0	1	0	0	0
B. rigida	-	0	?	0	1	0	0	0	0	1	1	0	0	1	0	0	0	2	0	1	0	0	1	1	0	0	0	0	0
B. triopterifolia	0	0	0	1	1	0	0	0	0	1	1	0	?	0	0	0	0	1	1	1	0	0	1	1	0	0	0	0	0
Bl. hypoleuca	0	0	0	0	1	0	0	1	0	0	0	1	2	0	1	0	0	1	2	1	1	0	1	1	0	1	0	1	0
D. aracaensis	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0	1	1	0	0	1	1	0	1	0	0	0
D. cordata	0	0	0	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	1	1	0	0	1	1	0	1	0	0	0
D. vestita	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0
A. latifolia	0	0	1	0	1	0	?	0	0	2	1	0	1	0	0	0	0	2	2	0	0	0	1	1	2	0	0	1	0
C. genipifolia	0	0	1	0	1	0	2	0	0	4	1	0	1	0	0	0	0	2	2	0	0	0	1	1	2	0	0	1	0
P. arborea	0	0	1	0	1	0	0	0	1	1	5	0	2	0	0	0	0	2	0	0	0	0	1	1	1/2	0	0	1	0

(Continued)

Table 2. Continued.

Species/characters	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	28	29
P. hatschbachii	0	0	1	0	1	0	2	0	1	1	5	0	1	0	0	0	0	2	5	0	0	0	1	1	1/2	0	0	1	0
P. pyroidea	0	0	1	1	1	0	1	0	1	1	5	0	2	0	0	0	0	2	5	0	0	0	1	1	1/2	0	0	1	0
G. brasiliensis	0	0	0	0	1	0	0	0	0	0	5	0	0	0	0	0	1	1	0	0	0	0	0	-	0	1	1	0	1
V. glaucophylla	0	0	0	0	1	1	2	0	0	0	5	0	1	0	0	0	0	1	0	0	0	0	0	-	0	0	0	0	1
S. correae	0	0	0	1	1	0	0	0	0	0	5	0	0	0	0	1	1	1	0	0	0	0	1	0	0	2	0	0	1
S. elegans	0	0	0	1	1	0	0	0	0	-	-	0	-	-	-	-	1	2	0	0	0	0	0	-	0	2	0	0	1
S. tricarpa	0	0	0	1	1	0	0	0	0	0	5	0	0	0	0	0	1	2	0	0	0	0	1	0	0	2	0	0	1
L. lactescens	0	0	0	1	1	0	2	0	0	4	3	0	0	0	0	1	1	2	4	0	0	0	1	0	0	1	0	0	1
L. longifolia	0	0	0	1	1	0	0	0	0	2	4	0	0	0	0	1	1	2	4	0	0	0	1	0	0	2	0	0	1
G. langlassei	-	0	0	0	1	0	2	0	0	0	5	0	0	0	0	0	1	-	-	0	0	0	0	-	0	0	1	-	1
G. oaxacana	-	0	0	0	1	0	2	0	0	1	5	0	0	0	0	0	1	2	0	0	0	0	0	-	0	1	1	0	1
G. mirandae	-	0	0	0	1	0	2	0	0	0	5	0	0	0	0	0	1	1	0	0	0	0	0	-	0	0	1	0	1
G. elegans	0	0	0	0	1	0	0	0	0	1	5	0	0	0	0	1	-	0	0	0	0	0	0	-	0	1	-	0	1
G. speciosa	-	0	0	0	0	0	2	0	0	0	5	0	0	0	0	1	1	1	0	0	0	0	0	-	0	1	1	0	1
G. mexiae	-	1	0	0	1	0	2	0	0	0	5	0	0	0	0	0	1	0	0	0	0	0	0	-	0	-	1	-	1
G. multicaulis	-	0	0	0	1	0	2	0	0	0	5	0	1	0	0	0	1	1	0	0	0	0	0	-	0	1	1	0	1
G. glandulosa	-	0	0	0	1	1	2	0	0	4	5	0	0	0	0	1	0	1	0	1	0	0	0	-	0	1	1	0	1
G. glauca	-	0	0	0	0	0	2	0	0	1	5	0	0	0	0	0	1	1	0	0	0	0	0	-	0	0	1	0	1
G. angustifolia	-	1	0	0	0	0	2	0	0	4	5	0	0	0	0	0	1	1	0	0	0	0	0	-	0	2	1	0	1
G. vestita	0/1	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	-	0	0	1	0	1
G. gracilis	-	0	0	0	1	0	0/1	0	0	0	5	0	1	0	0	0	1	1	0	0	0	0	0	-	0	0	1	0	1
S. membranacea	-	0	0	0	1	0	1	0	0	1	5	0	1	0	0	0	-	1	0	0	0	0	0	-	0	2	-	0	1
L. pendula	-	0	0	0	1	0	2	0	0	1	3	0	1	1	0	1	0	2	4	1	0	0	1	0	0	0	-	0	1

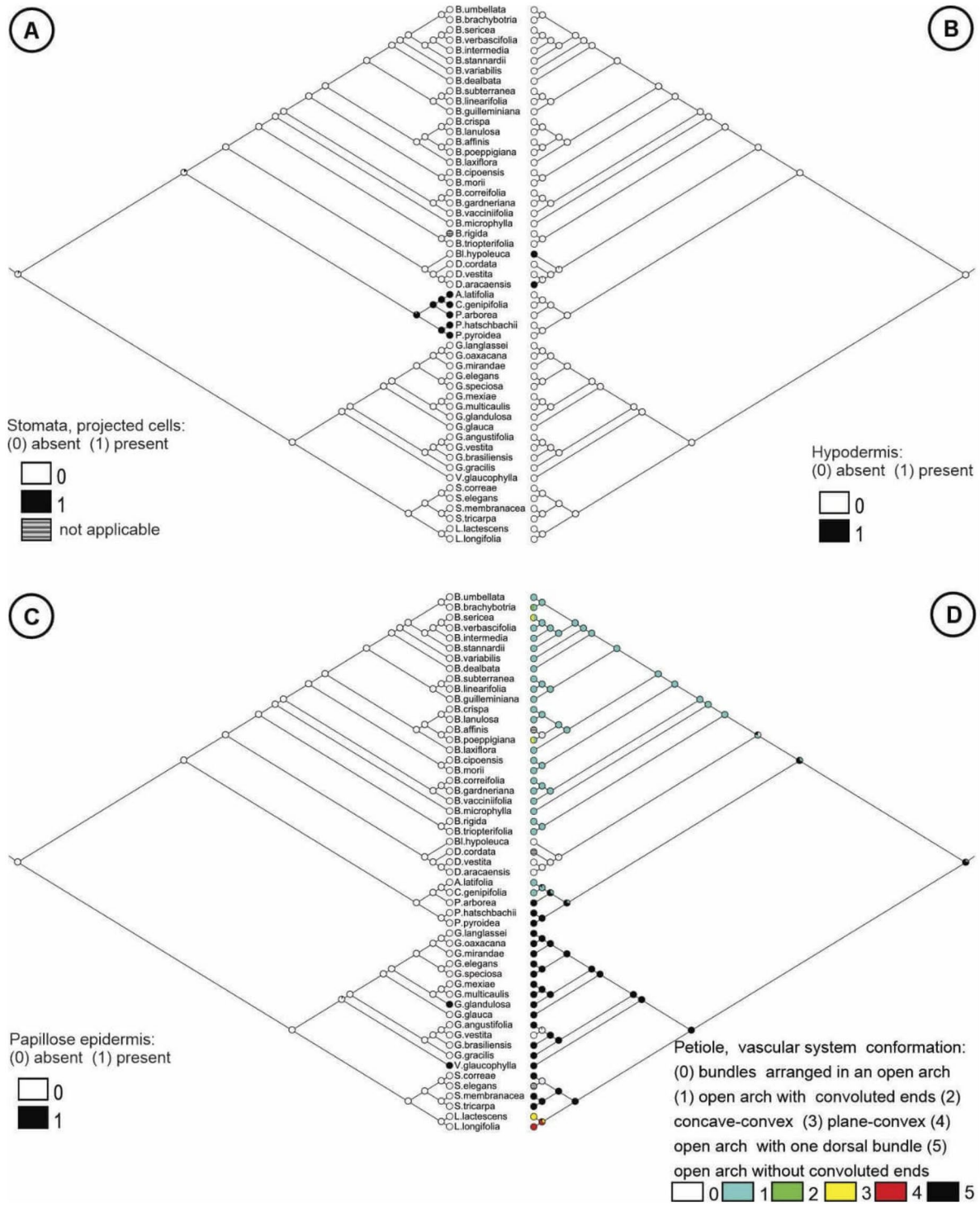

Figure 1. Anatomical characters recovered as synapomorphies potentially useful for the systematic of Byrsonimoid *s.l.* A, Stomata, projected cells. B, Presence of hypodermis. C, Presence of papillose epidermis. D, Petiole vascular system conformation.

(Fig. 5I), *Galphimia brasiliensis*, *G. glauca*, *G. gracilis*, *G. langlassei*, *G. mexiae*, *G. mirandae*, *G. multicaulis*, *G. speciosa*, *Spachea correae*, and *Verrucularia glaucophylla* (Fig. 5J); and irregular circular in *Coleostachys genipifolia* (Fig. 5K), *Galphimia angustifolia*, *G. glandulosa*, and *Lophantera lactescens*.

The biconvex contour of the midrib represents the ancestral condition for Byrsonimoid *s.l.* (55% probability). Being present at most of the evaluated species of *Byrsonima* (Fig. 6A, B), in all species of Acmantheroids (Fig. 6C), of *Lophanthera* (Fig. 6D), *S. elegans*, and *S. tricarpa* (Galphimioids). The plane-convex contour was observed in *Byrsonima triopterifolia* (Fig. 6E), *Blepharandra hypoleuca* (Fig. 6F), *Galphimia brasiliensis*, *Spachea mebranacea*, *S. correae*, *S. herberth-smith*, and *Verrucularia glaucophylla* (Fig. 6G) and the most of the

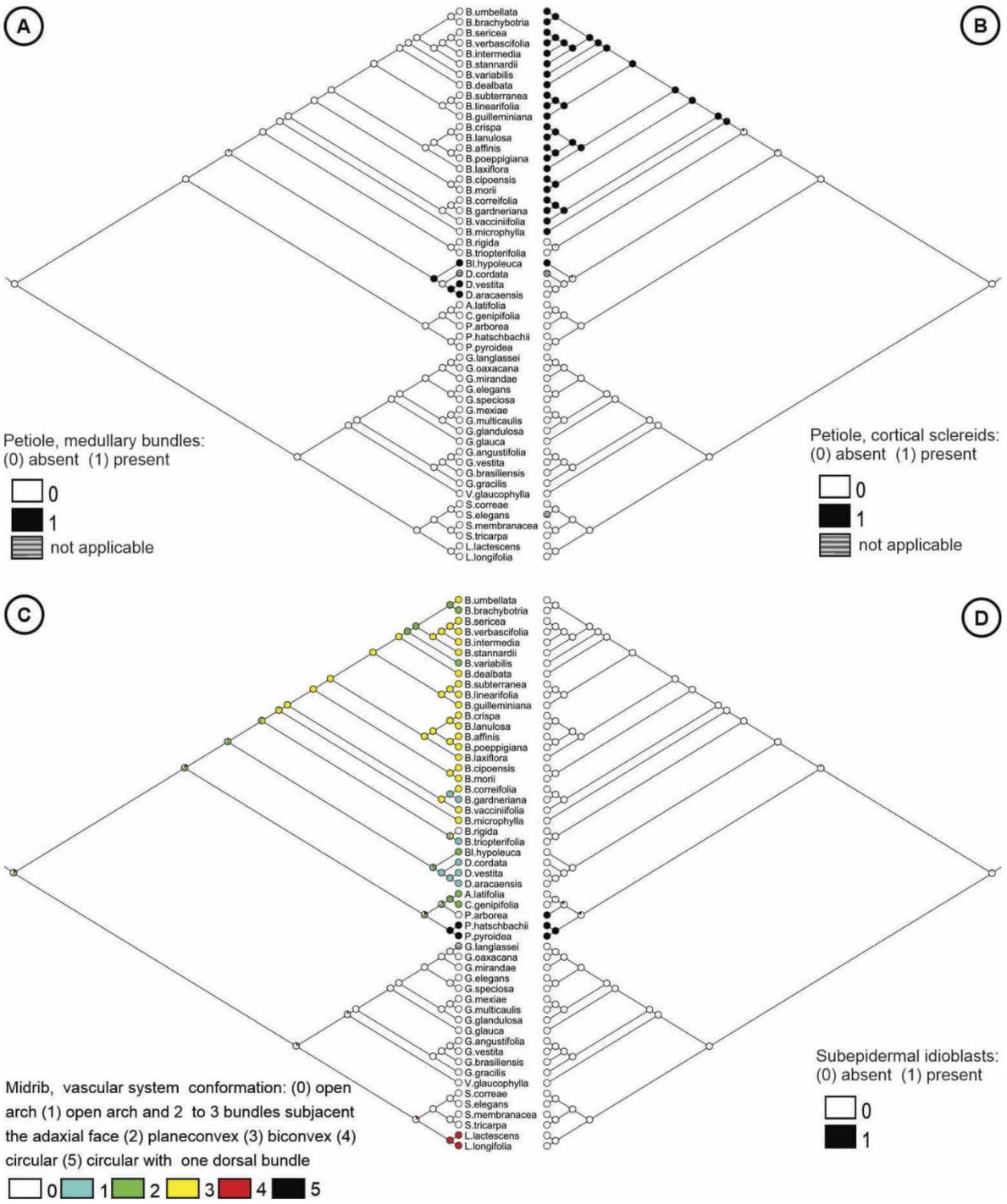

Figure 2. Anatomical characters recovered as synapomorphies potentially useful for the systematic of Byrsonimoid *s.l.* A, Petiole medullary bundles. B, Petiole cortical sclereids. C, Midrib vascular system conformation. D, Subepidermal idioblasts.

evaluated species of *Galphimia*; concave–convex contour for the analysed species of *Diacidia* (Fig. 6H), *Galphimia elegans*, and *G. mexiae*.

Although the three types of margin curvature show the same probability for the common ancestor of Byrsonimoid *s.l.*, the leaf margin straight occur for most species of Byrsonimoids (Fig. 7A-C), *Galphimia glauca*, *G. gracilis*, *G. langlassei*, *G. mirandae*, *G. vestita*, and *Verrucularia glaucophylla*; followed by slightly curved in *Blepharandra hypoleuca* (Fig. 7D), *Byrsonima blanchetiana*, *B. bumellifolia*, *B. brachybotrya*, *B. clausseniana*, *B. crispa*, *B. intermedia*, *B. laxiflora*, *B. microphylla*, *B. morii*, *B. umbellata*, *D. aracaensis* (Fig. 7E), *D. cordata*, *G. brasiliensis*, *G. elegans*, *G. glandulosa*, *G. multicaulis*, *G. oaxacana*, *G. speciosa*, and *L. lactescens* (Fig. 7F) and revolute margin in *B. affinis*, *B. correifolia*, *B. lanulosa* (Fig. 7G), *B. linearifolia*, *L. longifolia*, and species of *Spachea*, such as *S. tricarpa* (Fig. 7H).

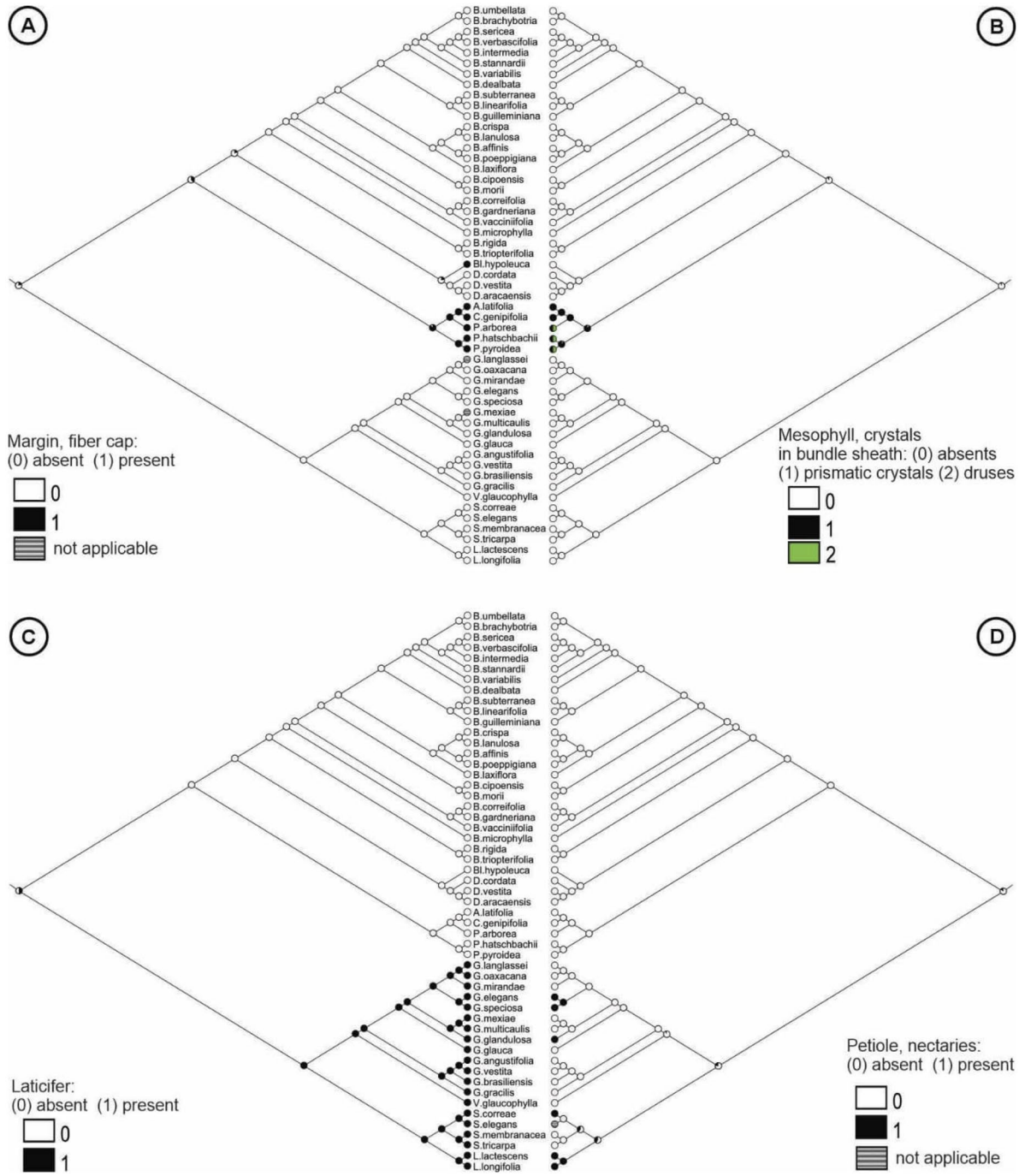

Figure 3. Anatomical characters recovered as synapomorphies potentially useful for the systematic of Byrsonimoid *s.l.* A, Fibre cap in margin. B, Crystals in bundle sheath of mesophyll. C, Presence of laticifers. D, Nectaries on the petiole.

Epidermal features

Trichomes are typically malpighiaceous (T-shaped) and occur in all studied species of Acmantheroids, Byrsonimoids *s.s.*, and Galphimioids (Fig. 8A). In *A. latifolia*, *Blepharandra*, *C. genipifolia*, *Diacidia*, *G. brasiliensis*, *Lophathera*, *Pterandra*, *Spachea*, and *V. glaucophylla*, the trichomes are T-shaped bear a short peduncle (Fig. 8A). In *Byrsonima*, variations in the length of the peduncle and the arrangement of the branches of the trichome are observed for some species. Trichomes in 'Y' and 'T' shapes with long peduncle were observed for *Byrsonima subterranea* (Fig. 8B) and *Byrsonima verbascifolia* (Fig. 8C) and 'Y' and 'T' shapes, with short peduncle in *Byrsonima correifolia* (Fig. 8D) and *Galphimia vestita*. In the other species of Byrsonimoid *s.l.*, only 'T' trichomes with a short peduncle were observed, as illustrated in *Byrsonima verbascifolia* (Fig. 8C) and *Pterandra pyroidea* (Fig. 8A). Most probably the common ancestor for Byrsonimoid *s.l.* had T-shaped trichomes on the leaves (99.7% probability).

In Acmantheroids, Byrsonimoids *s.s.* (*Byrsonima* and *Blepharandra*,) and the most species of Galphimioids, the stomata occur on the same epidermal level (Fig. 8E), probably the ancestral

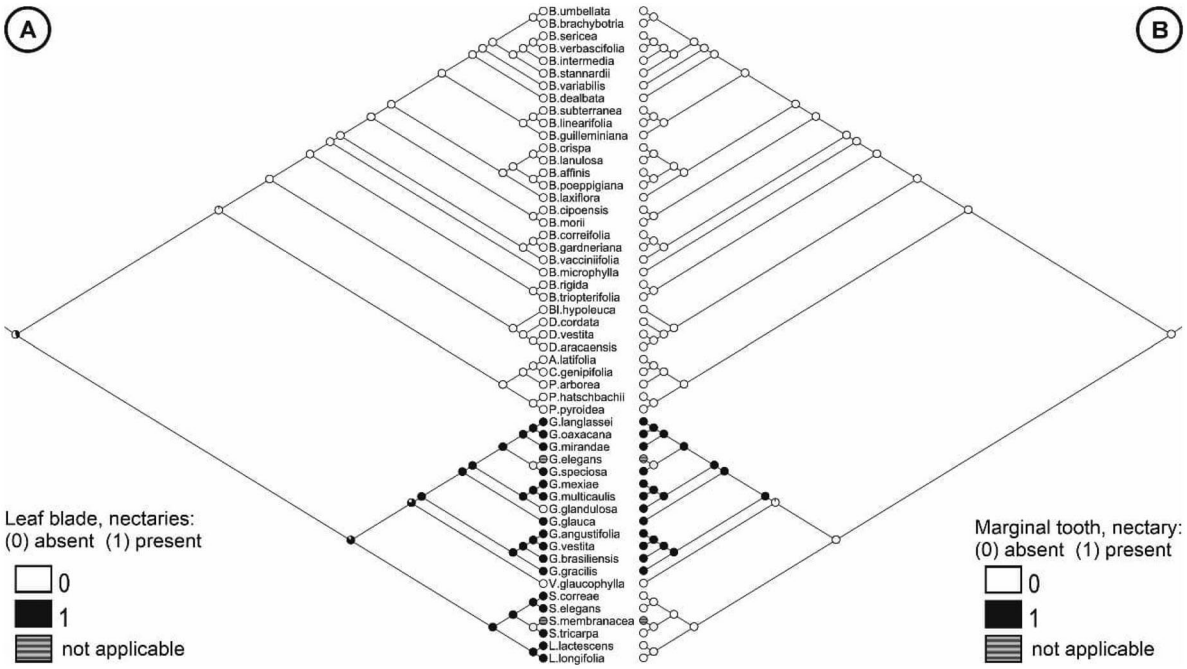

Figure 4. Anatomical characters recovered as synapomorphies potentially useful for the systematic of Byrsonimoid *s.l.* A, Nectaries on the leaf blade. B, Marginal tooth with nectary.

condition of Byrsonimoid *s.l.* (95.4% probability). In only *Diacidia* species (Fig. 8F), *Galphimia angustifolia*, *G. glauca*, and *G. speciosa* stomata below the other epidermal cell's level were present, a possible homoplastic synapomorphy for the genera. The ancestral condition of Byrsonimoid *s.l.* are stomata at the same level as the epidermis (95.4% probability).

Stomata occur only on the abaxial surface (hypostomatic leaves) in all genera of the Acmantheroids, Byrsonimoids *s.s.* and Galphimioids (Fig. 8G, H) and this represents the ancestral condition for Byrsonimoid *s.l.* (99.2% probability). However, amphistomatic leaves occur only in *Galphimia angustifolia* and *G. mexiae* (Fig. 8I). The stomata occur with subsidiary cells projected over the guard cells in Acmantheroid (Figs 1A, 8J), pointed out as a possible synapomorphy for the clade. Stomatal ridges occur for most of the analysed species (Fig. 8E), being absent in *A. latifolia*, *Byrsonima arctostaphylloides*, *B. blanchetiana*, *B. gardneriana*, *B. rigida*, *B. subterranea*, *Blepharandra hypoleuca*, *C. genipifolia*, *Diacidia aracaensis*, *D. cordata* (Fig. 8F), *D. vestita*, *Galphimia* species, *P.* arborea, *S. membranacea*, and *V. glaucophylla*. For Byrsonimoid *s.l.*, the presence of ridges has a 50.9% probability as being the ancestral condition.

The epidermis is uniseriate with mucilaginous idioblasts in all species of Byrsonimoid (Figs. 8G), *G. brasiliensis*, *G. elegans*, *L. longifolia*, *P. arborea*, *P. sericea*, and *Spachea*. This condition is reconstructed as ancestral for Byrsonimoid *s.l.*, with 83.3% of probability. The presence of mixed idioblasts, containing mucilage and phenolic compounds, was observed in *Byrsonima verbascifolia* (Fig. 8H), *B. umbellate*, *Galphimia gracilis*, and *Pterandra pyroidea*. Content was not observed in the epidermis of *C. genipifolia*, in the other species of *Galphimia*, *L. lactescens*, *P. hatschbachii*, and *V. glaucophylla*. In addition, discontinuous hypodermis occurs at the height of bundle sheath extension for *Blepharandra hypoleuca* (Fig. 8K) and single-layer hypodermis in *Diacidia aracaensis* (Fig. 8L), both potential synapomorphies for these genera (Fig. 1B). In the other genera, hypodermis is lacking (Fig. 8G). The presence of a papillose epidermis on the abaxial surface of the leaf for *Verrucularia glaucophylla* (Figs 1C, 8M) forms crypts where stomata are posited. In *Galphimia glandulosa*, the epidermis has some papillose cells, and these do not project over the stomata. The presence of papillose epidermis represents a homoplastic synapomorphy for the two taxa.

Characterization of the vascular system and mesophyll: focusing on its structural and relevant taxonomical traits

The vascular system of the petiole is collateral for all genera analysed (Fig. 9A, B) and the vascular system conformation showed a significant diversity. In *Blepharandra hypoleuca* (Fig. 9A) and *Diacidia* species, the vascular system conformation is formed by several bundles arranged in an open arc with medullary bundles, representing a homoplastic synapomorphy for the clade (94.7%); an open arch without convoluted ends was observed in *Galphimia* (Fig. 9C), *Pterandra* (Fig. 9B), *Spachea*, and *V. glaucophylla*. The open-arch type with convoluted ends represents a homoplastic synapomorphy for the clade formed by *A. latifolia* (Fig. 5B), *C. genipifolia* (Fig. 5J, 87.8%), and for *Byrsonima* (99.7%). In *Lophanthera longifolia* (Fig. 5E), the presence of an open arch accompanied by a dorsal vascular bundle emerges as an autapomorphy (59.5%), while *L. lactescens* and *L. pendula* is distinguished by a plane-convex closed arch (Fig. 6D). Only the petiole of *Byrsonima brachybotria* (Fig. 5C), *B. poeppigiana* (Fig. 5D), and *B. sericea* shows variation in conformation in the same taxon, with a concave–convex closed arch type observed in *B. brachybotria* (Fig. 5C) and a plane-convex closed arch type observed for the last two species (Fig. 5D). Thus, the vascular system conformation distinguishes *Blepharandra* (Fig. 9A) and *Diacidia* (Fig. 5F, I) from *Byrsonima* (Fig. 5C, D, H) (Byrsonimoid *s.s*); *A. latifolia* and

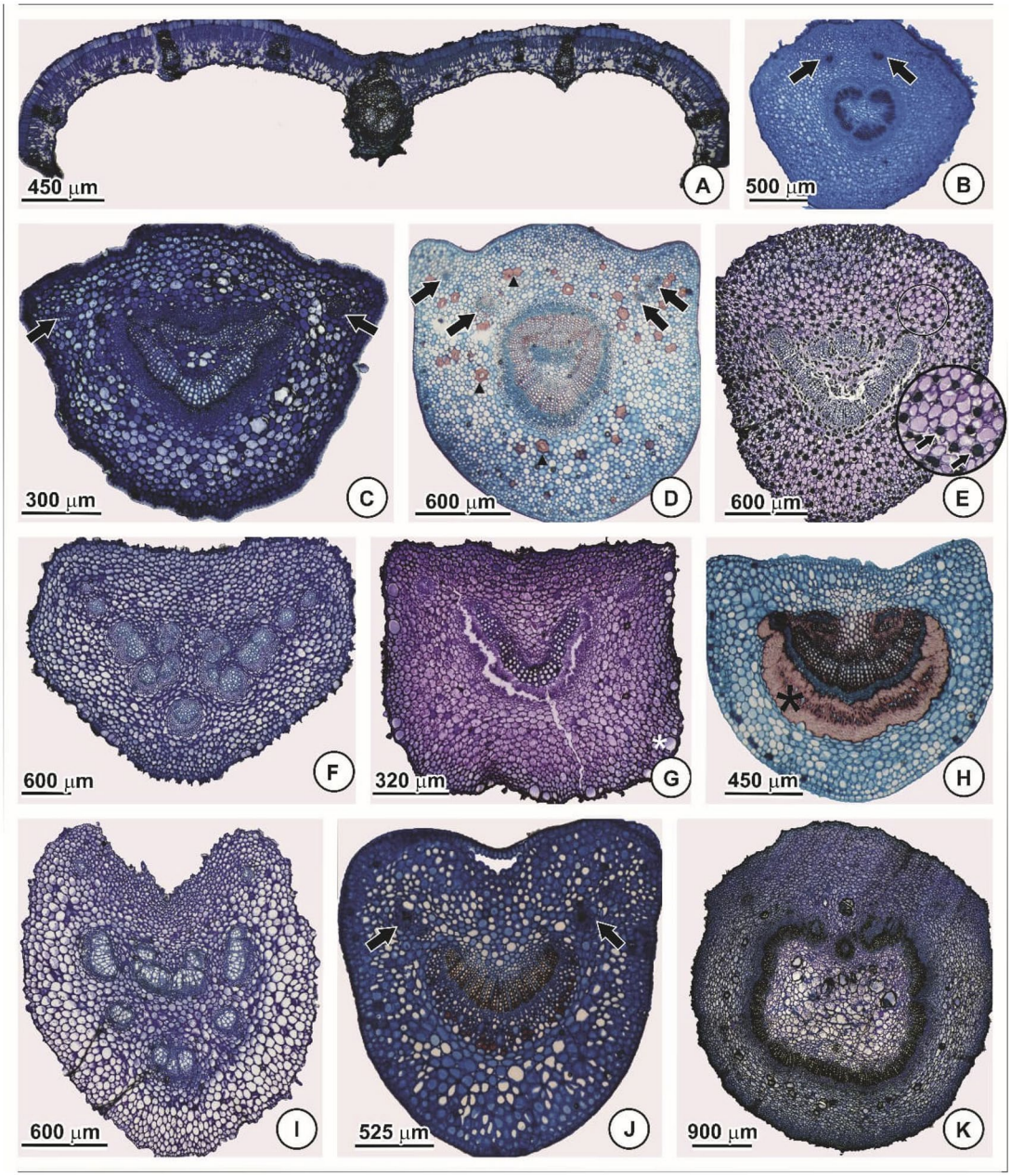

Figure 5. Leaf anatomy description of Byrsonimoid, based on the cross-section of the leaf blade (A) and petiole (B–K). A, Ericoid leaf of *Byrsonima linearifolia*. B, Biconvex petiole and one pair of accessory bundles (arrows) in *Acmanthera latifolia*. C, Biconvex petiole with one pair of accessory bundles (arrows) in *Byrsonima brachybotria*, note the concave–convex closed arch type. D, Biconvex contour with a central protuberance in *Byrsonima poeppigiana*, note one pair of the accessory bundles (arrows) and dispersed sclereids (arrowheads) in the cortex. E, Biconvex petiole with an open arch accompanied by a dorsal vascular bundle of conformation in *Lophanthera longifolia*, note the phenolic idioblasts (arrows). F, Plane-convex petiole of *Diacidia aracaensis*; G, Plane-convex petiole of *Pterandra sericea* with one pair of accessory bundles (arrows), which is amphicribal, note the subepidermal cortical idioblasts (asterisk). H, Plane-convex petiole of *Byrsonima rigida* with fibre sheath (asterisk) to the phloem in the vascular system. I, Concave–convex petiole of *Diacidia vestita*. J, Concave–convex petiole with a pair of accessory bundles (arrows) in *Verrucularia glaucophylla*, note an open arch accompanied by a dorsal vascular bundle. K, Circular petiole with open arch with convoluted ends of conformation in *Coleostachys genipifolia*: note one pair of accessory bundles. Abbreviations: Xy, xylem; Ph, phloem.

C. genipifolia (Fig. 5K) from *Pterandra* (Figs 5G, 9B) (Acmantheroid); *Lophanthera* (Fig. 5E) species from other genera of Galphimioid (Figs 5J, 9C). The estimated ancestral condition for Byrsonimoid *s.l.* is the open-arch conformation without convoluted ends (Fig. 1D, 76.1% probability).

With respect to the petiole accessory bundles, the probability is equal for the three-character states to be the ancestor of Byrsonimoid *s.l.* The accessory bundles of the petiole are absent in *Byrsonima rigida* (Fig. 5H), *Lophanthera*, *Spachea correae*, and the most species of *Galphimia*. All remaining species present

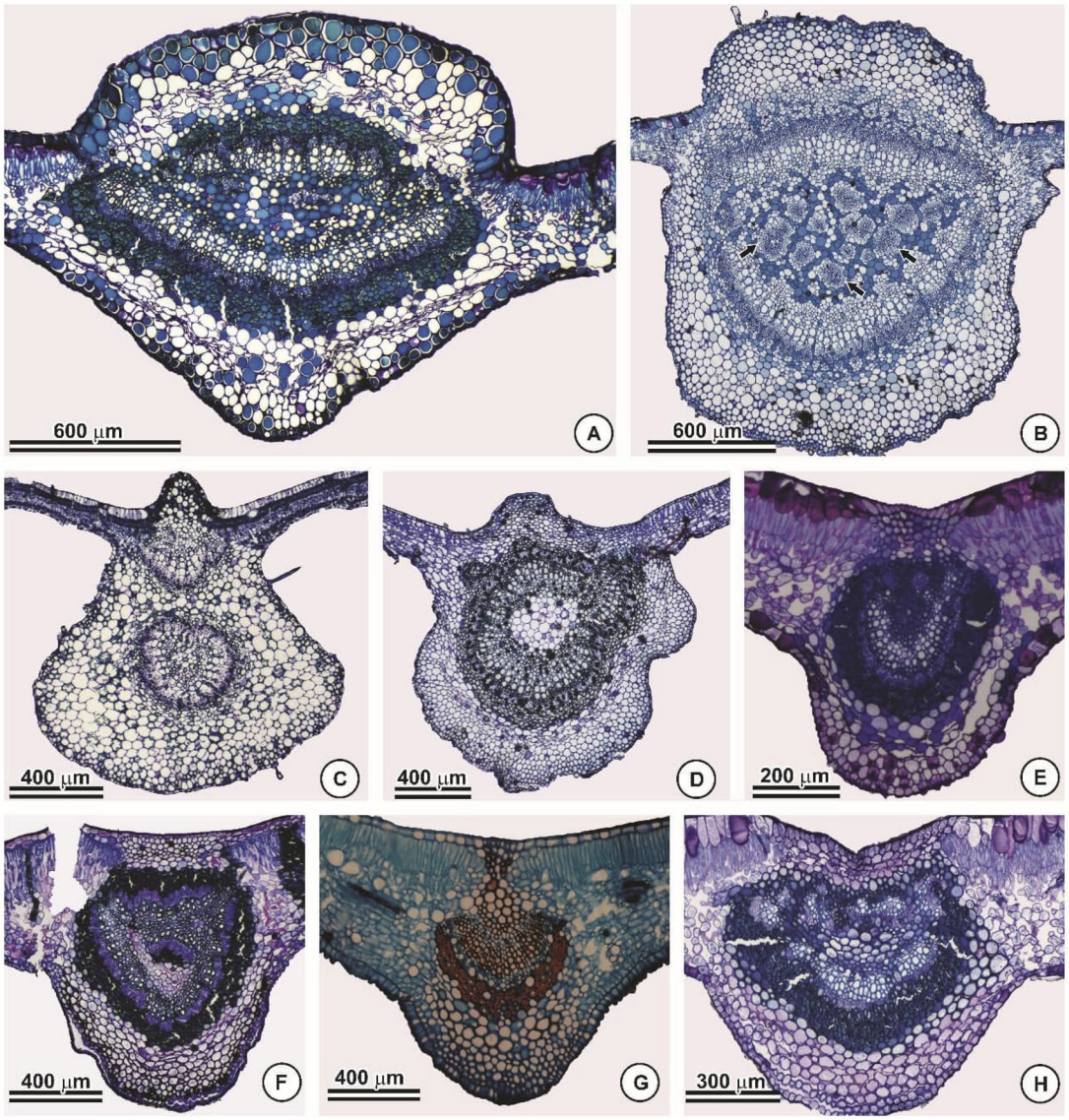

Figure 6. Details of midrib anatomy in Byrsonimoids. A, Biconvex midrib with open arch conformation in *Byrsonima umbellata*. B, Biconvex vascular system with medullary bundles (black arrows) of *Byrsonima verbascifolia*. C, Circular conformation with an open arch consisting of a dorsal bundle of midrib in *Pterandra pyroidea* midrib. D, Biconvex midrib with a circular conformation of the vascular system in *L. lactescens*. E, Epidermal and cortical mucilaginous idioblasts *Byrsonima triopterifolia*. F, Plane-convex midrib of *Blepharandra hypoleuca*. G, Plane-convex midrib with an open arc of vascular system conformation in *Verrucularia glaucophylla*. H, Concave–convex midrib and mucilaginous epidermis of *Diacidia cordata*. Abbreviations: Xy, xylem; Ph, phloem.

accessory bundles, which are amphicrival. The number of accessory bundles varies, being organized in one pair in *A. latifolia* (Fig. 5B), *C. genipifolia* (Fig. 5K), *P. hatschbachii*, and *P. sericea* (Fig. 5G), for most species of *Byrsonima* (Fig. 5C), all species of *Diacidia* (Fig. 5F, I), *Galphimia gracilis*, *G. multicaulis*, *Spachea herberth-smith*, *S. membranacea*, and *Verrucularia glaucophylla* (Fig. 5J). In *Byrsonima bumellifolia*, *B. cipoensis*, *B. lanulosa*, *B. laxiflora*, *B. microphylla*, *B. morii*, *B. vaccniifolia*, *B. variabilis*, *B. poeppigiana* (Fig. 5D), *P. arborea*, and in *P. pyroidea* (Fig. 9B) the accessory bundles occur in more than one pair; and in 3–5 pairs in *Blepharandra hypoleuca* (Fig. 9A). In only *Lophanthera* (Figs 5E, 6D), *Galphimia brasiliensis* (Fig. 9C), *Spachea correae*, and *S. tricarpa* accessory bundles are absent. Medullary bundles in the petiole, found in *Diacidia* (Fig. 5F, I) and *Blepharandra hypoleuca* (Fig. 9A), represent a potential synapomorphy for the clade formed by these two genera (Fig. 2A, 96.1%). The presence of scattered cortical sclereids emerged in Byrsonimoid *s.s.* (Fig. 5D), being lost in the clade formed by *B. rigida* and *B. triopterifolia*, a sister group to the other *Byrsonima*, and in the clade formed by *Diacidia* (Fig. 5F, I). Although the absence of this condition is considered the most likely for the ancestor of the group (Fig. 2B, 95.6%). In Acmantheroid and Galphimioid, the sclereids are absent in petiole (Fig. 9C). In *Byrsonima rigida* the pericycle is differentiated in a fibre sheath (Fig. 5H), representing a possible autapomorphy for this species. Druses were absent only in *Byrsonima brachybotrya* (Fig. 5C) for Byrsonimoid *s.s.* In *B. triopterifolia*, mucilaginous idioblasts also occur dispersed in the petiole cortex and absence of sclereids in the same region (Fig. 9D).

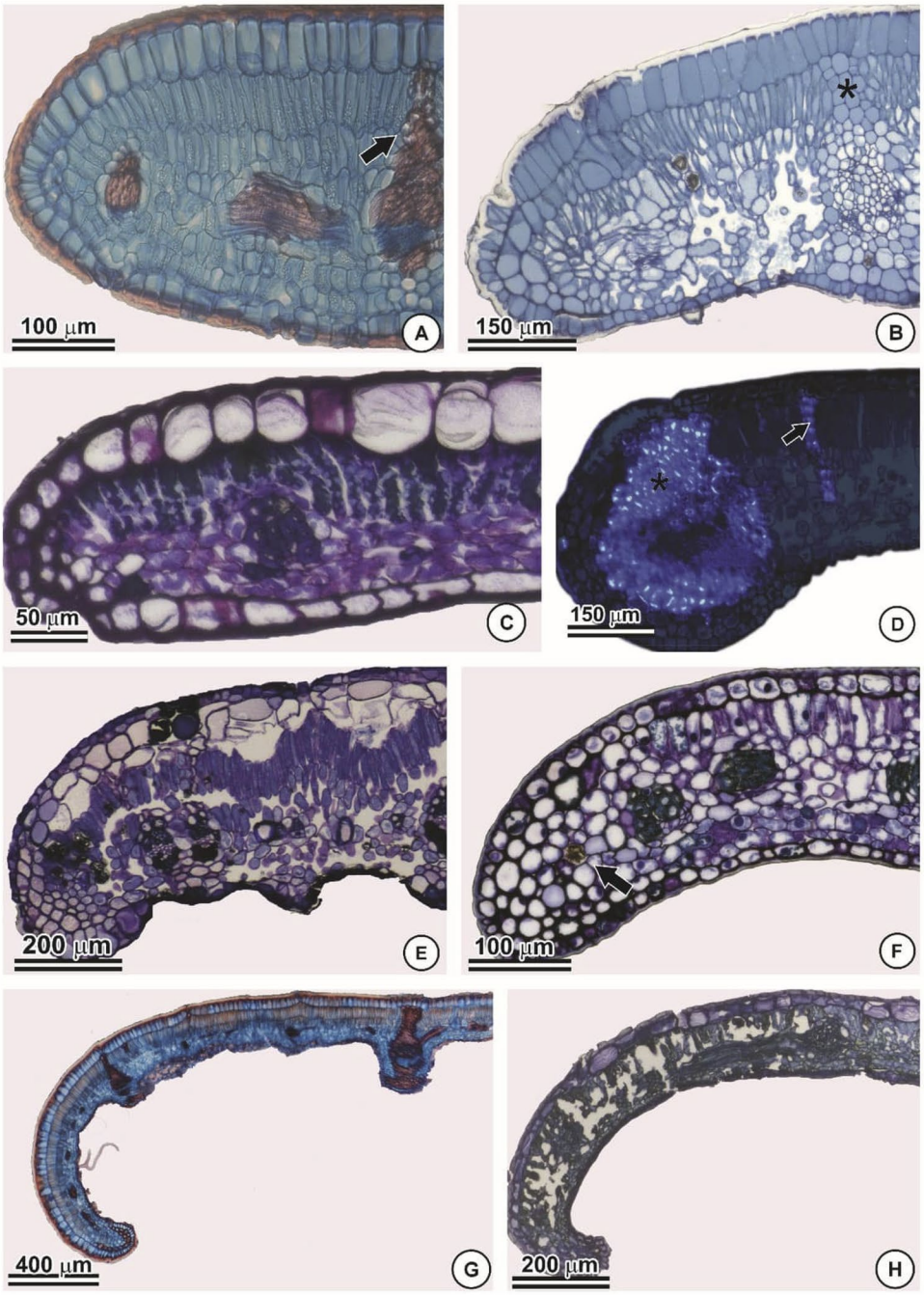

Figure 7. Margin anatomy of Bysonimoids. A, Straight margin of *Byrsonima gardneriana*, note the lignified extension of the sheath (black arrow) of the vascular bundle. B, Straight margin of *Byrsonima stanardii*, note the parenchymal extension of the sheath (asterisk) of the vascular bundle. C, Straight margin of *Spachea herbeth-smithii*. D, Fibre cap and sclerified beam extension in polarized light in *Blepharandra hypoleuca*, note the lignified extension of the sheath (black arrow) of the vascular bundles. E, Slightly curved margin of *Diacidia aracaensis*, note the hypodermis in the adaxial surface of the epidermis. F, Slightly curved margin and druses (black arrow) in *Lophanthera lactescens*. G, Revolute margin in *Byrsonima lanulosa*. H, Revolute margin in *Spachea tricarpa*.

In *Galphimia gracilis*, *Lophanthera*, *Spachea*, and *Verrucularia*, the petiole presents phenolic idioblasts (Fig. 5E).

The vascular system in the midrib is collateral for all genera (Fig. 6A–H), with variation in conformation according to species and the probabilistic indices are inconclusive for reconstructing the ancestral condition in the lineage. The plane-convex vascular system, formed by two arches, occurs in *Acmanthera latifolia*, *Blepharandra hypoleuca* (Fig. 6F), *Byrsonima brachybotrya*, *B. bumellifolia*, *B. variabilis*, and *Coleostachys genipifolia*. Open arch with 2–3 bundles underlying the adaxial face were registered for *B. gardneriana*, *B. tripterifolia* (Fig. 6E), *Diacidia aracaensis*, *D. cordata* (Fig. 6H), and *D. vestita*. Open arch typically occurs in *Byrsonima rigida* (Fig. 9E) and *Galphimia*, *Spachea*, *V. glaucophylla* (Fig. 6G) from Galphimioids, and *Pterandra arborea* (Fig. 9F).

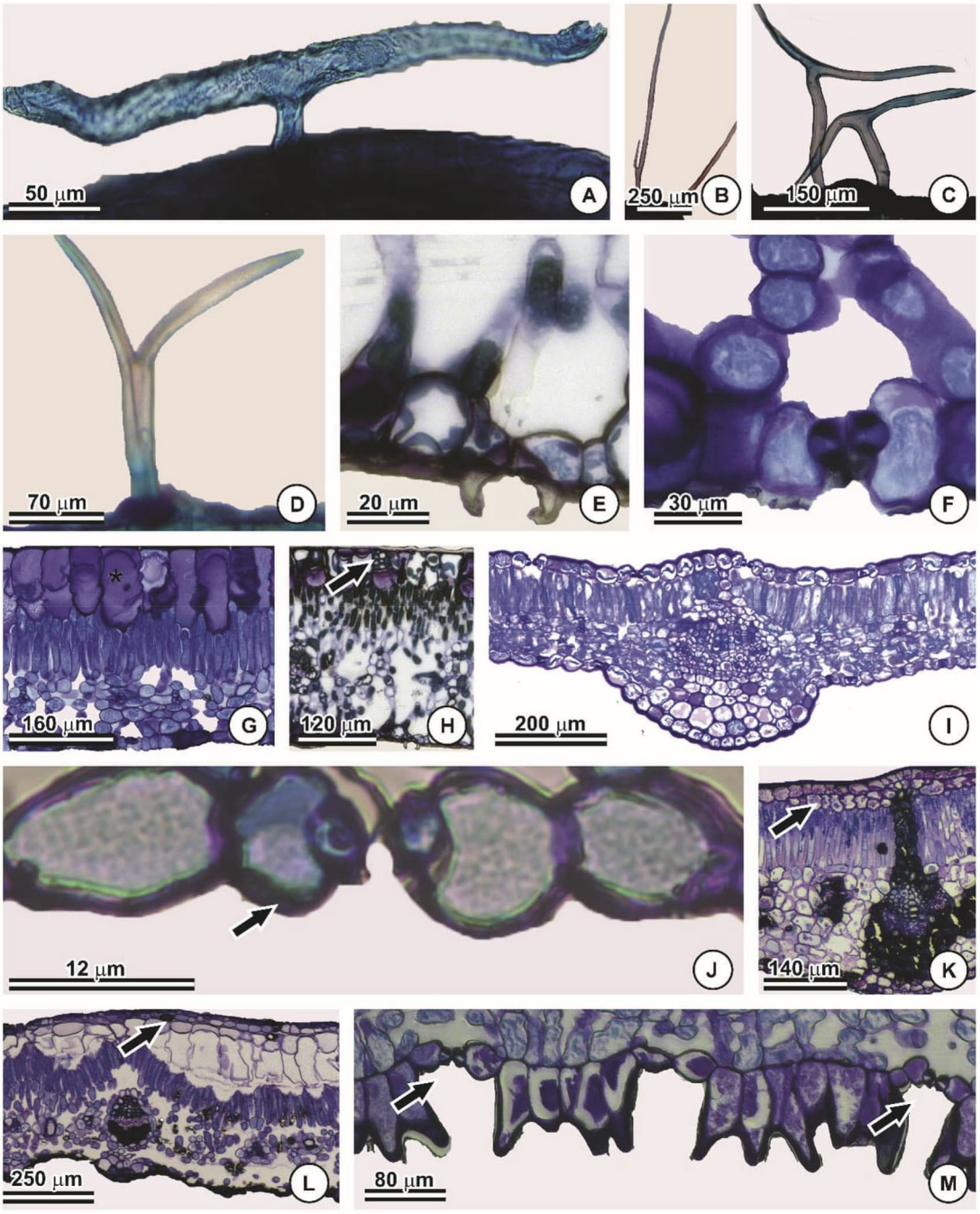

Figure 8. Details of epidermis in Byrsonimoids. A, 'T' trichome of *P. pyroidea*. B, Y-shaped trichome with long peduncle in *Byrsonima subterranea*. C, T-shaped trichomes of *B. verbascifolia*. D, Y-shaped trichomes with short peduncle in *B. correifolia*. E, Stomata on the abaxial surface with ridges in *B. verbascifolia*. F, Stomata below the level of epidermis and without ridges in *Diacidia cordata*. G, Mucilaginous epidermis (asterisk) and dorsiventral mesophyll of *D. cordata*. H, Epidermis with mixed idioblasts (arrow), stomatal ridges, and dorsiventral mesophyll of *B. verbascifolia*. I, Amphistomatic leaf in *Galphimia angustifolia*. J, Stomata in the abaxial surface of *Pterandra hatschbachii*, note subsidiary cells projected (black arrow). K, Discontinuous hypodermis (black arrow) of *Blepharandra hypoleuca*. L, Hypodermis (black arrow) of *Diacidia aracaensis*. M, Papillose epidermis on the abaxial surface of *Verrucularia glaucophylla*, note the stomata in crypts (black arrows).

In other species of *Pterandra* present a circular conformation with an open arch consisting of a dorsal bundle (Fig. 9G), pointed out as a possible synapomorphy (Fig. 2C, 99.9%). *Lophanthera* presents circular conformation (Fig. 6D), representing a possible synapomorphy (Fig. 2C, 99.5%). For the other analysed species of Byrsonimoid *s.s.*, the conformation is biconvex, formed by two open arcs (Fig. 6A, B). In *Blepharandra hypoleuca* (Fig. 6F) *Byrsonima affinis*, *B. crispa*, *B. guilleminiana*, *B. laxiflora*, *B. poeppigiana*, *B. sericea*, *B. stanardii*, *B. subterranea*, *B. variabilis*, and *B. verbascifolia* (Fig. 6B) vascular bundles occur in the midrib medullary region, appearing in different taxa in Byrsonimoid *s.s.* In Acmantheroids and Galphimioids, no found were vascular

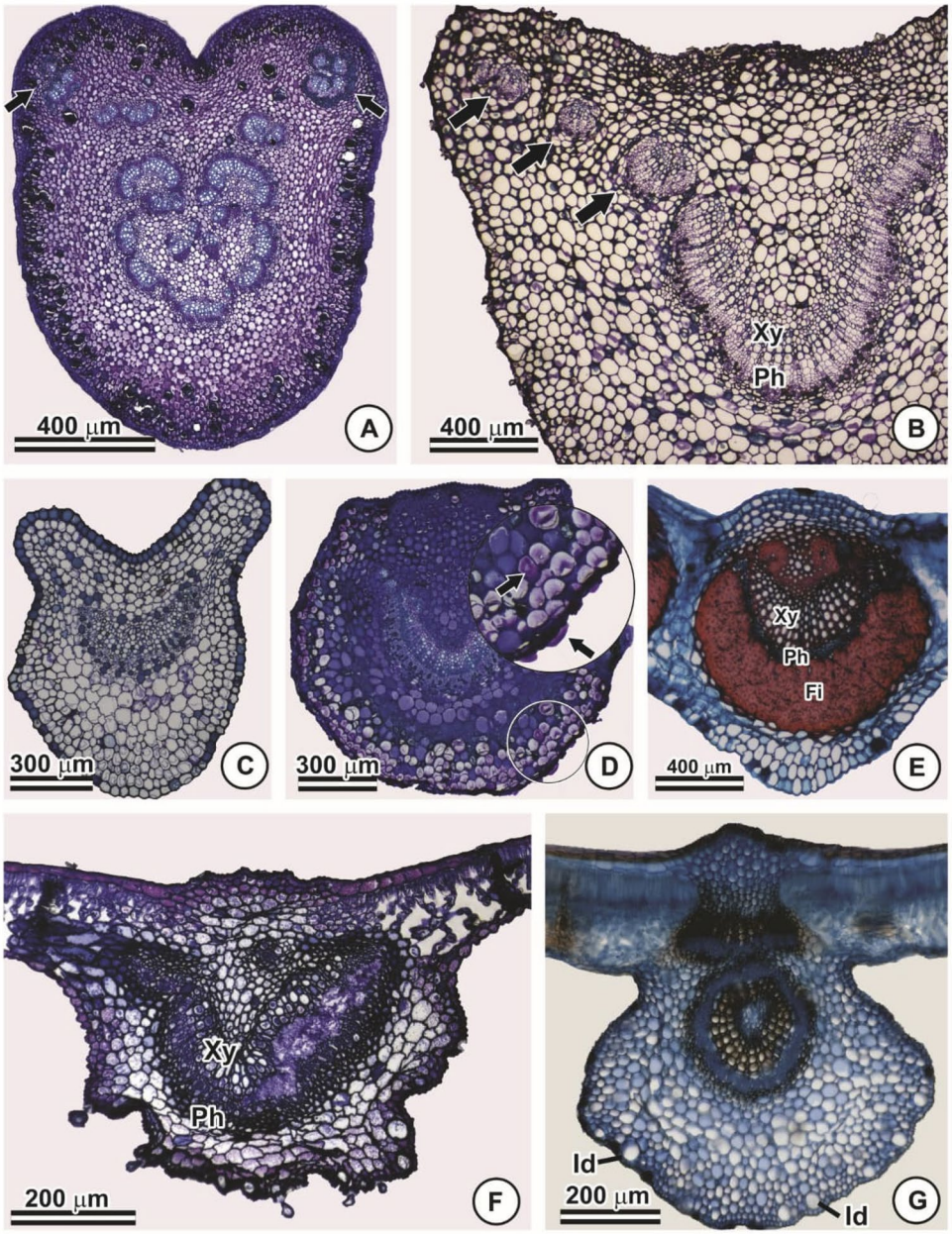

Figure 9. Details of the petiole in Byrsonimoid. A, Details of the petiole in *Blepharandra hypoleuca*, note the vascular bundles (black arrow). B, Accessory bundles (arrows) in the petiole of *Pterandra pyroidea*. C, Petiole anatomy of *Galphimia brasiliensis* evidencing the absence of sclereids and druses. D, Open-arch vascular system and pericycle differentiated in fibres (black arrow) in the midrib of *Byrsonima rigida*. E, Open-arch vascular system of the midrib of *Pterandra arborea*. F. Circular conformation with an dorsal bundle of vascular system of *Pterandra sericea*, note subepidermals idioblasts. G, Petiole anatomy of *Byrsonima triopterifolia*, note the mucilaginous idioblasts in cortical region. Abbreviations: Xy, xylem; Ph, phloem; Fi, fibres.

bundles in the midrib medullary region (Fig. 6C, D, G). In Acmantheroids, Galphimioids, and most species of Byrsonimoids *s.l.* do not present pericycle differentiated in fibres in the midrib (Fig. 6B), and represents the ancestral condition of the Byrsonimoid *s.l.* (78.9% probability). In *Blepharandra hypoleuca* (Fig. 6F), *Byrsonima rigida* (Fig. 9E), *B. umbellata* (Fig. 6A), *B. triopterifolia* (Fig. 6E), *Diacidia aracaensis*, *D. cordata*, and *G. glandulosa*, differentiated pericycle occurs in a fibre sheath. This condition appeared at least three times in Byrsonimoid *s.s.* and once inside Galphimioid.

Related to idioblasts, in *Galphimia gracilis*, *S. membranacea*, and *P. pyroidea*, phenolic idioblasts with granular and dense content occur in the cortex of the midrib (Fig. 6C). The presence of subepidermal idioblasts in midrib, mesophyll, and margin whose chemical nature is unknown is typical of *Pterandra* species (Figs 5G, 9G), pointed out as a possible homoplastic synapomorphy in *P. arborea* and the clade constituted by the other species of genus (Fig. 2D, 87.3%).

The bundles immersed in mesophyll are collateral: for the larger calibre bundles a parenchymatic sheath extension was observed

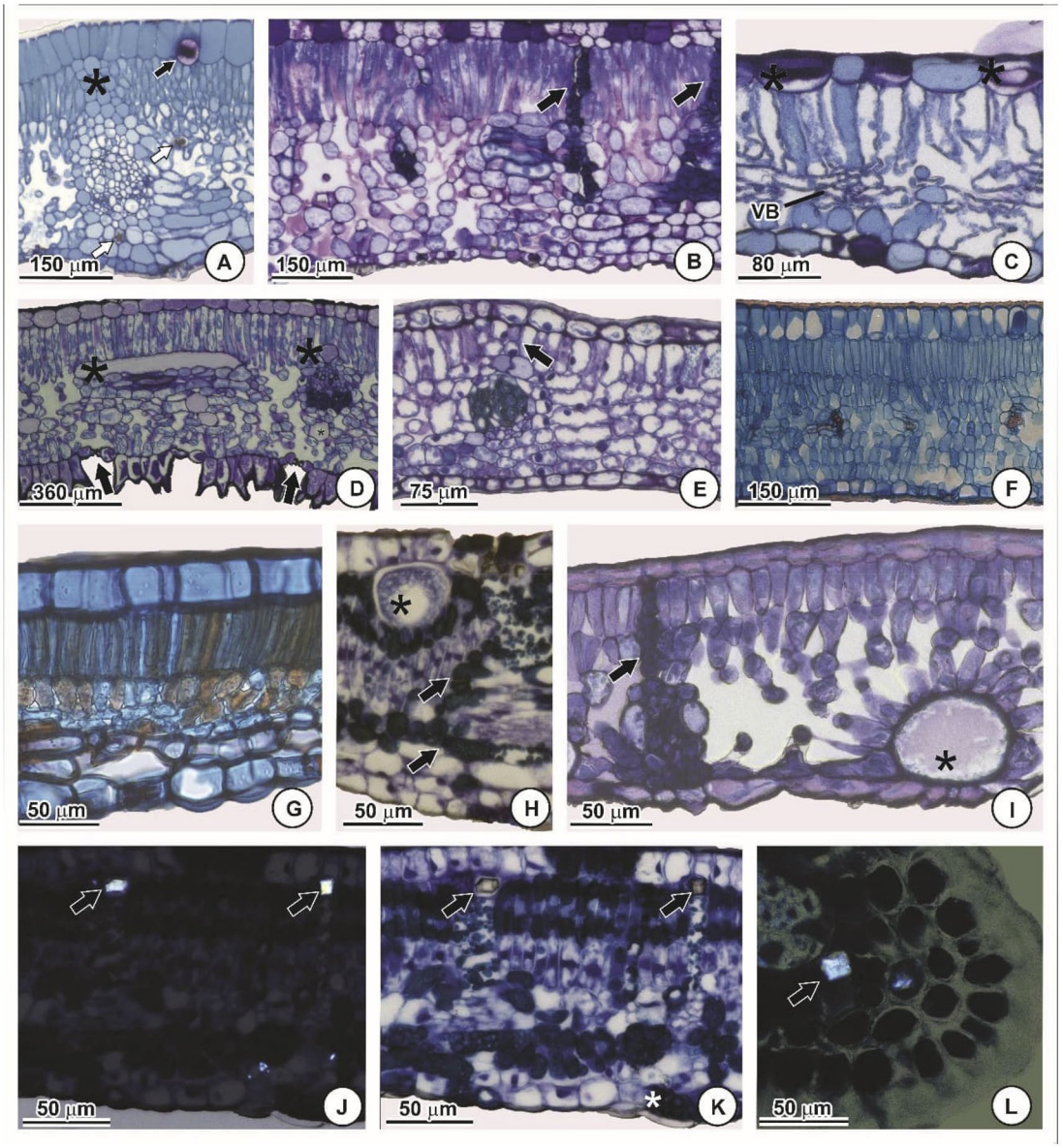

Figure 10. Mesophyll of Byrsonimoids. A, Extension of parenchymal sheath (asterisk) of the bundle in mesophyll and mucilaginous epidermis (arrow) in *B. stanardii*, note the presence of druses (white arrow). B, Mesophyll of *Blepharandra hypoleuca*, note the lignified sheath extension of the vascular bundle (black arrow). C, Dorsiventral mesophyll of *G. brasiliensis*, note the absence of the sheath extension of the vascular bundle. D, Dorsiventral mesophyll of *V. glaucophylla*, note the absence of a sheath extension of the vascular bundle and laticifers (asterisk) in mesophyll. E, Dorsiventral mesophyll of *Lophanthera lactescens*, note the extension of the parenchymal sheath (arrow) of the bundle. F, Isobilateral mesophyll of *Byrsonima gardneriana*. G, Mesophyll with one-stratified palisade parenchyma in *A. latifolia*; H, Dorsiventral mesophyll with phenolic idioblasts (black arrow) and large subepidermal idioblast with unidentified content (asterisk) in *Pterandra pyroidea*. I, Subepidermal idioblast with unidentified content (asterisk) in mesophyll of *P. arborea*, note the bundle sheath extension (black arrow). J, Mesophyll highlighted with polarized light in *P. pyroidea* showing the prismatic crystals (black arrows). K, Isobilateral mesophyll with prismatic crystals (black arrows) in *P. pyroidea*, note the phenolic idioblasts on epidermis (asterisk) and mesophyll. L, Single crystal (arrow) in polarized light at the *Byrsonima morii* margin.

in *Byrsonima bahiana*, *B. sericea*, *B. stanardii* (Fig. 10A), *B. subterranea*, *Lophanthera*, *Spachea tricarpa*, and *S. correae*. The sheath extension is lignified in Acmantheroid clade and the other species of Byrsonimoid, including *Blepharandra* (Fig. 10B) and *Diacidia*. The loss of sheath extension is punctual in *Byrsonima daelbata*, *Spachea elegans*, *S. membranacea*, and in the clade formed by *Galphimia* species (Fig. 10C) and *Verrucularia glaucophylla* (Fig. 10D). The most likely ancestral condition for Byrsonimoid *s.l.* is the presence of sheath extension (77.9% probability) and the parenchimatic type (72.9% probability).

The mesophyll is dorsiventral for Acmantheroid, and most of the analysed species of Bysonimoid and Galphimioid (Fig. 10A–E), being reconstructed as the ancestral condition for Byrsonimoid *s.l.* (95.3% probability). The isobilateral mesophyll occurs in at least four clades within *Byrsonima: B. arctostaphylloides*, *B. correifolia*, *B. bahiana*, *B. brachybotrya*, *B. cipoensis*, *B. gardneriana*

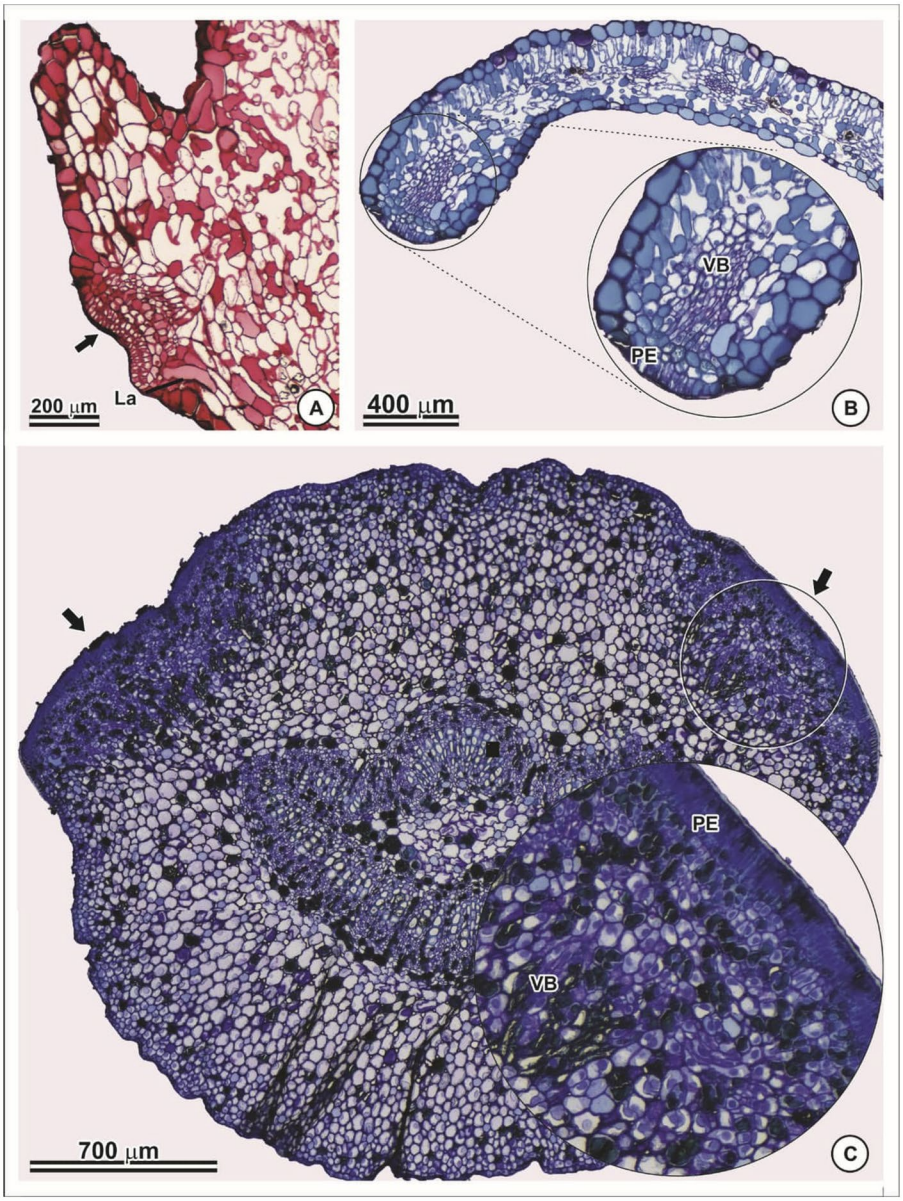

Figure 11. A, PAS reaction showing the nectary (black arrow) in the longitudinal section of the margin in *Galphimia brasiliensis*, note the laticifer near the nectary. B, Details of the nectary in cross-section of *Galphimia brasiliensis* showing palisade epidermis (PE) and vascular bundle (VB). C. Acropetiolar nectaries (one pair) in *Lophanthera lactescens*, note the PE and VB.

(Fig. 10F), *B. laxiflora*, *B. microphylla*, and *B. morii*. In Acmantheroid, the mesophyll layers are significant among taxa, consisting of one-stratified palisade parenchyma in *Acmanthera latifolia* (Fig. 10G) and *Coleostachys genipifolia*, and two or more layers of palisade parenchyma for *Pterandra* species (Fig. 10H–K).

The anatomical structure of the leaf margin is similar among *Byrsonima* species (Fig. 7A, B) and Galphimioids (Fig. 7C, F), with interrupted palisade parenchyma at the leaf blade edge. In *Blepharandra hypoleuca* (Fig. 7D) and Acmantheroid (Fig. 7H, 88.2%), a fibre hubcap occurs in the margin, representing homoplastic synapomorphy (Fig. 3A). The common ancestor of Byrsonimoid *s.l.* did not contain a fibre cap at the margin (Fig. 3A, 79.2% probability).

Mineral inclusions and secretory structures: position, description, and evolutionary relevance

Druses occur dispersed in the mesophyll of Acmantheroids and Byrsonimoids (Fig. 10A), except for *Byrsonima bahiana*, *B. brachybotrya*, *B. lanulosa*, *B. vacciniifolia*, and Galphimioids, where such structures are absent. In Byrsonimoid, prismatic crystals occur dispersed only in *Byrsonima morii* (Fig. 10L). In Acmantheroids, the subepidermal prismatic crystals were also observed in the sheath extension on both sides of the leaf for *Pterandra* species (Fig. 10J, K), pointed out as a possible synapomorphy for the clade formed by *P. hatschbachii* and *P. pyroidea* (Fig. 3B, 90.9%), also occurring in *P. arborea*.

In Galphimioid, the presence of laticifers, as reported for mesophyll of *Verrucularia glaucophylla* (Fig. 10D) and associated with the nectariferous parenchyma of *Galphimia brasiliensis* (Fig. 11A), is considered a possible synapomorphy for the group (Fig. 3C), observed in all evaluated species of the clade. Laticifers are absent in leaf of Acmantheroids and Byrsonimoids (Fig. 3C).

In Galphimioids, the analysed species belonging to *Galphimia elegans* (Fig. 11A, B), *G. glandulosa*, *G. speciosa*, *Lophanthera* (Fig. 11C), and *Spachea correae* there is one pair of acropeciolar nectaries, sessile, with a flat surface, being constituted by a palisade epidermis, vascularized, and nectariferous parenchyma placed below to the epidermis, where phenolic idioblasts with granular and dense content occur sparsely (Fig. 11C). These leaf nectaries emerge as a potential synapomorphy for Galphimoids (Figs 3D, 4A). In most species of *Galphimia* there are marginal protuberances at the base of the leaf blade, like a tooth, with a nectary at its base (Fig. 11A): probably a synapomorphy (Fig. 4B, 99.8%). The nectary is sessile with a concave or plane surface, constituted by a palisade secretory epidermis, which is vascularized by bundles from the leaf midrib to the subepidermal secretory parenchyma (Fig. 11B). In other taxa of Galphimioid, the nectaries are scattered in the leaf blade. In Acmantheroids and Byrsonimoids *s.s.*, no nectaries were found on the petiole and leaf blade, representing the likely ancestral condition for Byrsonimoid *s.l.* (Fig. 4A, 83.9% probability for eglandular petioles and 55.8% probability for leaf blade without nectaries).

DISCUSSION

In recent decades, phylogenetic studies of the Malpighiaceae have assessed the evolutionary history related to its infrafamilial classification. These studies allowed proposing new arrangements at the infrafamilial level, which should be described and defined on the basis of morphological characters. In this context, we encode anatomical characters in a comparative and robust approach in the Byrsonimoid *s.l.* group, one of the lineages well-supported in the phylogenetic hypothesis of Malpighiaceae. This innovative approach may contribute to our understanding of the anatomical diversification for Byrsonimoid *s.l.* and encoded characters may lead to new evolutionary insights into anatomical studies for the Malpighiaceae family.

The anatomical features related to petioles are considered valuable for taxonomy due to their low plasticity even when exposed to environmental pressures (Metcalfe and Chalk 1979). The patterns observed in this study, such as the contour, the vascular system conformation, presence, and number of accessory bundles proved useful for distinguishing between *Byrsonima* and the other evaluated genera. This observation is consistent across different genera in the family, including *Banisteriopsis*, *Heteropterys* (Araújo *et al.* 2010), and *Stigmaphyllon* (Guimarães *et al.* 2016). All these studies consider petiole characteristics useful in distinguishing taxa.

The intraspecific anatomical variations in the vascular system conformation of *Byrsonima brachybotria*, *B. sericea*, and *B. poeppigiana*, may be attributed to the long petioles of these species. The ancestral condition of Byrsonimoid *s.l.*, open arch without convoluted ends of petiole vascular system, evolved in other lineages in the family, as is the case of the petioles of *Banisteriopsis* (Stigmaphylloid, Araújo *et al.* 2020) and *Amorimia* (Malpighioid, Mello *et al.* 2019); This also applies to the open arch type with convoluted ends, which is characteristic of *Byrsonima* and in the clade formed by *Acmanthera* + *Coleostachys*. This feature has been described in *Glandonia* (Mcvaughioid, Guesdon *et al.* 2018) and *Bronwenia* (Stigmaphylloid, Vilarinho *et al.* 2023). Cortical sclereids on the petiole appear to have appeared in Byrsonimoid *s.s.*, while the absence of this trait is interpreted as an evolutionary loss for the clade consisting of *Byrsonima rigida* + *B. triopterifolia*, which are sister group to the other *Byrsonima* species, and the genus *Diacidia*. These findings align with data from Santos *et al.* (2020), which indicate that these evaluated *Byrsonima* species differ from *D. aracaensis* due to the presence of cortical sclereids. The authors also noted the existence of a fibrous sheath near the phloem, a condition similar to the differentiated pericycle found in *Byrsonima rigida*., suggesting that this feature may be more prevalent within *Byrsonima*.

The presence of medullary bundles in the petiole, a novelty found in the *Diacidia* + *Blepharandra* clade, is unprecedented in the Malpighiaceae family and may have originated in populations that gave rise to this clade, which is restricted to northern South America (Anderson 1981, Davis and Anderson 2010). These evolutionary traits may challenge the previously interpreted evolutionary history in Byrsonimoid *s.s.*, which was solely based on morphological markers. Overall, considering all the anatomical synapomorphies of the petiole, our results reinforce the importance of petiole characters in phylogenetic studies within the Malpighiaceae family.

The anatomy of the leaf margin in the Malpighiaceae family has not been thoroughly explored in taxonomic studies, with limited attention given in the studies by Almeida *et al.* (2017) and Mello *et al.* (2019). However, certain anatomical patterns have been identified that aid in distinguishing various taxa. For instance, the presence of a marginal fibre hubcap has been proposed as a probable synapomorphy for the genus *Amorimia* (Mello *et al.* 2019).

In this study, we identified two notable marginal characteristics in the Byrsonimoid group: margin curvature and the presence of marginal fibres hubcap. The fibre cap on the margin was also identified as a homoplastic synapomorphy in Acmantheroids and *Blepharandra hypoleuca*. Our findings support the notion that leaf margin anatomy can reveal evolutionary patterns, aligning with the discussion put forth by Mello *et al.* (2019).

The leaf epidermis of Malpighiaceae species exhibits a variety of informative anatomical features (Araújo *et al.* 2010, 2020, Araújo and Meira 2016, Santos *et al.* 2020, 2023, Vilarinho *et al.* 2023). These characteristics include the presence and type of trichomes, the presence and content of idioblasts, the presence and the number of epidermal layers, the types of stomata, and the presence of stomatal ridges. Recognizing these patterns can help differentiate species and contribute to phylogenetic interpretations within the Byrsonimoid group. In the study by Almeida *et al.* (2024), the authors recognized only three diagnostic characters for the group (treated as the subfamily Byrsonimoideae). Here, we point out numerous anatomical characters and consider the leaf as the important organ in taxonomic classification proposals.

Stomata on both sides of the leaf have been documented only once in the Malpighiaceae literature. However, *in vitro* cultivation studies of *Byrsonima verbascifolia* (L.) DC. showed that the

explants exhibited this characteristic, probably due to the controlled environmental conditions of the study (Vasconcelos-Filho 2008). By contrast, when collected from their natural habitat, the leaves of this species are typically hypostomatic, as noted in this study and in Santos *et al.* (2020). Thus, amphistomatic leaves are regarded as an evolutionary novelty in the Galphimioid lineage, representing convergent evolution in both *Galphimia angustifolia* and *G. mexiae*.

We observed a predominance of T-shape trichomes in the evaluated species, which highlights the widespread occurrence of malpighiaceous trichomes across various genera within the Malpighiaceae family (Metcalfe and Chalk 1950, Gates 1982, Araújo *et al.* 2010). This ancestral trait in Byrsonimoid *s.l.* can be reflected in Malpighiaceae as a whole, particularly considering its presence in different lineages within the family. Taxa with glabrous leaves may represent evolutionary losses of these trichomes. In addition, the Y-shaped trichomes found in a few species of *Byrsonima* and one species of *Galphimia* demonstrate the utility of this characteristic for distinguishing species within each genus, as noted in previous studies (Araújo *et al.* 2010, Santos *et al.* 2020). The predominance of T-shaped trichomes was also observed in *Acridocarpus* (Acridocarpoid), with only *A. spectabilis* possessing the Y-shaped trichome (Santos *et al.* 2023). Considering the hypothesis of an ancestral condition, the Y trichome might represent a more recent development within these lineages. In *Byrsonima*, the structural diversity of trichomes may be linked to the wide geographic distribution of the taxa (Francener 2020). Future studies should aim to generate hypotheses that connect the presence of different tector trichomes in the leaves with the distribution of these taxa across various phytophysiognomies.

The presence of mucilaginous epidermis is identified as an ancestral trait in Byrsonimoid *s.l.* particularly in all species of *Byrsonima*. This finding contradicts the structure of a biseriate epidermis, as suggested by Araújo *et al.* (2010) and, most recently, by Santos *et al.* (2020). In these studies, the samples were processed using double staining techniques, which probably led to the washing away of the epidermal contents and a subsequent misinterpretation of the epidermis type described in *Byrsonima*. In addition, the unique occurrence of epidermal idioblasts containing phenolic and mucilaginous compounds in Malpighiaceae family, as seen in *Byrsonima verbascifolia* and *B. umbellata*, helps to differentiate these two species from other representatives of the genus. This highlights the necessity for specialized techniques to accurately describe such secretory idioblasts, which is crucial for recognizing these patterns in the taxa being studied.

The presence of hypodermis in the *Blepharandra* + *Diacidia* clade can be interpreted as an evolutionary novelty that has not been previously described in the Malpighiaceae family. It should not be confused with the bistratified epidermis found in, for example, *Byrsonima* (Araújo *et al.* 2010, Santos *et al.* 2020) and *Banisteriopsis* leaves (Araújo *et al.* 2020). The clarification is important due to the misinterpretations discussed earlier and the structural differences between the two cases. In addition, the continuity of the hypodermis helped differentiate *Blepharandra hypoleuca* (discontinuous hypodermis), which has a discontinuous hypodermis, from *Diacidia*, which has a continuous hypodermis. This distinction is significant as it serves as an autapomorphy for *Blepharandra* and a synapomorphy for *Diacidia*. Despite a comprehensive molecular and morphological framework, a recent synopsis of Malpighiaceae could not establish synapomorphies for *Blepharandra* and *Diacidia* (Almeida *et al.* 2024). The leaf anatomical traits we describe here therefore fill a key systematic gap and provide a foundational contribution for delimiting these genera in future phylogenetic classifications.

Phenolic idioblasts are beneficial for delimitation taxa, as demonstrated in the case of *Amorimia* W.R.Anderson. The absence of this feature played a key role in distinguishing it from phylogenetically related genera (Mello *et al.* 2019). While the presence these idioblasts is commonly reported in Malpighiaceae (Metcalfe and Chalk 1950), many previously described genera lack these idioblasts (Araújo *et al.* 2010, Guimarães *et al.* 2016, Mello *et al.* 2019). This includes the sole species of *Coleostachys*, highlighting the significance of epidermis characteristics in distinguishing taxa.

The occurrence of leaves with papillose epidermis in the Malpighiaceae family is rare; However, this trait plays a significant role in distinguishing taxa within Galphimimoids. The presence of epidermal papillae has evolved at least twice in Byrsonimoid *s.l.* (specifically in *V. glaucophylla* and *G. glandulosa*). This trait has also been reported in species of the Amazonian genus *Glandonia* (Mcvaughioid) (Guesdon *et al.* 2018) and described as an adaptive trait by Anderson (1981). Therefore, the papillose epidermis on leaves represents a homoplastic synapomorphy, and its occurrence may be associated with Amazonian environments. There is a geographic disjunction between the two species of *Verrucularia: V. piresii* is found in the Amazon, near Venezuela, while *V. glaucophylla* is located in centre of Bahia (Brazilian state). Both species exhibit a papillose epidermis (Anderson 1981). If we consider the hypothesis of an Amazonian origin of the papillose epidermis, the papillae on the leaves of *V. glaucophylla* may indicate a conserved condition within the genus, suggesting that the populations of this taxon migrated from the Amazon and became relictual in Bahia. Furthermore, the papillae found in *Galphimia glandulosa* are smaller and occur in lower quantities, which could be interpreted as a transition from the ancestral condition observed in *Verrucularia*, its sister group.

Stomatal ridges are frequently mentioned in anatomical studies focused on taxonomy (Araújo *et al.* 2010, Santos *et al.* 2020). In the Malpighiaceae family, the presence of stomata ridges in the ancestor of Byrsonimoid *s.l.* and in other groups might suggest that these structures were also present in the family's hypothetical common ancestor. A notable feature distinguishing Acmantheroids from other groups within Byrsonimoid *s.l.* is the presence of subsidiary cells covering the guard cells. This feature that may have appeared more frequently in other more distant lineages within the Malpighiaceae, as highlighted by Araújo *et al.* (2010) in their observations of species such as *Banisteriopsis* (Stigmaphylloid) and *Heteropterys* (Heteropterys clade). In addition, stomata located below the epidermal level were observed in *Diacidia* and certain species of *Galphimia*, which is a distinguishing characteristic in the present study. This trait may have evolved four times in Byrsonimoid *s.l.* and represent a novel development for the family. In the proposal by Almeida *et al.* (2024), the tribe Acmanthereae (here treated as the Acmantheroids clade) is

recognized for presenting characters related to leaf vascularization and carpel conation. Our anatomical data expand the recognition of this group in taxonomic studies.

The contour and vascular system conformation of midrib are potentially useful traits to distinguish species and genera within the Malpighiaceae family (Araújo *et al.* 2010, Almeida *et al.* 2017, Guesdon *et al.* 2019, Mello *et al.* 2019, Santos *et al.* 2020). The biconvex contour pattern identified as an ancestral condition in Byrsonimoid *s.l.* has also been noted in other clades within the family, such as *Barnebya* W.R.Anderson (Barnebya clade, Matos *et al.* 2022), *Banisteriopsis*, *Bronwenia*, *Diplopterys*, and *Stigmaphyllon* (Stigmaphylloid, Vilarinho *et al.* 2023).

In this study, we found that the midrib vascular system patterns can differentiate *Lophanthera* from Galphimioids; as well as *Diacidia* from *Blepharandra hypoleuca* (Byrsonimoid *s.s.*) and *Pterandra* from other Acmantheroids. Notably, the vascular system with a circular shape and a dorsal bundle in the vein distinguishes the clade formed by *Pterandra hatschbachii* + *P. pyroidea* (including the unsampled species *P. sericea*) from *P. arborea*, which is placed in the clade with the other Acmantheroids.

The diversity of midrib vascular system conformation in Byrsonimoid *s.l.* is promising for systematic interpretations and facilitates the distinction of taxa within each genus. It also supports the recovery of two synapomorphies, within the genera *Lophanthera* and *Pterandra*. Similar findings were noted in Stigmaphylloids, where the open-arch vascular system of the midrib serves a synapomorphy for the clade (Vilarinho *et al.* 2023).

Regarding the pericycle differentiated into fibres, Mello *et al.* (2019) observed this feature in the veins of species from *Amorimia* and *Mascagnia s.s.*, although information on evolutionary interpretations was limited. Our data suggest the occurrence of fibre sheaths in unrelated taxa within Byrsonimoid *s.s*. In the case of *Byrsonima*, one of the most diverse genera within Byrsonimoid *s.s.* (Anderson *et al.* 2006), the anatomical diversity found in the midrib it is useful to distinguish species, which is consistent with the findings in recent studies (Santos *et al.* 2020).

The mesophyll type is a characteristic frequently included in identification keys, helping to distinguish different taxa. This is showed in studies on *Byrsonima* (Araújo *et al.* 2010, Santos *et al.* 2020), *Banisteriospis* (Araújo *et al.* 2010, 2020), and *Heteropterys* (Araújo *et al.* 2010). In our work, the mesophyll type is conserved in most of the genera evaluated, exhibiting a dorsiventral mesophyll, which is considered the ancestral condition in Byrsonimoid *s.l.* However, some *Byrsonima* species display isobilateral mesophyll, may be linked to genera that show higher taxa diversity within Malpighiaceae, as seen in *Banisteriopsis*, where 30 out of the 42 analysed species bear this trait (Araújo *et al.* 2020). Except for the monospecific genera, *Dinemandra* A.Juss and *Dinamagonum* A.Juss, isobilateral mesophyll is the only type observed in Ptilochaetoids (Lima *et al.* 2021). While the mesophyll type could be interpreted as distinguishing character for these taxa, it does not significantly contribute to evolutionary interpretations within Malpighiaceae.

The bundle sheath extension has not been thoroughly investigated in previous studies and should be regarded as an important feature in systematic due to the significant differences observed among the evaluated groups. The parenchymatic sheath extension, which is an ancestral condition for Byrsonimoid *s.l.*, is nearly absent in Byrsonimoid *s.s.*, with only three taxa of *Byrsonima* having parenchyma extension. The sclerified sheath extension type is a characteristic feature of the Acmanteroids, as well as in most sampled *Byrsonima* species and all species of *Diacidia* and *Blepharandra hypoleuca*. Thus, the type of bundle extension (whither sclerified or parenchymal) in the mesophyll may not be a reliable criterion for the taxonomy of all groups in the Malpighiaceae family. The absence of extension may be indicated an evolutionary loss in *Galphimia*, as well as in certain species of *Byrsonima* and *Spachea*. In other genera of Malpighiaceae there is variation of this character among individuals of each species, which could limit its utility in comparative anatomical studies. For instance, the constant variation observed among individuals in the *Banisteriopsis* taxa restricts this characteristic to merely its presence or absence within the genus (Araújo *et al.* 2020). Therefore, while the types of bundle sheath extension show a promise for evolutionary interpretations, they require careful consideration in future research.

In the phylogenetic hypothesis, *P. arborea* emerges in a clade alongside *A. latifolia* and *C. genipifolia*, while the remaining species of *Pterandra* form a separate sister group clade. The species in the first clade are closely geographical distributed, predominantly in the northern region of the country: *A. latifolia* is found in the states of Amazonas and Roraima (Farronay *et al.* 2019); *C. genipifolia* is located in Amapá and Pará, extending into Maranhão in Northeastern region (Almeida and Hall 2016); and *P. arborea* occupies Amazonas, Amapá, and Pará state. By contrast, the other *Pterandra* species are located in the Midwest region: *P. hastchbachii* is restricted to Mato Grosso, while *P. pyroidea* can be found in the Federal District, Goiás, and extending into Minas Gerais state in the south-east region (Anderson 1997b). This phylogenetic arrangement probably reflects geographic distribution, with *Pterandra* species exhibiting disjunct populations forming a clade from the other taxa sampled Acmantheroids. Furthermore, the unique structure of the vascular system observed exclusively in *P. sericea*, which differentiates it from the other species of the genus, may highlight this phylogenetic arrangement. In addition, the characteristics considered exclusive to *Pterandra*, such as the presence of prismatic crystals in the sheath extension of bundles and subepidermal idioblasts of unknown nature, could be interpreted as symplesiomorphies that have been lost in the clade formed by *P. arborea* and the other genera of Acmantheroid.

Leaf nectaries represent a synapomorphy for the Galphimioid group (Byrsonimoid *s.l.*), but these leaf glands have developed multiple times within Malpighiaceae family, appearing in both Paleo- and Neotropical lineages. This is evident in species of *Acridocarpus* (Acridocarpoid) (Santos *et al.* 2023), *Mcvaughia* (Mcvaughioid) (Almeida *et al.* 2019), *Banisteriopsis*, *Bronwenia*, *Diplopterys*, *Stigmaphyllon*, (Stigmaphylloid) (Vilarinho *et al.* 2023), and *Bunchosia* (Tristellateioid) (Silva *et al.* 2025). This supports the idea that the presence and location of nectaries in leaves can improve systematic approaches within Malpighiaceae. This corroborates the findings of Almeida *et al.* (2024) in describing the genus *Byrsonima* with eglandular leaves. The authors also point to the occurrence of glands in the bracteoles as diagnostic for the tribe Galphimieae (here treated as the Galphimia clade).

Our findings may expand knowledge about the treatment of this tribe (*sensu* Almeida *et al.* 2024) with the anatomical synapomorphies recorded for the Galphimia clade.

Interestingly, all the aforementioned anatomical studies reveal a consistent structure of leaf nectaries. This structure comprises a palisaded secretory epidermis, several layers of secretory parenchyma beneath the epidermis, and associated vascularization (Araújo and Meira 2016, Nery *et al.* 2017, Almeida *et al.* 2019, Vilarinho *et al.* 2023). This discovery indicates that the anatomy of leaf nectaries in Malpighiaceae is conserved across the family.

In *Galphimia brasiliensis*, nectaries are described as projections located at the base of the leaf (Castro *et al.* 2001). However, these authors did not mention the presence of laticifers associated with the nectariferous parenchyma, which our study has observed in several species of *Galphimia*. This finding regarding the association of laticifers with nectaries in Malpighiaceae family is novel. A similar observation was made in Euphorbiaceae (Malpighiales), where species of *Croton* L. were studied (Vitarelli *et al.* 2015). The authors suggested that latex may play a role in the transfer of sugars from the phloem to the nectary.

The exclusive presence of laticifers in Galphimioids was initially interpreted as a synapomorphy for the Malpighiaceae family (Vega *et al.* 2002) and our findings support this interpretation for Byrsonimoid *s.l.* However, laticifers have also been described in other lineages within this family, specifically in the genus *Stigmaphyllon* (Stigmaphylloid) and in the remaining group of genera *Tetrapterys* (*Tetrapterys s.s.*) (Pace *et al.* 2019). One of the features leading to the segregation of *Glycophyllum* R.F.Almeida from *Tetrapterys* was the absence of laticifers (Almeida and Van den Berg 2021). These authors viewed this as an example of homoplastic evolution within the family, suggesting that laticifers have evolved independently at least three times in Malpighiaceae (Pace *et al.* 2019). Laticifers appear to have evolved independently and represent relevant traits in the systematics of Malpighiaceae, being present sporadically in certain groups. Our results reinforce the necessity for histochemical tests and ontogenetic studies to confirm the presence and type of laticifers in these groups, to understand the ancestral condition of these secretory structures within the family.

CONCLUSION

The comparative analysis of leaf anatomy offers valuable characteristics for distinguishing species, genera, and, especially, informal groups within the Malpighiaceae family, such as Byrsonimoid *s.l.* Key features include the presence of hypodermis, leaf secretory structures, the arrangement of the vascular system in the petiole and midrib, cortical sclereids in the petiole, and a fibre cap at the leaf margin. These trats are considered evolutionary novelties (either synapomorphies or autapomorphies) and may aid in developing hypotheses about the diversification of clades within Byrsonimoid *s.l.*

The reconstruction of ancestral characteristics suggests that the leaves of the ancestor of Byrsonimoid *s.l.* were probably hairy, featuring T-shaped trichomes. These leaves probably did not contain laticifers or nectaries and had a mucilaginous epidermis. They were hypostomatic with the stomata were located at the same level as the epidermis. The mesophyll was dorsiventral with an extension of the parenchymatic sheath surrounding the bundles. In addition, the midrib was biconvex, exhibiting an open-arch vascular system without convoluted ends and an absence of fibre cap along the margin.

The application of specialized techniques for identify secretory idioblasts, particularly within the *Byrsonima* genus, has challenged the previously held belief of a non-continuous biseriate epidermis in this genus, as noted in various literature sources. In addition, the anatomical analysis of leaf margin revealed promising anatomical patterns that can be interpreted in an evolutionary context for the Malpighiaceae family, providing valuable insights for this purpose.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Botanical Journal of the Linnean Society* online.

CONFLICTS OF INTEREST

None declared.

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DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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