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The taxonomic value of leaf anatomy for species *Byrsonima*: a difficult *genus* of Malpighiaceae Juss.

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ABSTRACT

We studied the leaf anatomy of 15 species of *Byrsonima* and a single species of *Diacidia* as an outgroup to test the relevance of leaf anatomical characters to the complicated taxonomy of this genus. We present complete anatomical descriptions for petioles and leaf blades, together with an anatomical matrix, dendrogram and an identification key for the studied taxa. The most informative characters were the presence of stomatal crest, trichome type, epidermis type, contour and conformation of the vascular system in the diaphragm and medium petiole and number of accessory bundles. The aforementioned characters revealed that the anatomy of the petioles in the genus differs from that of petioles in the outgroup taxon with regard to vascular system conformation and the absence of cortical sclereids. Furthermore, the anatomy of the leaf blade appears to be quite informative in *Byrsonima*, especially in synonymized species. The anatomical features evaluated here have great value for *Byrsonima* taxonomy. These features may be used in an evolutionary approach to the group, especially for proposing a new classification system based on morphoanatomical characters of the genus.

Keywords: murici, plant anatomy, similarity analysis, taxonomy

Introduction

Malpighiaceae is a pantropical plant family comprising 75 genera and ca. 1300 species, with most of them (75 %) being endemic to the Neotropics, especially in the Brazilian territory where 45 genera and 530 species occur (Davis & Anderson 2010; BFG 2018; Almeida *et al.* 2016). *Byrsonima* is the second largest genus of the family with ca. 135 tree and shrub species (Anderson *et al.* 2006; Almeida *et al.* 2016) and is phylogenetically related to *Blepharandra* and *Diacidia* (Davis & Anderson 2010). The species of *Byrsonima* are popularly known as murici in Brazil, and as nanche in the remaining Latin-America countries, where its fruits are largely consumed *in natura* by human populations (Anderson *et al.* 2006).

Byrsonima was divided into two subgenera, *Byrsonima* and *Macrozeuma*, based on the connectors morphology (equal in height to the locules or more) and the color of the anthesis petals (yellow or white / pink, respectively) (Niedenzu 1901; 1928). The first taxonomic revisions of *Byrsonima* were published in the early twentieth century by Niedenzu (1897; 1901), alongside with an infrageneric classification with several sections and subsections based exclusively on leaf features. However, the new molecular

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phylogeny for *Byrsonima* point out the polyphyletism of the subgenera *Byrsonima* and *Macrozeugma* (Davis & Anderson 2010) and evidences the absence of a classification system that reflects the evolutionary history of the genus *Byrsonima*.

Although being taxonomically complicated, the genus *Byrsonima* can be recognized by its terminal inflorescences, elongated thyrses with 1-3-flowered cincinni; cucullate lateral petals and fleshy drupaceous fruits (Fig. 1) (Anderson *et al.* 2006; Almeida *et al.* 2016). However, *Byrsonima* does not have the characteristic leaf glands of Malpighiaceae, making it difficult to distinguish species in absence of fertile material (Anderson 1981).

Therefore, anatomical data may be relevant for a taxonomic approach in the group, considering plant anatomy has been historically used as an important tool in the biological classification of plants. It provides additional informative features which helps to understand its morphological evolution (Metcalfe & Chalk 1979; Endress *et al.* 2000). Leaf anatomy is widely used as an aid in flowering plants taxonomy, especially in groups with taxonomic problems, as in Malpighiaceae (Metcalfe & Chalk 1957; Gomes *et al.* 2009; Araújo *et al.* 2010; Almeida-Jr *et al.* 2013; Almeida *et al.* 2017; Guesdon *et al.* 2018; Almeida *et al.* 2019; Araújo *et al.* 2020). Even though leaf anatomy of *Byrsonima* species has been explored in previous studies, few have listed features that can be attributed to a new classification system for the genus (Araújo *et al.* 2010; Silva 2014).

We present the anatomical study of leaves from 15 species of *Byrsonima*, including representatives of the two subgenera according to Niedenzu (1901; 1928), in order to test the taxonomic relevance of leaf anatomy in the group. *Diacidia aracaensis* was also included to provide a comparative standard. In addition, we provide a matrix with the relevant anatomical data for future studies and a dendrogram obtained from the morphological matrix.

Materials and methods

The selected species of *Byrsonima* represent distinct infrageneric categories established in classic taxonomical studies (Niedenzu 1901; 1928) described in Table 1. *Diacidia aracaensis* was selected as an outgroup due to its phylogenetic proximity to *Byrsonima* (Davis & Anderson 2010). *Byrsonima correifolia*, *B. gardneriana* and *B. sericea* fully expanded leaves (collected in Pai Cormo village, Pedro II city, Piaui state, Brazil; São Brás village, Pedro II city, Piauí state, Brazil; Lagoa do Boi village, Pastos Bons city, Maranhão state, Brazil, respectively) were fixed in the field using FAA (formaldehyde, acetic acid and 50 % etanol; 1:1:18 by volume) (Johansen 1940) and the control material was deposited in a herbarium (HAF / UESPI).The remaining species were taken from herborized material from different herbariums, as shown in Table 1. Herborized leaves were boiled in distilled water until full submersion for a maximum of 10 minutes, kept in a 2 % potassium hydroxide solution for two hours in room temperature, washed in distilled water several times, dehydrated in an ethanol series, and kept in 70 % ethanol (Smith & Smith 1942). *Byrsonima cydoniifolia* A.Juss. and *B. orbignyana* A.Juss. are currently synonyms (Anderson *et al.*2006), but were separately analyzed, since we recognized distinct patterns on their leaf anatomy. All specimens were manually sectioned using a razor blade

Table 1. List of species and vouchers used in this study.

Subgenera (Niedenzu 1901; 1928)	Sections (Niedenzu 1901; 1928)	Species	Collector / number or Accession number
Byrsonima	*Eriolepsis*	*Byrsonima crassifolia* (L.) Kunth	TEPB25696; TEPB26727; TEPB14611
		Byrsonima cydoniifolia A.Juss.	A. Francener 1246 (SP); TEPB32026; TEPB32019
		Byrsonima guilleminiana A. Juss.	A. Francener 1240 (SP); 1129 (SP)
		Byrsonima orbignyana A. Juss.	HAF2731
		Byrsonima pachyphylla A.Juss.	R.F. Almeida 730 (HUEFS), A. Francener 1147 (SP)
		Byrsonima verbascifolia (L.) DC.	TEPB547, TEPB6073, TEPB5642
	Sericolepsis	*Byrsonima chrysophylla* Kunth	A. Francener 1324 (SP); A. Francener 1231 (SP); A. Francener 1346 (SP)
		Byrsonima crispa A.Juss.	A. Francener 1329 (SP)
		Byrsonima intermedia A.Juss.	HAF03460; HAF03235; HAF03457
		Byrsonima laxiflora Griseb.	A. Francener 1186 (SP); 1201 (SP)
		Byrsonima sericea DC.	TEPB14609, TEPB17024, TEPB3652, HAF04357, HAF04407
		Byrsonima spicata Rich. *ex* Kunth	T.A.P. Santos 19 (MFS)
Macrozeugma	*Colobotheca*	*Byrsonima correifolia* A.Juss.	HAF03545; HAF03546; HAF03547; TEPB17115; TEPB27035; TEPB22793
		Byrsonima gardneriana A.Juss.	TEPB20333; HAF745; HAF2727
		Byrsonima ligustrifolia A.Juss.	TEPB26729, TEPB26730
Outgroup	______	*Diacidia aracaensis* W.R.Anderson	G.T. Prance 29105 (US)

Photograph records were taken using a Nikon® E100 photomicroscope, projecting the micrometric scale on the same optical conditions. The classification of vascular bundles contour follows Howard (1979), trichome classification follows Theobald *et al.* (1979), and Metcalfe & Chalk (1979), and stomata classification also follow Metcalfe & Chalk (1979).

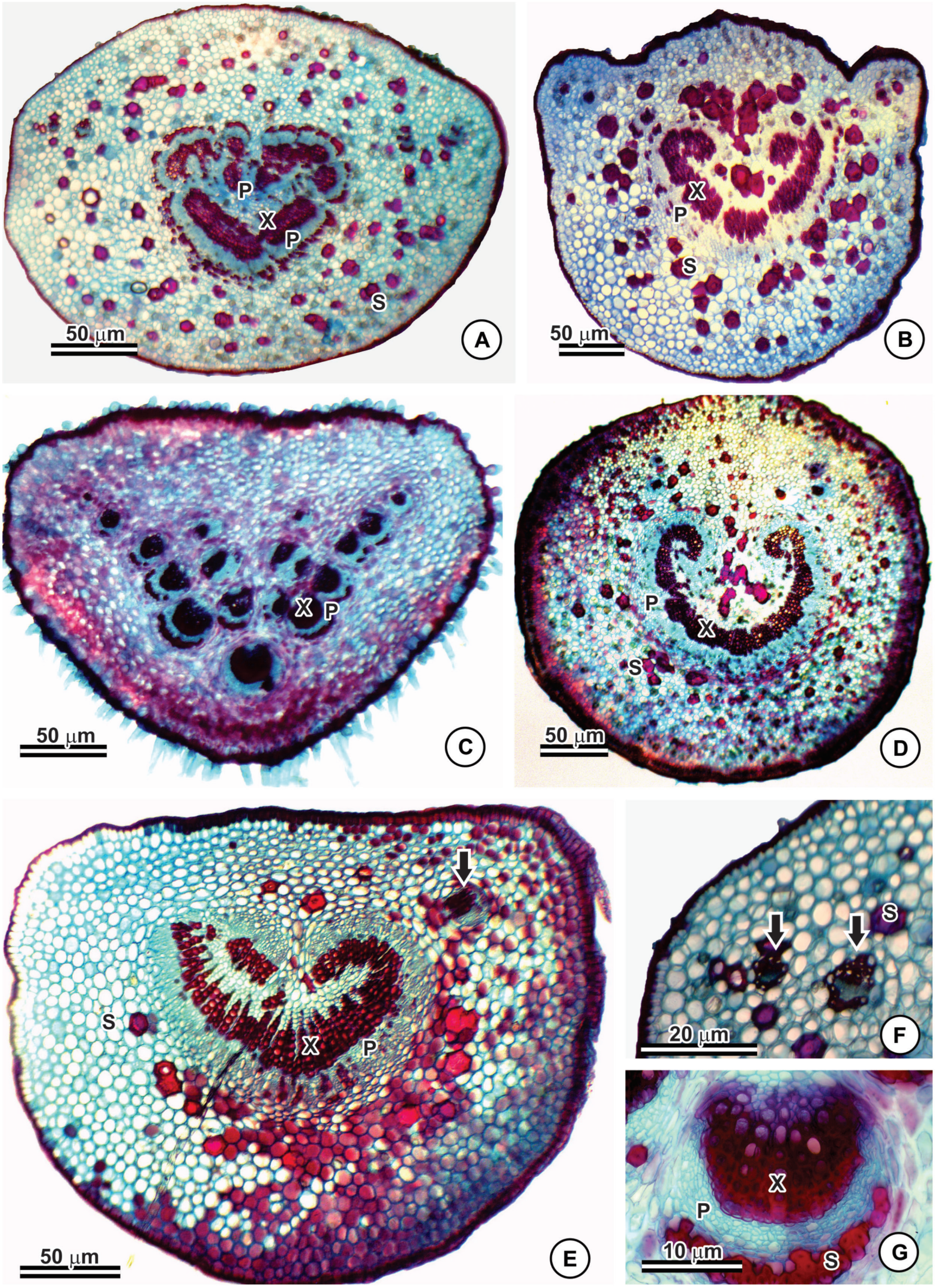

Figure 1. Petiole anatomy in *Byrsonima* and *Diacidia*. Species **A.** biconvex petiole contour in *Byrsonima cydoniifolia*. **B.** biconvex petiole contour with two lateral protuberances in *B. spicata*. **C.** plane-convex petiole contour in *Diacidia aracaensis*. **D.** circular petiole contour in *B. crispa*. **E.** plane-convex petiole contour in *B. gardneriana*. **F.** details of accessory bundles (black arrow) of petioles in *B. cydoniifolia*. **G.** collateral vascular bundle in *D. aracaensis*. Abbreviations: P= phloem; X= xylem; S= sclerenchyma.

on the middle third of petioles, midrib, and semi limb, followed by immersion in 50 % sodium hypochlorite, and staining in basic fuchsin and Astra blue (Roeser 1972). Epidermal dissociations were obtained from leaf fragments immersed in chromic and nitric acids solution 1:1 (v/v) for 24 hours to dissociate the epidermis (Johansen 1940). Temporary slides were mounted in 50 % glycerin and sealed with colorless varnish.

All the *Byrsonima* species and *Diacidia aracaensis* were compared according to their analyzed structural characteristics using a presence/absence matrix (Tab. 2). This matrix used Jaccard's index and an average group linkage technique (also known as unweighted pair-group method using arithmetic averages, UPGMA) with software Past 3.19 (Hammer *et al.* 2001). The definition of the maximum similarity values was made using the software RANDMAT 1.0. A total of two thousand replications were used (a = 1 %).

Results

The petiole contour is biconvex in *Byrsonima ligustrifolia*, *B. intermedia*, *B. cydoniifolia* (Fig. 1A), *B. crassifolia*, *B. orbignyana*, *B. sericea*, *B. verbascifolia*; petioles with two adaxial protuberances in *B. chrysophylla* and *B. spicata* (Fig. 1B); contour plane-convex in *B. correifolia, B. gardneriana*, *B. guilleminiana*, *B. laxiflora*, *B. pachyphylla* and *D. aracaensis* (Fig. 1C); and circular contour in *B. crispa* (Fig. 1D). The main vascular system of petioles is collateral in all species of *Byrsonima* and the conformation of bundles is an open arch with convoluted extremities (Fig. 1A, B, E), followed by a pair of accessory bundles and the occurrence of dispersed druses and sclereids in the cortical region in most species (Fig. 1D). There are more than one pair of accessory bundles in *B. crispa*, *B. chrysophylla*, *B. cydoniifolia, B. intermedia, B. laxiflora B. pachyphylla*, *B. sericea* and *B. spicata* (Fig. 1F). The occurrence of medullary phloem in petioles was exclusive from *B. crassifolia, B. chrysophylla, B. cydoniifolia* (Fig. 1A) and *B. intermedia*. In *Diacidia aracaensis*, the vascular system consists of bundles arranged in an arch and several medullary bundles (Fig. 1C), all collateral (Fig. 1G).

The midribs contour is plane-convex in *B. correifolia* (Fig. 2A), *B. intermedia* and *D. aracaensis*, and biconvex in the remaining species (Fig. 2B, C). In all species bundles are collateral (Fig. 2A), but the vascular system conformation varies, being biconvex in two open arches surrounded by fibers with no medullary phloem in *B. correifolia* (Fig. 2A); concave-convex in two arches interleaved by sclerenchyma in *B. ligustrifolia* (Fig. 2B); biconvex in two arches surrounded by fibers and interrupted by medullary phloem in *Byrsonima crassifolia*, *B. crispa*, *B. chrysophylla*, *B. cydoniifolia*, *B. guilleminiana*, *B. intermedia*, *B. laxiflora*, *B. pachyphylla*, *B.*

Table 2. Morphological matrix of binary characters (absence/presence) including 15 species of *Byrsonima* and its outgroup. 1. Non-continuous bistratified epidermis on the adaxial surface, 2. Non-continuous biestratified epidermis on the abaxial surface, 3. Unistratified epidermis on both sides, 4. Laterocytic paracytic stomata, 5. Paracytic stomata, 6. Stomatal crests, 7. V-shaped trichomes, 8. Y-shaped trichomes, 9. T-shaped trichomes, 10. Dorsiventral mesophyll, 11. Isobilateral mesophyll, 12. Extension of the parenchymal sheath in the bundles in the mesophyll, 13. Extension of the sclerified sheath in the bundles in the mesophyll, 14. Plane-convex midrib in cross-section, 15. Biconvex midrib in cross-section, 16. Midrib with biconvex vascular system, 17. Midrib with plane-convex vascular system, 18. Midrib with concave-convex vascular system, 19. Midrib with vascular system with 3-4 dorsal bundles and an open arch, 20. Midrib with phloem in the medullary region, 21. Plane-convex petiole in cross-section, 22. Biconvex petiole in cross-section, 23. Circular petiole in cross-section, 24. Petiole with two lateral protuberances in cross-section, 25. Petiolar vascular system arranged in an arc with convoluted extremities, 26. Vascular system consisting of bundles arranged in an arch and several medullary bundles, 27. One pair of accessory bundles in the petiole, 28. More than a one pair of accessory bundles in the petiole, 29. Petiole with phloem in the medullary region, 30. Dispersed sclereids in the cortical region in the petiole, Abbreviations: *Byrsonima correifolia* - Bcor; *B. crassifolia* – Bcra; *B. crispa* – Bcri; *B. chrysophyla* – Bchr; *B. cydoniifolia*- Bcyd; *B. gardneriana* – Bgar; *B. guilleminiana*- Bgui; *B. intermedia* – Bint; *B. laxiflora*- Blax; *B. ligustrifolia* – Blig; *B. orbigyniana* – Borb; *B. pachyphylla*- Bpac; *B. sericea* – Bser; *B. spicata*- Bspi; *B. verbascifolia* – Bver e *Diacidia aracaensis*- Dara.

	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	30
Bcor	0	0	1	1	0	0	1	1	1	0	1	0	1	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	1
Bcra	1	0	0	1	0	1	0	0	1	0	1	0	1	0	1	1	0	0	0	1	0	1	0	0	1	0	1	0	1	1
Bcri	0	0	1	1	0	1	0	0	0	1	0	1	0	0	1	1	0	0	0	1	0	0	1	0	1	0	0	1	0	1
Bcrh	1	0	0	1	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	0	1	0	1	1	0	0	1	0	1
Bcyd	1	0	0	0	1	0	0	0	1	0	1	0	1	0	1	1	0	0	0	1	0	1	0	0	1	0	0	1	1	1
Bgar	0	0	1	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0	1	0	1	0	0	0	1	0	1	0	0	1
Bgui	1	0	0	1	0	1	0	0	1	0	1	0	1	0	1	1	0	0	0	1	1	0	0	0	1	0	1	0	0	1
Bint	1	1	0	0	1	1	0	0	1	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0	1	0	0	1	1	1
Blax	1	0	0	1	0	1	0	0	1	0	1	0	1	0	1	1	0	0	0	1	1	0	0	0	1	0	0	1	0	1
Blig	1	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	0	1	0	0	1
Borb	0	0	1	0	1	0	0	0	1	0	1	1	0	0	1	0	1	0	0	1	0	1	0	0	1	0	0	1	1	1
Bpac	1	0	0	1	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	0	0	0	1	0	0	1	0	1
Bser	1	0	0	1	0	1	0	0	1	0	1	0	1	0	1	1	0	0	0	1	0	1	0	0	1	0	0	1	0	1
Bspi	0	0	1	1	0	1	0	0	1	0	1	1	0	0	1	1	0	0	0	1	0	1	0	1	1	0	0	1	0	1
Bver	1	0	0	0	1	1	0	0	1	0	1	0	1	0	1	0	1	0	0	1	0	1	0	0	1	0	1	0	0	1
Dara	1	0	0	0	1	0	0	0	1	1	0	1	0	1	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0

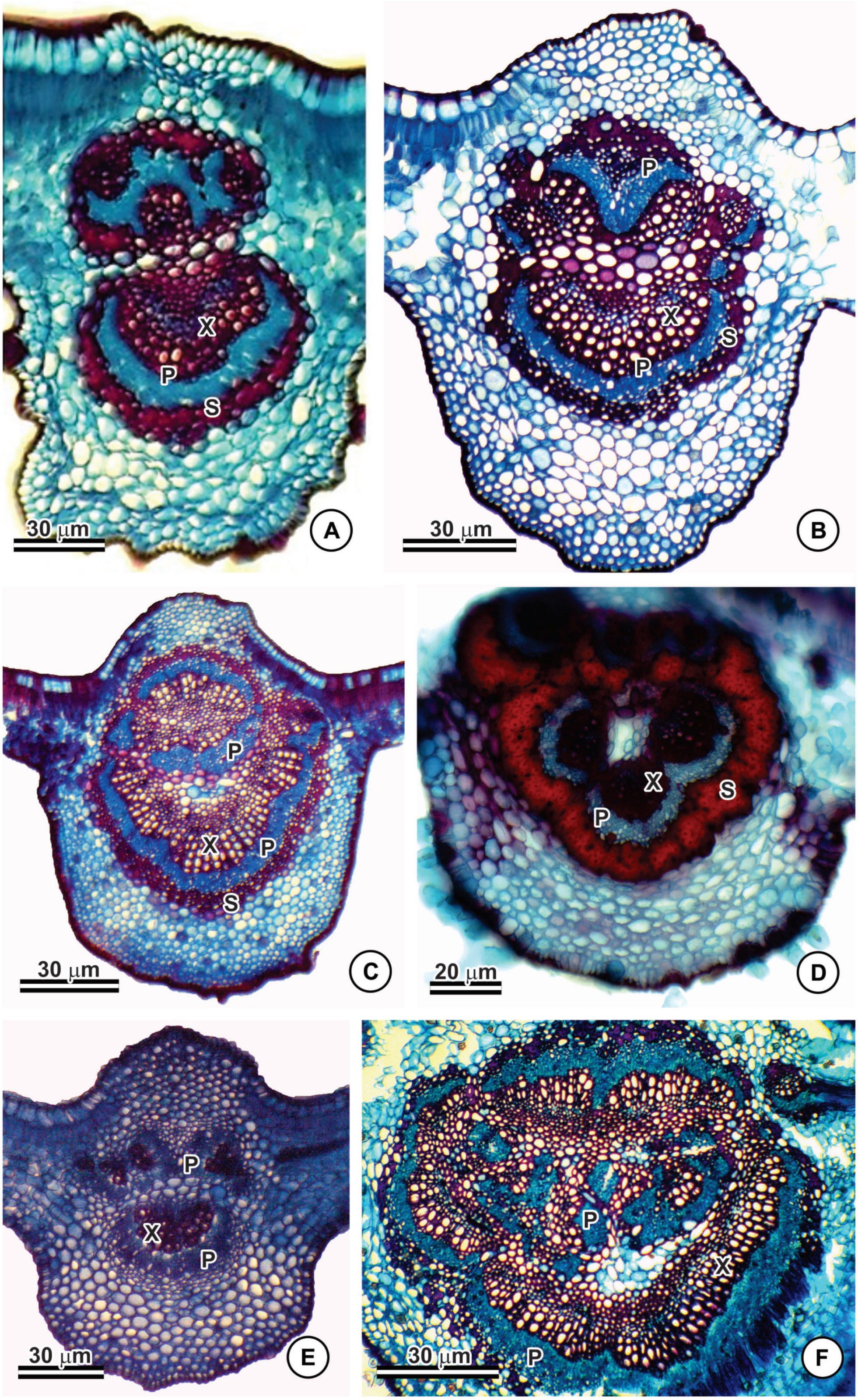

Figure 2. Midrib anatomy in *Byrsonima* and *Diacidia* species **A.** plane-convex midrib contour and biconvex vascular system with no phloem in *B. correifolia*. **B.** biconvex midrib contour and concave-convex vascular system in *B. ligustrifolia*. **C.** biconvex vascular system with medullar phloem *B. chrysophylla* midrib. **D.** midrib vascular system in *Diacidia aracaensis*. **E.** vascular system in open arch and three bundles in *B. gardneriana* midrib. **F.** plane-convex vascular system with medullar phloem in of *B. verbascifolia* midrib. Abbreviations: P= phloem; X= xylem; S= sclerenchyma.

sericea e *B. spicata* (Fig. 2C); collateral bundles adjacently aligned to the adaxial surface and in an arch facing the adaxial surface in *D. aracaensis* (Fig. 2D); three to four dorsal bundles and an open arch in *B. gardneriana* (Fig. 2E); and plane-convex in two arches surrounded by fibers and medullary phloem in *B. verbascifolia* (Fig. 2F) and *B. orbignyana*. In *Diacidia aracaensis*, the vascular system consists of bundles arranged in an arch and three dorsal bundles, all surrounded by fibers (Fig. 2D).

Leaves are hypostomatic with adaxial cells larger in dimension than those from the abaxial surface (Fig. 3A). The epidermis is unistratified in *B. correifolia*, *B. crispa*, *B. gardneriana* (Fig. 3A), *B. spicata* and *B. orbignyana* and the adaxial surface of the epidermis is bistratified non-continuous in most species, as *B. ligustrifolia* (Fig. 3B), except for *Byrsonima intermedia* which was the only species with bistratified non-continuous epidermis on both surfaces (Fig. 3C). Paracytic stomata are predominant in all species, and laterocytic paracytic stomata were observed in *Byrsonima correifolia*, *B. crispa*, *B. chrysophylla*, *B. crassifolia*, *B. guilleminiana*, *B. laxiflora*, *B. pachyphylla*, *B. sericea* and *B. spicata* (Fig. 3D). Stomatal crests are also found in *B. crassifolia*, *B. crispa* (Fig. 3E), *B. intermedia*, *B. guilleminiana*, *B. laxiflora*, *B. sericea*, *B. spicata* and *B. verbascifolia*; while not observed in other species. Trichome scars are observed in *Byrsonima crassifolia*, *B. crispa*, *B. chrysophylla*, *B. cydoniifolia*, *B. gardneriana*, *B. guilleminiana*, *B. intermedia*, *B. ligustrifolia* (Fig. 3F), and *B. sericea*. No trichomes were found in *Byrsonima crispa* and *B. ligustrifolia* (Fig. 3F), only trichome scars. Y-T-V-shaped trichomes, with peduncles varying in size, were recorded in *B. correifolia* (Fig. 3G, H, I). T-shaped trichomes were recorded in *B. crassifolia* (Fig. 3I), *B. chrysophylla*, *B. cydoniifolia*, *B. guilleminiana*, *B. laxiflora*, *B. orbignyana*, *B. pachyphylla*, *B. sericea*, *B. spicata*, *B. verbascifolia*, *B. intermedia* and *Diacidia aracaensis*.

Two types of mesophyll were observed in the studied species: isobilateral in most species, such as *Byrsonima gardneriana* (Fig. 3A) and dorsiventral mesophyll in *B. crispa*, *B. ligustrifolia* (Fig. 3B) and *D. aracaensis*. Vascular bundles in the mesophyll are collateral and circled by a parenchymal sheath in all species, while larger bundles show the same sheath extension in *B. crispa*, *B. chrysophylla*, *B. gardneriana*, *B. ligustrifolia*, *B. pachyphylla*, *B. spicata* and *D. aracaensis* (Fig. 3J). In the remaining species, the sheath extension occurs facing the adaxial surface and is sclerified (Fig. 3K).

Based on the anatomical diversity, petiole and midrib features were used to elaborate an identification key for the studied species, presented below.

Identification key for the studied species of ***Byrsonima*** *and* ***Diacidia***

1. Vascular system with sclereids dispersed in the petioles 2
1’. Vascular system with no sclereids dispersed in the petiole .... ***Diacidia aracaensis***
2. Petiole with circular contour .... ***Byrsonima crispa***
2’. Petiole with plane-convex to biconvex contours 3
3. Petiole with plane-convex contour 4
3’. Petiole with biconvex contour 8
4. Vascular system of the midrib with three or four dorsal bundles and with an open arch .... ***Byrsonima gardneriana***
4’. Vascular system of the midrib with no dorsal bundles and no open arch facing the same surface 5
5. Vascular system of the midrib biconvex with two arches and no medullar phloem .... ***Byrsonima correifolia***
5’. Vascular system of the midrib biconvex with two arches and with medullary phloem 6
6. One pair of accessory bundles associated to the vascular system of petioles .... ***Byrsonima guilleminiana***
6’. More than one pair of accessory bundles associated to the vascular system of petioles 7
7. Presence of stomatal crests on the leaves .... ***Byrsonima laxiflora***
7’. Absence of stomatal crests on the leaves .... ***Byrsonima pachyphylla***
8. Presence of two lateral protuberances in the petiole contour 9
8’. Absence of two lateral protuberances in the petiole contour 10
9. Presence of stomatal crests on the leaves .... ***Byrsonima spicata***
9’. Absence of stomatal crests on the leaves .... ***Byrsonima chrysophylla***
10. Presence of phloem in the medullary region of petioles 11
10’. Absence of phloem in the medullary region of petioles 14
11. One pair of accessory bundles associated to the vascular system of petioles .... ***Byrsonima crassifolia***
11’. More than one pair of accessory bundles associated to the vascular system of petioles 12
12. Unistratified epidermis .... ***Byrsonima orbignyana***
12’. Bistratified epidermis 13
13. Non-continuous epidermis only on the adaxial surface .... ***Byrsonima cydoniifolia***
13’. Non-continuous epidermis on both surfaces .... ***Byrsonima intermedia***
14. Vascular system of the midrib biconvex with two arches with medullary phloem .... ***Byrsonima sericea***
14’. Vascular system of the midrib plane-convex or concave-convex 15
15. Vascular system of the midrib plane-convex with two arches with medullary phloem .... ***Byrsonima verbascifolia***
15’. Vascular system of the midrib concave-convex .... ***Byrsonima ligustrifolia***

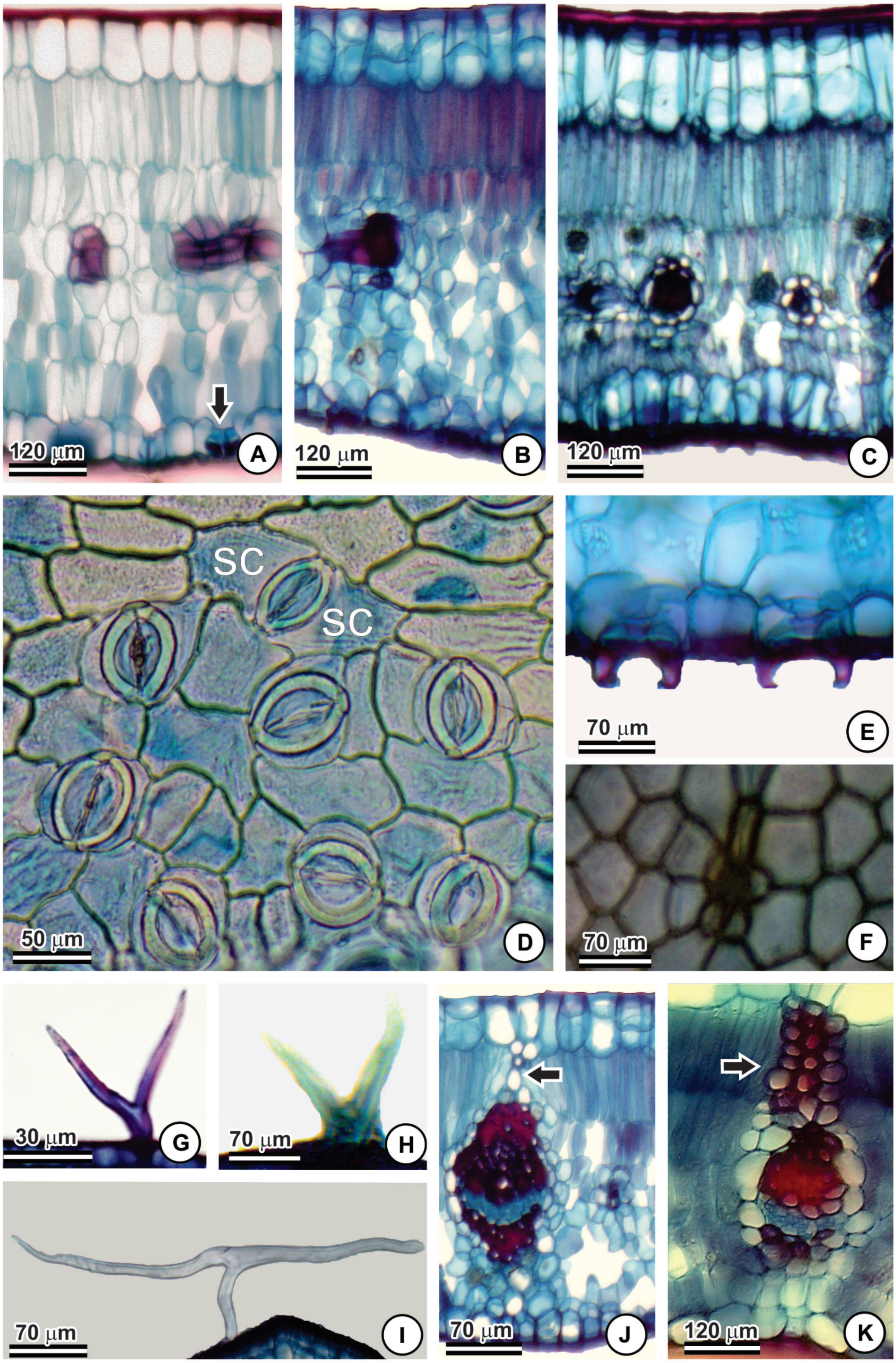

Figure 3. Leaf blade anatomy of *Byrsonima* and *Diacidia*. **A.** Isobilateral mesophyll with unistratified epidermis and stomata (black arrow) on the abaxial surface of *B. gardneriana*. **B.** Dorsiventral mesophyll with bistratified epidermis of *B. ligustrifolia*. **C.** *B. intermedia* mesophyll showing the bistratified non continuous epidermis on both surfaces of the leaf blade. **D.** paracytic and paracytic laterocytic stomata in *B. spicata*. **E.** stomatal crests in *B. spicata*. **F.** hair scar in *B. ligustrifolia*. **G.** Y-shaped hair in *B. correifolia*. **H.** V-shaped hair in *B. correifolia*. **I.** T-shaped hair of *B. crassifolia*. **J.** *B. ligustrifolia* parenchymal extension (black arrow) and vascular bundles sheath **K.** Sclerified leaf sheath extension (black arrow) of the vascular bundle in *B. correifolia* mesophyll. Abbreviation: SC= subsidiary cells.

Similarity analysis

A similarity analysis with UPGMA (Fig. 4) organizes all the evaluated species of *Byrsonima* in a group different from *Diacidia aracaensis*. This difference is attributed to the absence of sclereids dispersed in the cortical region of the petiole in *Diacidia aracaensis* in the vascular system present in the outgroup. Furthermore, the group formed by *Byrsonima* species is consistent in the analysis and point only *Byrsonima correifolia*, *B. gardneriana* and *B. ligustrifolia* for less similarity than the other species, especially because they do not present phloem in the medullary region of the median vein. The highest degree of similarity appears between *B. laxiflora*, *B. sericea* and *B. guilleminiana* (group 1) due to the similarity of the epidermis, especially due to the presence of stomatal crests and trichomes only in T and also the extension of the sclerified sheath of the bundles in the mesophyll; the second group shows the high similarity of *B. chrysophylla* and *B. spicata* (group 2), due to the presence of parenchymal extension of the bundles in the mesophyll and the exclusive presence of petiole with two lateral protuberances in its contour.

Discussion

In the last sixty years, several studies were published on the morphological and classification aspects of *Byrsonima*, focusing on leaves indumentum, bracts and bracteoles, petal color, and anther shape (Niedenzu 1901; 1928). However, no authors explored the anatomy of vegetative organs as an aid to the taxonomy of the genus.

Based on leaf blade and petiole anatomical analyses, we have identified several informative characters that can be used to support a new classification in *Byrsonima* in future studies. The anatomy of petioles shows little influence of environmental pressure and might represent great taxonomic value in different taxa (Metcalfe & Chalk 1957). The four types of petiole contour found in our study corroborates the results presented by Silva *et al.* (2011), and studies on different genera, such as *Stigmaphyllon* (Guimarães *et al.* 2016), and *Banisteriopsis* (Araújo *et al.* 2020). The variation evidenced in this feature makes it a useful tool in the circumscription of species and genera in Malpighiaceae, especially in *Byrsonima*, the second largest and most taxonomic challenging genus in the family according to Almeida *et al.* (2016).

Petiole vascular system presents a similar conformation in all studied species of *Byrsonima*, except for *Diacidia*. Araújo *et al.* (2010) had already highlighted the similarity in conformation of bundles in species of *Byrsonima*, but the authors observed some variation on this structure within species of the genus. Studies exploring the conformation of vascular bundles in the petioles of Malpighiaceae are promising and may contribute with valuable taxonomic characters at the generic level (Araújo *et al.* 2010; Guimarães *et al.* 2016). In *Byrsonima*, the anatomy of petioles evidenced relevant data in the elaboration of an identification key and may subsidize future taxonomic and phylogenetic studies in the family. Nonetheless, it is important to highlight the

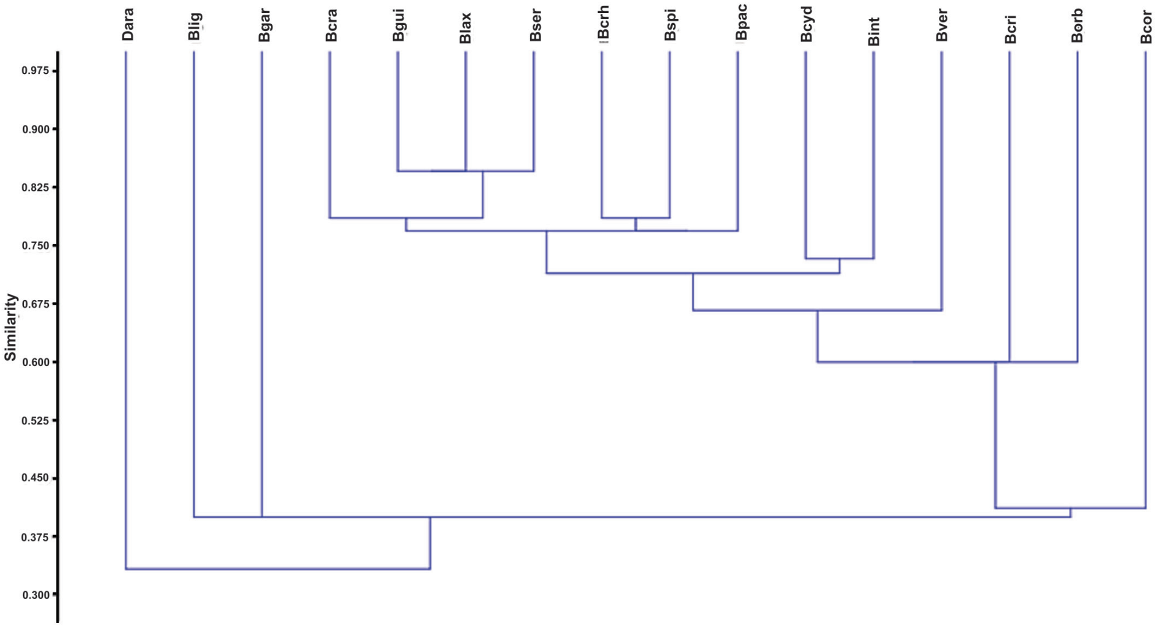

Figure 4. Cluster analysis (UPGMA) of *Byrsonima* species. Abbreviations: *Byrsonima correifolia* - Bcor; *B. crassifolia* – Bcra; *B. crispa* – Bcri; *B. chrysophyla* – Bchr; *B. cydoniifolia*- Bcyd; *B. gardneriana* – Bgar; *B. guilleminiana*- Bgui; *B. intermedia* – Bint; *B. laxiflora*- Blax; *B. ligustrifolia* – Blig; *B. orbigyniana* – Borb; *B. pachyphylla*- Pac; *B. sericea* – Bser; *B. spicata*- Bspi; *B. verbascifolia* – Bver e *Diacidia aracaensis*- Dara.

need for standardization of the analyzed region in case of long petioles, since differences may occur. This fact would explain the data divergences in the conformation of the vascular system recorded in *B. gardneriana* and *B. sericea* in our study from the results obtained by Silva *et al.* (2011). The midrib contour showed variations already observed by previous authors in some species of Malpighiaceae, and in *Byrsonima* this variation was corroborated as important in the delimitation of species (Higuchi 2007; Araújo *et al.* 2010; Silva 2014). The presence of medullary phloem in the midrib is another distinctive character for *Byrsonima* and it has been used to differentiate genera and species in Malpighiaceae (Araújo *et al.* 2010; Silva 2014). The vascular system conformation in the midrib has also been regarded as an important feature by several authors due to the variation or constancy within species, representing a great phylogenetic signal in the genus (Araújo *et al.* 2010; Almeida *et al.* 2019; Mello *et al.* 2019; Araújo *et al.* 2020).

Paracytic stomata are widely distributed in Malpighiaceae (Metcalfe & Chalk 1957), and its occurrence in *Byrsonima* is corroborated by several authors (Higuchi 2007; Vasconcelos-Filho *et al.* 2008; Araújo *et al.* 2010; Silva 2014). On the other hand, the occurrence of other types of stomata is rare in the family, with only Ferreira *et al.* (2015) reporting anomocytic and tetracytic stomata in *Byrsonima coccolobifolia*. The presence of paracytic laterocytic stomata in *Byrsonima* evidenced in this study contributed to differentiate the analyzed species. Future studies sampling a larger dataset in the genus may evidence its relevance to the taxonomy of *Byrsonima*. Trichome scars were also observed in leaves of *B. gardneriana* by Silva *et al.* (2011), besides the presence of Y-T-shaped trichome in *B. intermedia* and T-shaped trichome in *B. orbignyana*. In anatomical and histochemical analysis made by Higuchi (2007) in leaves of *Byrsonima* species, the presence of long Y-shaped tector trichomes in *B. basiloba* and T-shaped in *B. crassifolia* was also reported. Additionally, the author did not observe tector trichomes in *B. fagifolia* and *B. intermedia*. However, young leaves of *Byrsonima* species analyzed by Attala (2004) showed trichomes in the adaxial surface, which probably provides protection during the process of leaf extension. Also, these same trichomes are later deciduous, leaving scars on mature leaves. For this reason, the lack of trichomes in *B. ligustrifolia* is justifiable, since trichome scars were only recorded on the leaf blades of expanded leaves.

Bistratified non-continuous epidermis on the adaxial surface was also observed by Araújo *et al.* (2010) in three from five analyzed species of *Byrsonima*, and by Silva *et al.* (2011) in two of three analyzed species of *Byrsonima*. On the other hand, all analyzed species by Higuchi (2007), Vasconcelos-Filho (2008) and Silva (2014) showed unistratified epidermis on both surfaces of the leaf blade. Bistratified epidermis was also found in *Banisteriopsis* and *Heteropterys* (Araújo *et al.* 2010), showing that this feature probably evolved multiple times independently in the family and it is an important character to differentiate species within genera in Malpighiaceae (Metcalfe & Chalk 1979). The type of mesophyll is widely used in identification keys elaborated from anatomical characters (Araújo *et al.* 2010; Silva 2014; Ferreira *et al.* 2015), and it has been an important aid to the taxonomy of Malpighiaceae. In relation to the bundles immersed in the mesophyll, the collateral type is characteristic of Malpighiaceae as described in several genera (Araújo *et al.* 2010; Silva 2014; Guimarães *et al.* 2016). The extension of the bundle sheath being sclerified or not has helped to distinguish species of *Byrsonima*, even though it has not been commonly described in anatomical studies of Malpighiaceae (Araújo *et al.* 2010).

Our findings are congruent with a recent anatomical study in *Banisteriopsis*, whose authors showed the taxonomic relevance of anatomical characters to support the monophyletism of informal groups, being promising in a new infrageneric classification (Araújo *et al.* 2020). In addition, characters such as biconvex contour of petioles with two protuberances, bistratified non-continuous epidermis on both surfaces of the leaf blade, paracytic laterocytic stomata, and medullary phloem in the petioles were reported here for the first time in *Byrsonima* and should be highlighted in future anatomical studies in Malpighiaceae.

The similarity analysis clearly distinguishes the *Byrsonima* species from its outgroup, and the anatomical data related to the vascular system justifies this distinction between the genera. Davis & Anderson (2010) recovered *Byrsonima* as a monophyletic group and the vascular system anatomy may contain potential synapomorphies in future studies based on molecular phylogeny. The most similar groups presented here include species placed in different infrageneric categories. These findings are consistent when the subgenera and their respective sections represent polyphyletic groups in *Byrsonima*, according to the molecular phylogeny by Davis & Anderson (2010). For instance, the species *B. laxiflora*, *B. sericea* (sect. *Sericolepsis*, subg. Byrsonima) and *B. guilleminiana* (sect. *Eriolepsis*, subg. *Byrsonima*) show great anatomical similarities and can be considered closely related in a future molecular analysis. Moreover, the analysis points out that *Byrsonima orbignyana* is distant from its synonym, *B. cydoniifolia*, due to anatomical differences as epidermis stratification, sheath extension of the mesophyll bundles and conformation of the midrib vascular system. Nonetheless, molecular studies using a greater sampling within the genus are needed in order to propose a new infragenetic classification and the anatomical characters described in this study may be a key element in the process.

Conclusions

In this study, we present the relevance of anatomical characters to *Byrsonima* taxonomy, especially in order to differentiate species and punctuate promising characters for a future infrageneric classification. Therefore, the following characters stand out taxonomically: stomatal crest, types of trichomes and epidermis, contour and conformation of the vascular system in the midrib and petiole, and number of accessory bundles. The petiole anatomy seems to be conserved in *Byrsonima* and midrib anatomy seems to be quite informative at species level. Several characters were first described in *Byrsonima*, such as bistratified epidermis on the adaxial surface of the leaves, laterocytic paracytic stomata and medullary phloem in the petioles. Furthermore, anatomical leaf studies are needed in order to establish a taxonomic circumscription of *Byrsonima* and closely related genera, as several differences have been recorded between *Byrsonima* and the outgroup (*Diacidia aracaensis*), and in the leaf anatomy of two species currently recognized as synonyms*, B. cydoniifolia* and *B. orbignyana*.

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