



Original Article

Leaf morphology and venation of *Psidium* species from the Brazilian Savanna



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ABSTRACT

The Brazilian Savanna biome has the largest plant diversity among savannas worldwide and is the predominant biome in Goiás state, Brazil. Among plant species previously catalogued in the Brazilian Savanna, the Myrtaceae family has received attention, as these plants show great economic potential for its medicinal properties, food and ecological importance, highlighting in this context the *Psidium* genus. In order to contribute to the differentiation of problematic taxonomic groups, such as Myrtaceae, and to quality control of its plant material, morphological and venation leaf studies of four species of *Psidium* were performed. For this purpose, leaf samples of *Psidium firmum* O.Berg., *P. myrsinites* DC., *P. laruotteanum* Cambes., and *P. guineense* Sw. were collected from Goiás State University, Anápolis Air Base, and Serra de Caldas Novas State Park and submitted to classical techniques for morphological and leaf venation characterizations. The results showed that *P. firmum* presents brochidodromous secondary veins, marginal last venation of the fimbrial type, an abaxial surface with a grooved midrib, flat secondary veins on both sides, an apex obtuse to mucronate, and a rounded base. *P. guineense* presents trichomes on both surfaces, a grooved midrib on the adaxial surface and a prominent midrib on the abaxial surface, which distinguishes this species from all other Myrtaceae species examined in the present study. The species *P. guineense* and *P. firmum* presented a set of differential leaf characteristics among the others taxa of the genus, clearly separating these plants in the morphological identification key.

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Introduction

The Brazilian Savanna is considered a hotspot (priority area) for global biodiversity conservation (Myers et al., 2000); for hosting an estimated number of 837 bird species, 161 mammal species, 150 amphibian species, and 120 reptile species (Silva and Bates, 2002); and for high concentrations of endemic plants (Mittermeier et al., 2005).

Among the great diversity of the Brazilian Savanna endemic fruits, we highlight those belonging to the Myrtaceae family. The ecological importance of this family in different biomes of Brazil has been confirmed in several floristic, phylogenetic, and phytosociological studies. According to Oliveira-Filho and Fontes (2000), in both the Brazilian Savanna and Atlantic Forest, the Myrtaceae family has a great diversity of species, representing 10–15% of the vegetal cover in these biomes.

One of most comprehensive surveys of the Brazilian Myrtaceae listed 1726 species (Berg, 1859). According to Landrum and Kawasaki (1997), this family is represented by 23 genera and 1000 species. Among these plants, 696 species are exclusive to Brazil (Arantes, 1997). In a more recent study in Brazil, Sobral et al. (2013) recorded 23 genera and approximately 997 species. In the Brazilian Savanna, Mendonça et al. (2007) listed 14 genera and 211 species.

Among the plant species of the Myrtaceae family previously catalogued in open areas of the Brazilian Savanna, the *Psidium* genus is distinguished by its great economic potential, reflecting its food and pharmacological uses (Souza and Lorenzi, 2008). In Brazil *Psidium guajava* L. stands out as the species of greatest economic interest of the *Psidium* genus. However, there has been growing interest in the “araçazeiros” (Medina et al., 2011), which encompass several species of *Psidium*. In the Central West region of Brazil, in addition to *Psidium guineense*, the following “araçás” species have also been observed: *Psidium laruotteanum* Cambes., *Psidium firmum* O.Berg., *Psidium myrsinites* DC., *Psidium sartorianum*, and *Psidium salutare* (Frazon et al., 2009).

With regard to the medicinal potential of Savanna “araçás”, a recent ethnobotanical survey has indicated the use of leaves and

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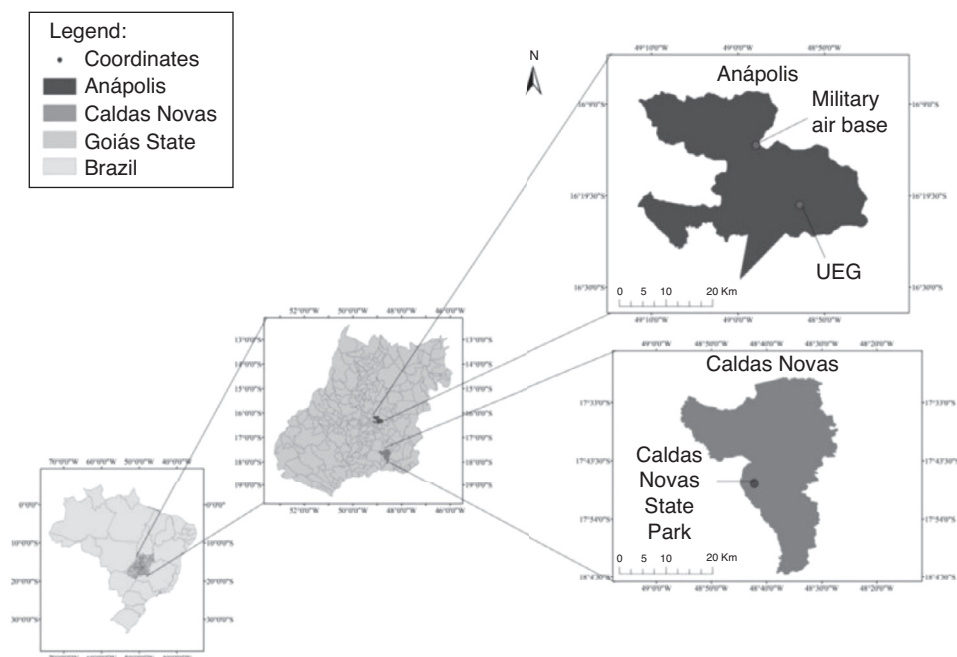


Fig. 1. Map of Brazil showing the locations of Goiás State, Anápolis and Caldas Novas municipalities and *Psidium* spp. collection sites.

Table 1

Geographic location, date of collection, geographic coordinates, and voucher number of *Psidium* species collected in the Savanna of the Goiás state.

Species	Local	Collection date	Geographic coordinates Longitude/latitude/altitude (m)	Voucher number
<i>Psidium firmum</i> O.Berg.	GSU	08/2013 02/2014 08/2014	16° 22' 53.977" / 48° 56' 41.779" / 1098	9211 ^a
<i>Psidium myrsinites</i> DC.	GSU	06/2013 02/2014 08/2014	16° 22' 42.058" / 48° 56' 40.488" / 1099	9212 ^a
	AAB	11/2013 06/2014 08/2014	16° 12' 52.991" / 48° 57' 55.868" / 1104	9213 ^a
<i>Psidium laruotteanum</i> Cambess.	GSU	06/2013 02/2014 08/2014	16° 22' 46.885" / 48° 56' 39.774" / 1100	9214 ^a
	AAB	11/2013 06/2014 08/2014	16° 12' 56.088" / 48° 57' 56.486" / 1114	9215 ^a
	SCNSP	08/2013 11/2013 02/2014	17° 28' 12.396" / 48° 24' 3.604" / 982	9218 ^a
<i>Psidium guineense</i> Sw.	AAB	06/2014 11/2013 06/2014 08/2014	16° 12' 45.507" / 48° 57' 23.651" / 1104	9217 ^a

GSU, Campus Anápolis of the Goiás State University; SCNSP, Serra de Caldas Novas State Park; AAB, Anápolis Air Base.

^a Herbarium of the Goiás State University (HGSU).

shoots by residents of urban areas, settlements and “quilombola” communities of the Goiás Savanna for the treatment of dysenteries (Campos, 2010). Data from the scientific literature reinforce the pharmacological potential of “araçás”, since relevant biological activities have been attributed to the fruits and leaves of these species, such as: antioxidant, antimicrobial, antiproliferative on human tumor cells and increased sleep induced by ketamine in mice (Fauth et al., 2002; Corrêa et al., 2011; Medina et al., 2011; Voss-Rech et al., 2011; Oliveira et al., 2012; Patel, 2012).

Several researchers, including Mc Vaugh (1968) and Barroso et al. (1991), have described the taxonomic problems involving Myrtaceae, stating that this family is a complex taxonomic group. According to Costa (2004), the difficulty of identifying

Brazilian Myrtaceae can be attributed to the speciation, resulting from hybridization and polyploidy, and the lack of morphological and anatomical studies for a better delimitation of the taxa. The *Psidium* species, in particular, have a great phenotypic plasticity due to the different environmental pressures to which they are subject, causing difficulties in the identification and delimitation of the species (Costa, 2009). They are found in diverse environments such as, semi-desert and restingas, with calcareous and sandy soils, among others (Brandão et al., 2002).

According to Frazon et al. (2009) in the Brazilian Savanna there are approximately thirteen native or introduced *Psidium* species, known as “araçazeiros”. In relation for habit, these species are characterized as sub-shrub or tree, reaching up to five meters in height.

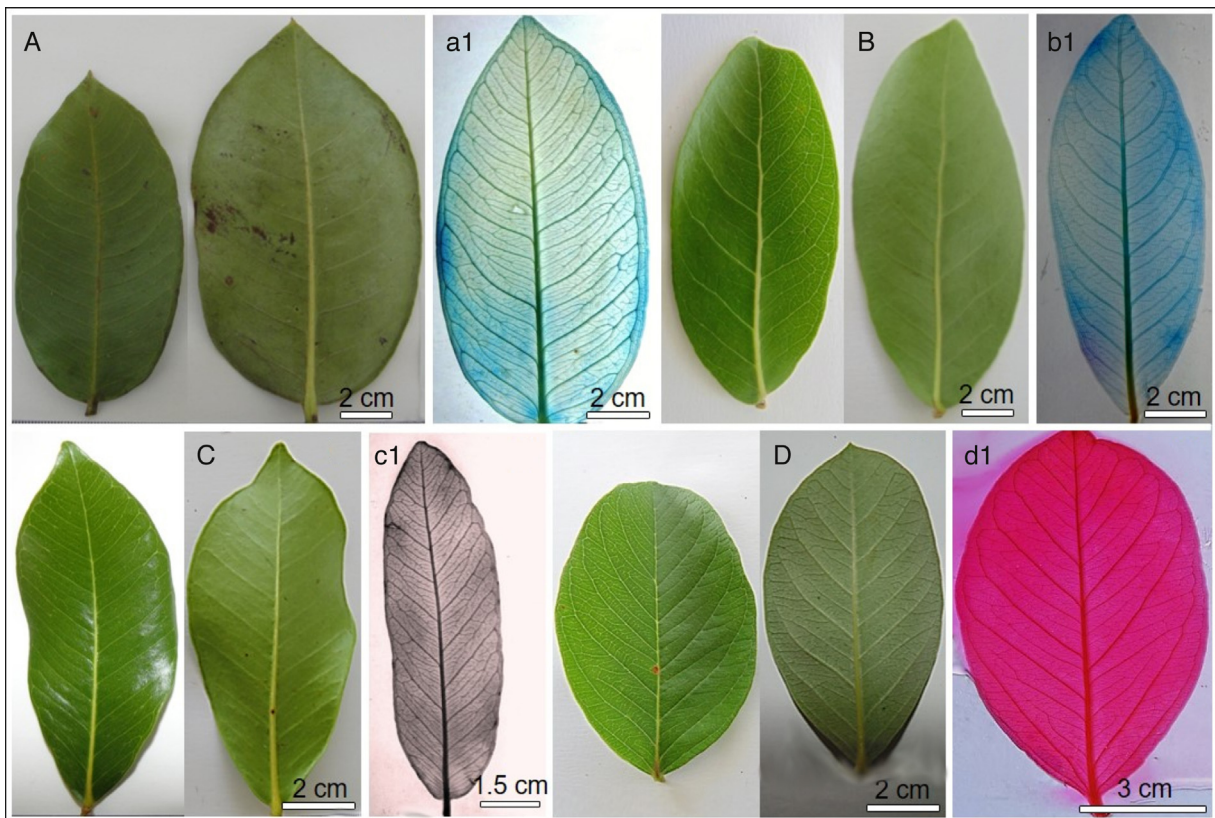


Fig. 2. *Psidium* spp. leaves: (A) *P. firmum*, adaxial and abaxial surfaces; (a1) *P. firmum* diaphanized leaf – brochidodromous secondary venation pattern; (B) *P. myrsinites*, adaxial and abaxial surfaces; (b1) *P. myrsinites* diaphanized leaf – camptodromous–brochidodromous secondary venation pattern; (C) *P. laruotteanum*, adaxial and abaxial surfaces; (c1) *P. laruotteanum* diaphanized leaf – camptodromous–brochidodromous secondary venation pattern; (D) *P. guineense*, adaxial and abaxial surfaces; (d1) *P. guineense* diaphanized leaf – camptodromous–brochidodromous secondary venation pattern.

Among the foliar morphological characteristics common to these species, we can highlight the simple, opposite, usually crossed, amphi or hypostomatic leaves, with abundant tector trichomes on the abaxial surface and rare in the adaxial surface, and camptodromous or brochidodromous secondary vein framework (Soares-Silva and Proença, 2008; Gomes et al., 2009; Campos, 2010).

Research concerning the aspects of leaf architecture can be as fundamental as the morphology of the reproductive organs used in systematic studies (Hickey and Taylor, 1991). According to Ellis et al. (2009), the study of leaf architecture is a relatively new approach that can provide important information on taxonomically complex families, such as Myrtaceae.

According to Arantes and Monteiro (2002), despite the high representation of Myrtaceae in different biomes, few taxonomic studies have been conducted, reflecting the diversity of species and taxonomic complexity of these plants. The morphological studies of this family, and especially of the *Psidium* genus, can contribute to ecological and phytosociological studies, providing subsidies to validate the taxonomic classification and recognition of new species (De-Carvalho, 2008).

Morphological references are extremely important, particularly for medicinal plants, so that the samples can be confirmed and to enable tests of authenticity. These tests are needed, because confusion between morphologically similar species is very common and can lead to the improper use of a particular species. Thus, the leaf architecture adds to the set of information that allows the accurate identification of plant species.

The aim of the present study was to describe the morphology and foliar venation of four species of *Psidium* collected from a Savanna biome in Goiás State, Brazil, to contribute to the identification and delineation

of these taxa, and to the quality control of the plant material.

Material and methods

Leaves of *Psidium* spp. were collected from the Brazilian Savanna areas in Goiás State, in 2013 and 2014, in the cities of Anápolis and Caldas Novas (Fig. 1; Table 1).

The botanical material comprised fully expanded adult leaves collected below the third node. For each individual, a voucher specimen was deposited in the Herbarium of Goiás State University – HGSU (Table 1), and the next characteristics were recorded for each voucher specimen: *P. firmum* O.Berg.: shrub, about 90 cm high; stem smooth, cylindrical, glabrous, and brown; *P. myrsinites* DC.: tree, about 3 m high; stem smooth cylindrical, glabrous, with irregular plate depressions that are detached from the trunk, slightly pink brown coloration; *P. laruotteanum* Cambess.: tree, about 3 m high; stem with cracked rhytidoma and sinuous and discontinuous crests, bark with dark grayish coloration; *P. guineense* Sw.: tree, about 5 m high; smooth cylindrical stem, yellowish gray coloration.

Leaf morphological characterization was performed by observations with the naked eye using a stereoscopic microscope, when necessary, according to Oliveira and Akisue (2003) and Ellis et al. (2009).

For venation pattern analyses, fresh leaves were collected and diaphanized according to Kraus and Arduin (1997). At least five whole leaves from each specimen were placed in a container with 20% sodium hydroxide for 24 h to remove chlorophyll. Subsequently, several washes with water and distilled water were performed, and the botanical materials were clarified using sodium

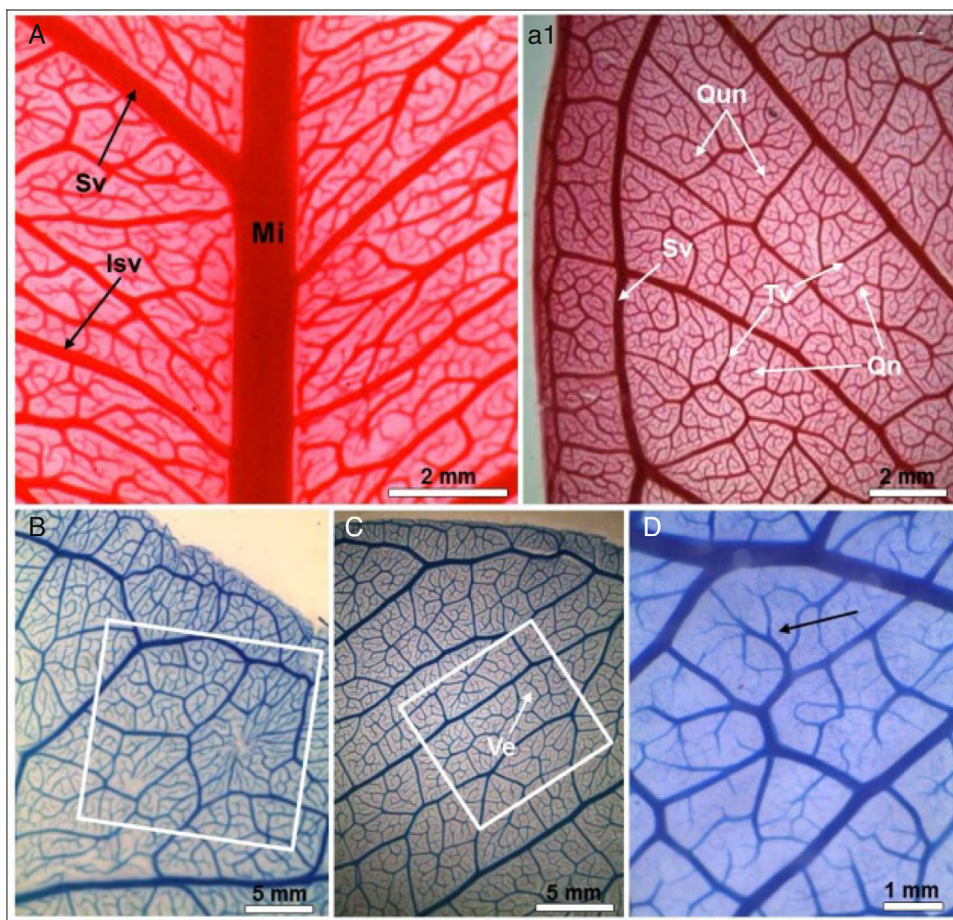


Fig. 3. *Psidium* spp. leaf vein characters. (A) *P. firmum*, intersecondary vein (arrow); (a1) Vein orders (until the 5th order) (arrows); (B) *P. myrsinites*, irregular areoles (square); (C) *P. laruotteanum*, freely ending veinlets (FEV) (arrow); (D) *Psidium guineense*, irregular areoles and freely ending veinlets (FEV) (arrow). Sv, secondary vein; Tv, tertiary vein; Qn, quaternary vein; Qun, quinternary vein; Ve, venule; Mi, midrib; lsv, intersecondary vein.

hypochlorite. Subsequently, dehydration was performed in a series of 70% (w/w), 90% (w/w) and absolute ethanol, incubating the plant material for 1 h in each solution. The leaves were stained with methylene blue/xylol (1:1) or safranin/xylol (1:1). Each leaf was immersed in xylol and immediately transferred to a glass plate containing resin, mounted on two plates and dried. Analyses were performed using a LEICA, EZ4D model stereoscopic microscope.

The pattern venation registration was generated from expansions of diaphanized leaves (using a photographic amplifier Durst M601), and smaller networks of the veins were recorded using a Olympus BX40 photomicroscope. Macro and microscopic characterizations of the leaf venation patterns were performed according to Cardoso and Sajo (2004, 2006) and Ellis et al. (2009).

Results and discussion

The leaves of the *Psidium* species analyzed in the present study were typically coriaceous, with short petioles and a primary pinnate type venation pattern, and characterized by only one midrib of a higher gauge.

The leaf laminates have several shapes, and elliptic or obovate shapes were primarily observed. The apex shapes were acute to acuminate in *P. firmum*, *P. laruotteanum*, and *P. myrsinites*. *P. guineense* displayed apex obtuse to mucronate shapes. The base shapes were acute to cuneate in *P. myrsinites* and *P. laruotteanum* and rounded in *P. firmum* and *P. guineense* (Box 1; Fig. 2).

Trichomes were present on the abaxial surface of the leaf lamina of all species. *P. guineense* displayed trichomes on both surfaces (Box 1).

The leaf midrib was more prominent on the abaxial surface in the four species. *P. firmum* (Fig. 2A) and *P. guineense* (Fig. 2D) presented grooved midribs on adaxial surface (Box 1).

The major secondary vein framework was the camptodromous–brochidodromous mixed venation pattern, followed by the brochidodromous pattern (Box 1; Fig. 2).

The brochidodromous venation pattern was observed in *P. firmum* (Fig. 2a1), consistent with the observation of Campos (2010) for this species. This venation pattern is formed when secondaries join in a series of prominent arches and loops of secondary gauge (Ellis et al., 2009). According to Soares-Silva and Proença (2008), brochidodromous is the typical venation pattern for *Psidium*. Cardoso and Sajo (2006) reported that the brochidodromous arches and loops might be more or less prominent depending on the closing angle of the secondary veins.

P. myrsinites, *P. laruotteanum*, and *P. guineense* (Fig. 2b1, c1, d1) present camptodromous–brochidodromous secondary venation patterns. This mixed venation pattern is characterized by secondary veins that anastomose to each other from the leaf base in two ways: the proximal secondaries connect to superjacent major secondaries via tertiaries without forming marked marginal loops of the secondary gauge; in the medium third of the leaf, the secondaries join in a series of prominent arches and loops, forming several closing angles, and in the apex, the arches are concaved and uniform (Ellis et al., 2009).

Box 1: Leaf morphological characters and venation of four species of *Psidium* spp.

Characters/species		<i>P. firmum</i>	<i>P. myrsinites</i>	<i>P. laruotteanum</i>	<i>P. guineense</i>
Leaf	Shape	Elliptic to oblong	Elliptic to obovate	Elliptic to obovate	Elliptic to obovate
	Apex	Acute to acuminate	Acute to acuminate	Acute to acuminate	Acute to mucronate
	Base	Rounded	Acute to cuneate	Acute to cuneate	Rounded
Petiole		Short-petiolate	Short-petiolate	Short-petiolate	Short-petiolate
Indument	Adaxial surface	Glabrous	Glabrous	Glabrous	Pilose
	Abaxial surface	Pilose	Pilose	Pilose	Pilose
Main vein salience	Adaxial surface	Grooved	Prominent	Prominent	Grooved
	Abaxial surface	Prominent	Prominent	Prominent	Prominent
Primary venation pattern		Pinnate	Pinnate	Pinnate	Pinnate
Secondary venation	Venation pattern	Brochidodromous	Camptodromous–brochidodromous	Camptodromous–brochidodromous	Camptodromous–brochidodromous
	Number of pairs	<13	<13	<13	<10
Vein (orders)	Salience adaxial surface	Flat	Prominent	Flat	Grooved
	Salience abaxial surface	Flat	Flat	Flat	Prominent
		Until the 5th order	Until the 5th order	Until the 5th order	Until the 5th order
Intersecondary veins		Present	Present	Present	Present
Intramarginal veins		Absent	Absent	Absent	Absent
Marginal ultimate venation		Fimbrial	Incomplete loops	Looped	Looped
Collector vein	Distance from the edge (mm)	<1	1–3	1–3	1–3
Venules	Simple/branching	Branching	Branching	Branching	Branching
Areoles		Present	Present	Present	Present

The camptodromous–brochidodromous secondary venation pattern is common for representatives of Myrtinae, Myrceinae and Eugeniinae (Costa et al., 1995; Farmacopeia Brasileira, 2002; Cardoso and Sajo, 2004, 2006). Costa et al. (1995) described the camptodromous–brochidodromous standard for six of the eleven *Eugenia* species occurring in the sandbank of Marica, Rio de Janeiro State in Brazil. In the Brazilian Pharmacopeia IV, this secondary venation pattern is registered to *P. guajava* L. (Farmacopeia Brasileira, 2002). Cardoso and Sajo (2004) identified the camptodromous–brochidodromous secondary venation standard in nine (9) *Eugenia* species from the hydrographic basin of Tibagi River, Paraná State, Brazil: *Eugenia arenosa* Mattos, *E. hamiltonii* (Mattos) Mattos, *E. handroana* D. Legrand, *E. hiemalis* Cambess., *E. neoverrucosa* Sobral, *E. pyriformis* Cambess., *E. ramboi* D. Legrand, *E. speciosa* Cambess., and *E. uniflora* L.

The arrangement of secondary veins relative to the midrib in the leaves of the studied species produces angles with divergences ranging from 45° to 75°. According to Cardoso and Sajo (2004), the divergence angles ranging from 45° to 60° are most striking in species with mixed venation patterns (camptodromous–brochidodromous), as recorded in the present study for *P. laruotteanum*, *P. myrsinites*, and *P. guineense*. The divergence angles cannot be considered to be taxonomic characters for distinguishing species, as in the present study, all species showed wide variations among the angles in leaves from the same individual.

The amount of secondary vein pairs for each species is shown in Box 1. *P. guineense* (Fig. 2d1) presents fewer secondary vein pairs than other species (8–10 pairs), thereby distinguishing this species. All species have veins until the 5th order (Fig. 3a1).

Note the presence of intersecondary veins in all species (Box 1; Fig. 3A). According to Ellis et al. (2009), the intersecondary veins originate from the midrib, running parallel to the major secondary veins, as characterized by an intermediate gauge between the secondary and tertiary veins. In the present study, no species showed intramarginal veins, which according to Cardoso and Sajo (2004), are the veins formed between brochidodromous arches and the leaf margin.

Spacing among the secondary veins occurred in decreasing order toward the apex. Irregular spacing was observed in *P. firmum*, *P. myrsinites*, and *P. guineense* (Fig. 2a1, b1, d1), and regular spacing was observed in *P. laruotteanum* (Fig. 2c1).

The configuration of the highest-order leaf veins in the margin or marginal ultimate venation was absent, showing incomplete loop, looped, and fimbrial types. In the incomplete loop type, the marginal ultimate vein recurves to form incomplete loops near the leaf margin; in the looped type, the tertiary veins anastomose, forming a series of arches near the edge; the fimbrial vein type occurs when close veins join at the edge and produce a continuous vein running along the margin (Cardoso and Sajo, 2006; Ellis et al., 2009).

In the present study, the marginal ultimate venation of the fimbrial type was observed only in *P. firmum* (Box 1; Fig. 4A). In *P. myrsinites* (Box 1; Fig. 4B), the incomplete loop type was observed, and in *P. laruotteanum* (Fig. 4C) and *P. guineense* (Fig. 4D) the looped types, which have a smaller gauge closer to the margin, were verified. According to Gomes et al. (2009) and Silva et al. (2008), in most surveys of *Psidium*, the marginal ultimate venation pattern is the looped type, formed by complete arches, consistent with the results obtained in the present study.

The marginal ultimate looped type venation pattern is common in most species of the Myrtaceae family, followed by fimbrial and incomplete loop types (Cardoso and Sajo, 2006; Oliveira et al., 2011). De-Carvalho (2008) registered the fimbrial and looped types in two and seven species, respectively, of the nine species of *Myrcia* DC. (Myrtaceae) studied in Federal District, Brazil.

Areoles are the smallest areas of the leaf tissue completely surrounded by veins of the fourth and fifth orders. These structures can have various shapes and arrangements, with or without venules (simple or ramified). The areoles are considered to be regular (perfect) when they have the same shape and size; irregular (imperfect) areoles vary in size and shape; and incomplete areoles have no limitations on the sides (Alvarez et al., 2006; Ellis et al., 2009).

The results of the present study revealed irregular areoles with freely ending veinlets (FEVs) of the dendritic ramified type for all *Psidium* species (Box 1; Fig. 3B–D). Costa et al. (1995) and Klucking (1988) highlighted that the formation of incomplete and irregularly shaped randomly distributed areoles is a common characteristic in the Myrtaceae family.

In summary, the leaves of the *Psidium* species analyzed in the present study are coriaceous, short-petiolated structures with a primary pinnate type venation pattern. The secondary venation pattern is brochidodromous, occurring more frequently the camptodromous–brochidodromous mixed pattern. The secondary

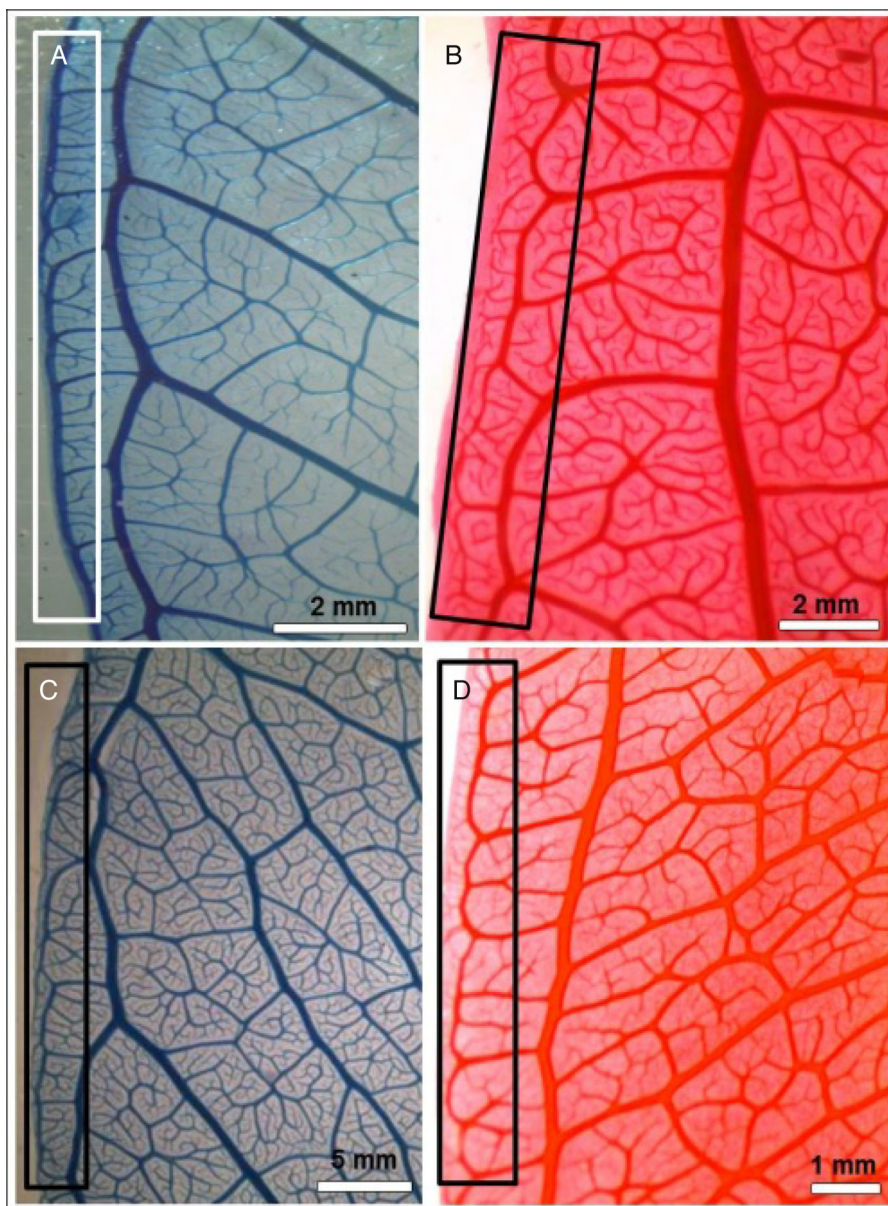


Fig. 4. *Psidium* spp. marginal ultimate venation. (A) *P. firmum*, marginal ultimate venation of fimbrial type (rectangle); (B) *P. myrsinites*, marginal ultimate venation of incomplete loop type (rectangle); (C) *P. laruotteanum*, marginal ultimate venation of looped type (rectangle); (D) *P. guineense*, marginal ultimate venation of looped type (rectangle).

Box 2: Morphological identification key for *Psidium* spp.

1	Shrubby habit; brochidromous secondary venation pattern; marginal ultimate venation fimbrial type	<i>P. firmum</i>
1	Tree habit; camptodromous–brochidromous secondary venation pattern; marginal ultimate venation looped type	2
2	Ridged adaxial surface with prominent midrib on the abaxial surface; pilose on both surfaces; apex obtuse to mucronate; base rounded	<i>P. guineense</i>
2	Adaxial surface with prominent midrib; glabrous adaxial surface and pilose abaxial surface; apex acute to acuminate; base acute to cuneate	3
3	Secondary veins prominent on the adaxial surface and marginal ultimate venation with incomplete loops	<i>P. myrsinites</i>
3	Secondary veins flat on the adaxial surface and marginal ultimate venation looped	<i>P. laruotteanum</i>

veins have different spacing patterns, with acute divergence angles to the midrib, a plane on the adaxial surface and are primarily prominent on the abaxial surface. All plants have intersecondary veins. The marginal ultimate venation of the looped type appears in most species and might appear incomplete and fimbrial. The areolas are well developed veins of the fourth and fifth orders, and have branched FEVs.

The different characters observed generated the following identification key for *Psidium* species (Box 2).

Reflecting the small number of species analyzed in the present study with respect to the biodiversity reported for Brazil, the need for additional comprehensive studies on *Psidium* species collected in other Brazilian biomes is evident. However, the morphological characters and leaf architecture were useful for distinguishing

Psidium species, further confirming that diaphanization is an inexpensive, fast and conclusive technique.

Notably, phenotypic variation reflecting the influence of the environment was not considered in this analysis, as the sites where the species were collected have similar microclimates.

Authors' contributions

EFO (Master student) contributed in collecting plant sample and identification, confection of herbarium, running the laboratory work, analysis of the data and drafted the paper. DGB contributed in collecting plant sample, confection of herbarium, running the laboratory work, analysis of the data and drafted the paper. MLS contributed in collecting plant sample and identification, confection of herbarium, analysis of the data and to critical reading of the manuscript. MHR contributed to critical reading of the manuscript. JAMP designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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