



Original Article

Anatomical and histochemical characterization of *Dipteryx odorata* and *Taralea oppositifolia*, two native Amazonian species



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ABSTRACT

Dipteryx odorata (Aubl.) Willd. and *Taralea oppositifolia* Aubl., Fabaceae: Dipterygeae, are two Amazonian species of great economic and pharmacological potential. The anatomy of these species, however, remains poorly studied. The aim of this work was to inventory leaf anatomical characteristics of *D. odorata* and *T. oppositifolia* and to locate and identify secretory structures and determine the main classes of metabolites they store. Vegetative branches were collected in Parque Ecológico de Gunma, Belém, state of Pará, Brazil. Some of the branches were destined for herborization while the remainder was submitted to standard protocols for anatomical analysis and histochemical tests. Both species were found to possess an unstratified epidermis, with *D. odorata* being amphistomatic and *T. oppositifolia* being hypostomatic, and dorsiventral mesophyll with spongy parenchyma and wide cellular space. The two species were also found to possess idioblasts and secretory cavities that produce a heterogeneous exudate consisting of polysaccharides, lipids, alkaloids and phenolic compounds. The species presented differences in leaf anatomy and chemical composition of the secretory structures, which may be useful in their differentiation.

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Introduction

Dipterygeae (Leguminosae, Papilionoideae) is an exclusively Neotropical clade of approximately 25 species of woody legumes distributed among four genera whose representatives occur in phytogeographical domains of the Amazon: *Monoptyeryx* Spruce ex Benth, *Pterodon* Vogel, *Dipteryx* Schreb, and *Taralea* Aubl. (Cardoso et al., 2013). Among these genera, *Dipteryx* and *Taralea* stand out due to the economic potential of their species. Although these genera share a number of vegetative characters, such as thick leaves, winged rachises, terminal appendices probable arising from the reduction of the terminal leaflet, 4–18 alternate leaflets, leaflet apexes ranging from cuspidate to obtuse and the presence of translucent punctuations, their fruits have distinctive morphological characteristics, including drupe-type fruit with late dehiscence

in *Dipteryx* and legume fruit with elastic dehiscence in *Taralea* (Barroso et al., 1999; Francisco, 2010).

Dipteryx odorata (Aubl.) Willd and *Taralea oppositifolia* Aubl. are popularly known in Brazil as “cumaru” or “cumaru-ferro” (Sousa et al., 2007; Carvalho, 2009) and “cumarurana” or “cumaru-amarelo” (Faria and Lima, 2002; Sousa et al., 2007), respectively. Both species have multiple uses, and have been used in medicine, pharmacology, and the perfume and cosmetics industries (Uchida and Campos, 2000; Bessa et al., 2001; Takemoto et al., 2001; Pesce, 2009; Breitbach et al., 2013), as well as in the timber sector (Sousa et al., 2007; Herrero-Jáuregui et al., 2012; Soriano et al., 2012). Almonds of *Dipteryx odorata* have also been used as a raw material in the production of biodiesel (Ramalingam et al., 2018).

Studies related to the anatomy of vegetative and reproductive organs involving genera of Leguminosae have provided new characters to support both taxonomic (Lackey, 1978; Leelavathi et al., 1980; Coutinho et al., 2016), and phylogenetic investigations such as, for example, morphological data combined with molecular markers for the reconstruction of phylogenies and the indication of possible synapomorphies for genera (Francisco, 2010). In gen-

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eral, anatomical studies and studies of the chemical compounds produced by these species have mainly emphasize wood (Gasson, 1999) and seeds (Bessa et al., 2001; Takemoto et al., 2001; Pesce, 2009), leaving little known about the anatomical and histochemical characterization of the leaves (Palermo et al., 2017; Silva et al., 2018). Nonetheless, anatomical and histochemical characterization is necessary for identifying potentially useful species and for determining sites of accumulation and/or secretion of biologically active products (Thadeo et al., 2009; Palermo et al., 2017), since species of *Dipteryx* and *Taralea* possess considerable pharmacological value.

In view of the above considerations, the aim of this study was to characterize the anatomy and histochemistry of the leaves of *D. odorata* and *T. oppositifolia*, looking for diagnostic characters to delimit the taxa since the occurrence and distribution of secretory spaces varies in the Dipterygeae clade and the species are morphologically similar.

Materials and methods

Study area

Material of the two species was collected in Parque Ecológico de Gunma – PEG ($1^{\circ}13'86''S$; $48^{\circ}17'41.18''W$), located in the municipality of Santa Bárbara, about 48 km from Belém, in the northeastern region of the state of Pará, Brazil. The park possesses approximately 580 ha with vegetation composed of “terra-firme” forest along with environments of igapós and várzeas (Almeida et al., 2009). The climate of the region is Af – tropical humid (Köppen classification), with a mean annual temperature of around $26^{\circ}C$, a minimum of $22^{\circ}C$ and a maximum of $31^{\circ}C$. Annual rainfall varies from 2500 to 3000 mm, with relative air humidity reaching about 85% (Sudam, 1984).

Plant material

Dipteryx odorata (Aubl.) Willd., Fabaceae, leaves composite, alternate, rachis winged and protruding without leaflets in the apical zone; leaflets sub-opposite, 3–4 pairs per pinna, ovate-lanceolate, margin entire, apex acuminate and base round, and translucent punctuations present.

Taralea oppositifolia Aubl., Fabaceae, leaves composite, alternate, opposite, rachis winged, appendix terminal; leaflets alternating, sub-opposed, rarely opposite, 4–8 pairs per pinna, elliptic, lanceolate, ovate to oval-elliptical, margin entire, apex acuminate to cuspidate, and translucent punctuations present.

Flowering branches of *D. odorata* and *T. oppositifolia* were collected in Parque Ecológico do Gunma with vouchers for the two species being deposited in Herbarium MG, with the registration numbers MG 20.0490 and MG 20.1435, respectively.

Structural and histochemical characterization

For anatomical and histochemical characterization, fully expanded leaflets and petiolules were collected from the third to the fourth node of the medial portions of vegetative branches (including the main rib, margin and region between the margin and main rib) and petiolules and fixed in FAA (formaldehyde, glacial acetic acid and 70% ethyl alcohol; 1:1:18 v/v; Johansen, 1940) for 24 h. Portions of these samples (3–4) were fixed in neutral-buffered formalin [NBF; Lillie (1965)] or ferrous sulphate in formalin [FSF; Johansen (1940)] for 48 h for the detection of lipophilic and phenolic substances, respectively. After fixation, the samples were washed in distilled water, dehydrated in a tertiary butyl series and embedded in paraffin (Johansen, 1940). Transverse and longitudinal sections (12–14 μm) were made using a semiautomatic rotary microtome (Leica RM 2245), stained with 1.5% alcoholic

safranin and 1% aqueous astra blue (Bukatsch, 1972), and mounted in Permount® synthetic resin.

The epidermis of leaflets was dissociated using the solution of Franklin (1945), followed by washing in water, staining with astra blue and 1% safranin (Bukatsch, 1972), and mounting in 1:1 v/v water/glycerin temporary medium (Purvis et al., 1964).

Histochemical tests of fresh samples were performed using PAS (Periodic Acid Schiff reagent) for total polysaccharides (McManus, 1946); Ruthenium red for the detection of acidic mucilages (Johansen, 1940); tannic acid/ferric chloride for mucilages (Pizzolato and Lillie, 1973); Lugol for starch (Johansen, 1940); Sudan black B for total lipids (Pearse, 1980); Nile blue for acidic and neutral lipids (Cain, 1947); NADI reagent for essential oils and oil resin (David and Carde, 1964); Dragendorff (Sverdsen and Verpoorte, 1983) and Wagner reagents (Furr and Mahlberg, 1981) for alkaloids, and ferric chloride for total phenolic compounds (Johansen, 1940).

For the control for tests for lipophilic substances, samples were stored in extraction solution (methanol/chloroform/water/HCl; High, 1985) for 48 h, fixed in neutral buffered formalin (NBF) and submitted to the aforementioned reagents and dyes. The control for the tests for hydrophilic substances was performed according to the respective techniques. Samples were also analyzed without any treatment (blank), for visualization of color *in natura*. The tests were applied to sections cut by free hand and then mounted in glycerin jelly (Kaiser, 1880).

Photographic documentation was performed using an AxioLab Zeiss microscope coupled to a Canon Powershot A640 digital camera.

Scanning electron microscopy (SEM)

For SEM analyses, leaflets samples were fixed in FAA, dehydrated in an ethanolic series and critical point dried with CO₂ (Bozzola and Russel, 1991). The leaf fragments were then glued to a metallic support, metalized with gold and examined under a LEO 1450 VP scanning electron microscope.

Results

Anatomical characterization of leaves

In frontal view, the adaxial leaf surface of *D. odorata* and *T. oppositifolia* exhibited epidermal cells with anticlinal (Fig. 1A) and sinuous (Fig. 1B) walls, respectively. The adaxial leaf surface of both species exhibited cells with sinuous anticlinal walls (Fig. 1C and D). The leaves of *D. odorata* and *T. oppositifolia* were found to be amphistomatic and hypostomatic, respectively. Both anomocytic (Fig. 1C) and paracytic stomatal types were present in *D. odorata*, while only paracytic stomata were present in *T. oppositifolia* (Fig. 1D). Stellate non-glandular trichomes were observed in *D. odorata* (Fig. 1G) while simple non-glandular trichomes were observed in *T. oppositifolia* (Fig. 1H). In cross section, both species were found to possess uniseriate epidermis on both faces, along with a thick cuticle (Fig. 1E and F).

Both species exhibited dorsiventral mesophyll with stratified palisade parenchyma and stratified spongy parenchyma with conspicuous intercellular spaces (Fig. 2C–F).

The leaf blade of the two species exhibited small-caliber vascular bundles surrounded by fibers forming an extensive vascular bundle sheath (Figs. 2A, C, and D). The vascular system of the central rib of both species was found to be composed of collateral-type vascular bundles surrounded by fibers forming a closed arch in *D. odorata* and an open arch in *T. oppositifolia*, which possess idioblasts and

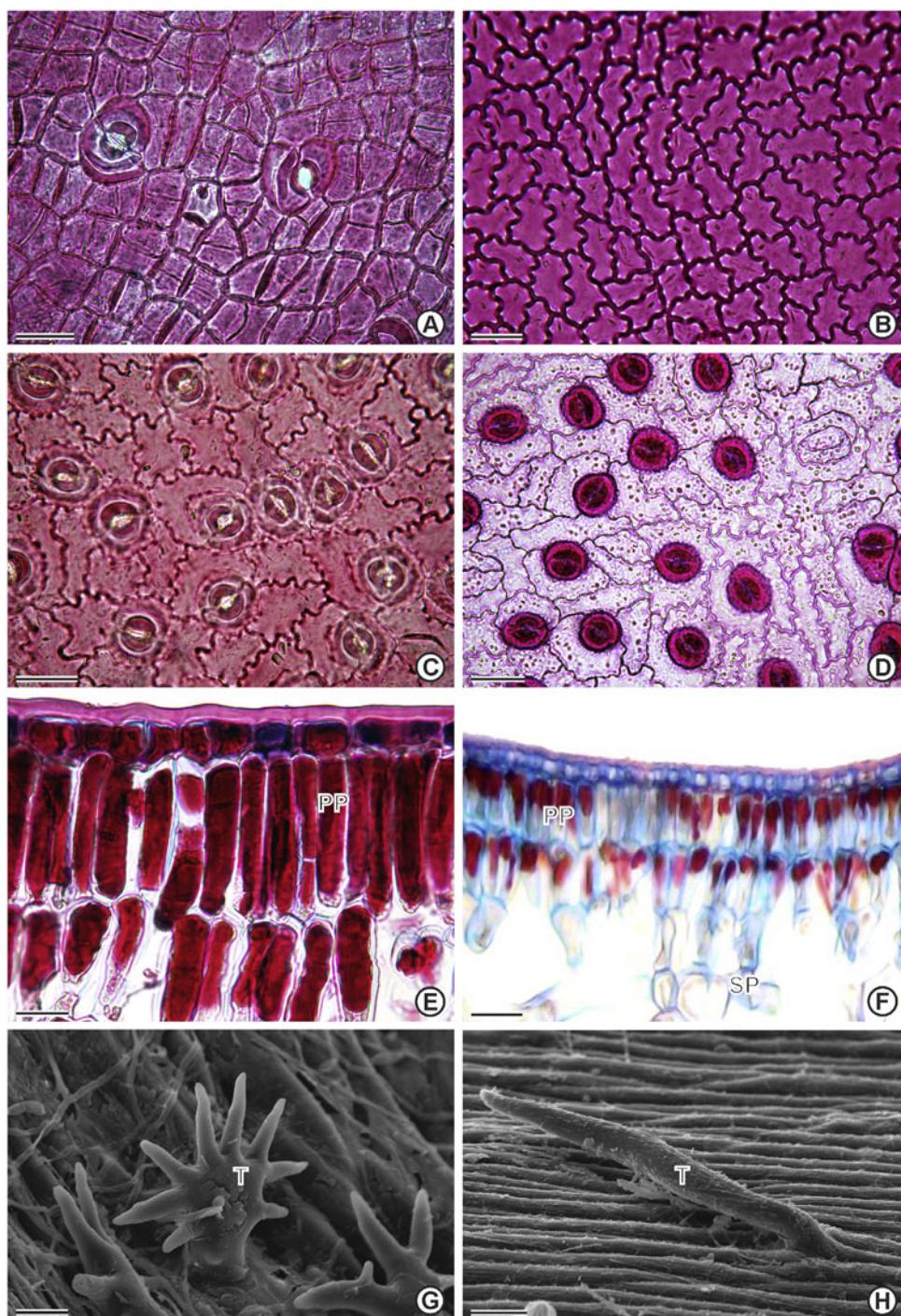


Fig. 1. Leaf anatomy of *Dipteryx odorata* (A, C, E and G) and *Taralea oppositifolia* (B, D, F and H) under light (A–F) and scanning electron microscopy (G and H). A. Adaxial face with straight epidermal cells and stomata; B. adaxial face with sinuous epidermal cells; C. abaxial face with sinuous epidermal cells and stomata; D. abaxial face with sinuous epidermal cells and stomata; E and F. detail of the epidermis and the palisade parenchyma; G. stellate non-glandular trichomes; H. simple non-glandular trichomes. Bars: 50 m (A, and C–F); 150 m (B); 13 m (G); 20 m (H).

secretory cavities in the cortical parenchyma and only idioblasts in the medullar parenchyma (Fig. 2G and H).

Leaf margins were curved in *D. odorata* (Fig. 2A) and straight in *T. oppositifolia* (Fig. 2B). Leaf margins of the latter species were observed to be completely sclerenchymatic and possessing secretory cavities.

The petiole of both species revealed a circular outline with fundamental tissue composed of several layers of parenchyma containing idioblasts and secretory cavities (Fig. 3A and B). All of these anatomical characteristics are summarized in Table 1.

Histochemistry of leaflet secretory structures

The secretory structures of the leaves of both species were found to comprise idioblasts and cavities, which are located in the cortical parenchyma of the central rib and the petiole (Figs. 2G, H and 3A, B). The mesophyll of *T. oppositifolia* also exhibited secretory cavities in the adaxial face (Fig. 2D). Idioblasts were revealed in red when stained by safranin (Figs. 2G and 3B).

Spherical epithelial cells were observed to delimit an isodiametric lumen in cavities (Fig. 3C–F). Histochemical tests of

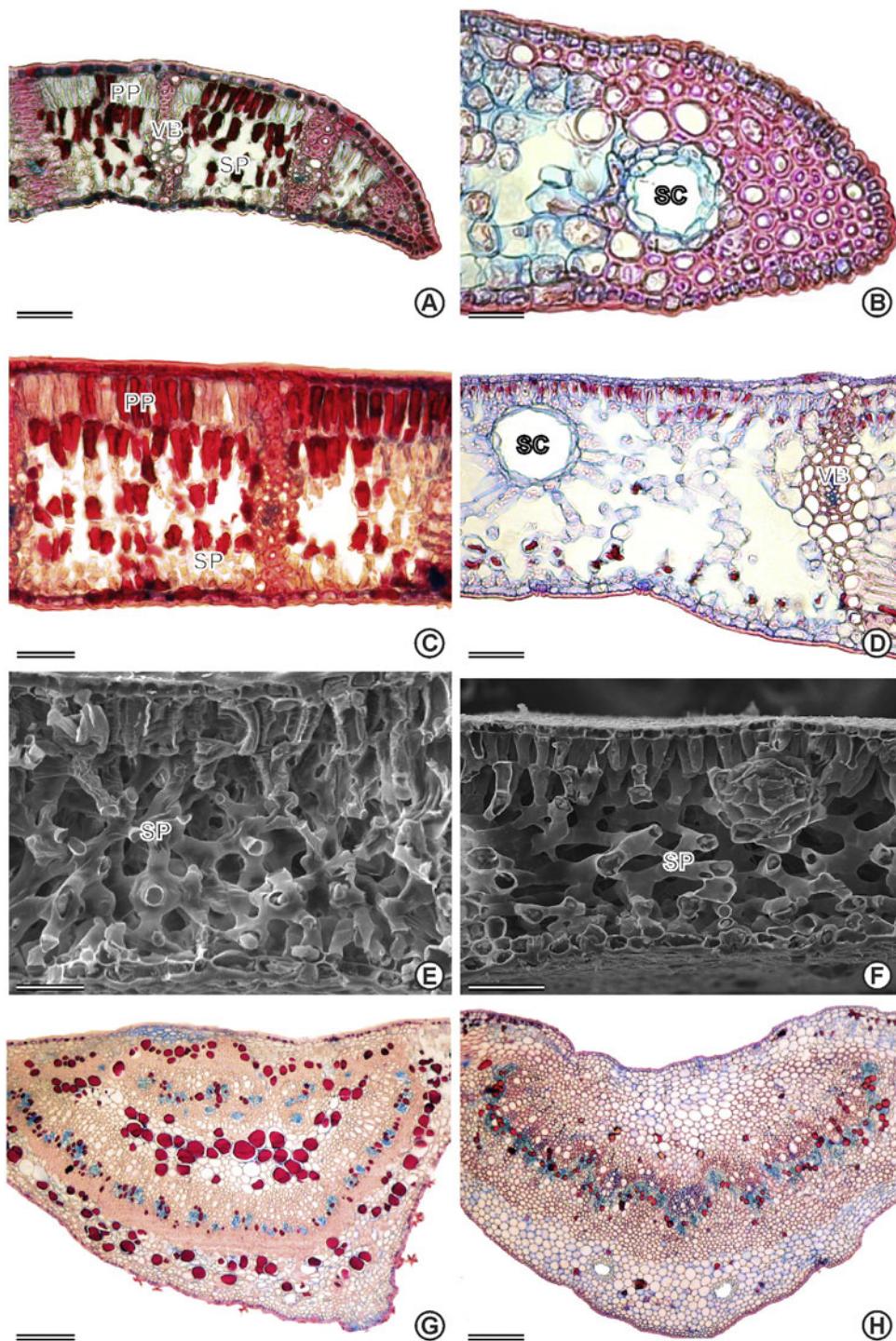


Fig. 2. Leaf anatomy of *Dipteryx odorata* (A, C, E and G) and *Taralea oppositifolia* (B, D, F and H) under light (A–D, G and H) and scanning electron (E and F) microscopy. A. Curved downward leaf margin; B. straight leaf margin with secretory cavity (SC); C. general view of mesophyll; D. general view of mesophyll with secretory cavity; E and F. detail of spongy parenchyma (SP); G and H. general view of central rib. Legend: (PP) palisade parenchyma; (VB) vascular bundle. Bars: 150 m (A, C and D); 50 µm (B); 60 m (E and F); 300 m (G and H).

idioblasts and secretory cavities revealed that they contain a series of chemical compounds, namely: total lipids (acidic and neutral), polysaccharides, alkaloids and phenolic compounds (Table 2). Secretions were seen preserved in idioblasts and cavities, with the latter being present both in epithelial cells and inside the lumen (Figs. 4A–F and 5A–F). The exudates present in the idioblasts possessed a dark brown coloration in both species, evidencing the presence of phenolic compounds (Fig. 5F).

Discussion

Among taxa of the Dipterygeae clade, the outline of the epidermal cells of both leaf surfaces varies from rectilinear to sinuous (Silva et al., 2018), as observed in the present study. Cells with straight and sinuous walls have been widely reported among others species of Fabaceae, such as *Aldina heterophylla* Spruce ex Benth. (Araújo and Mendonça, 1998), *Bauhinia microstachya*

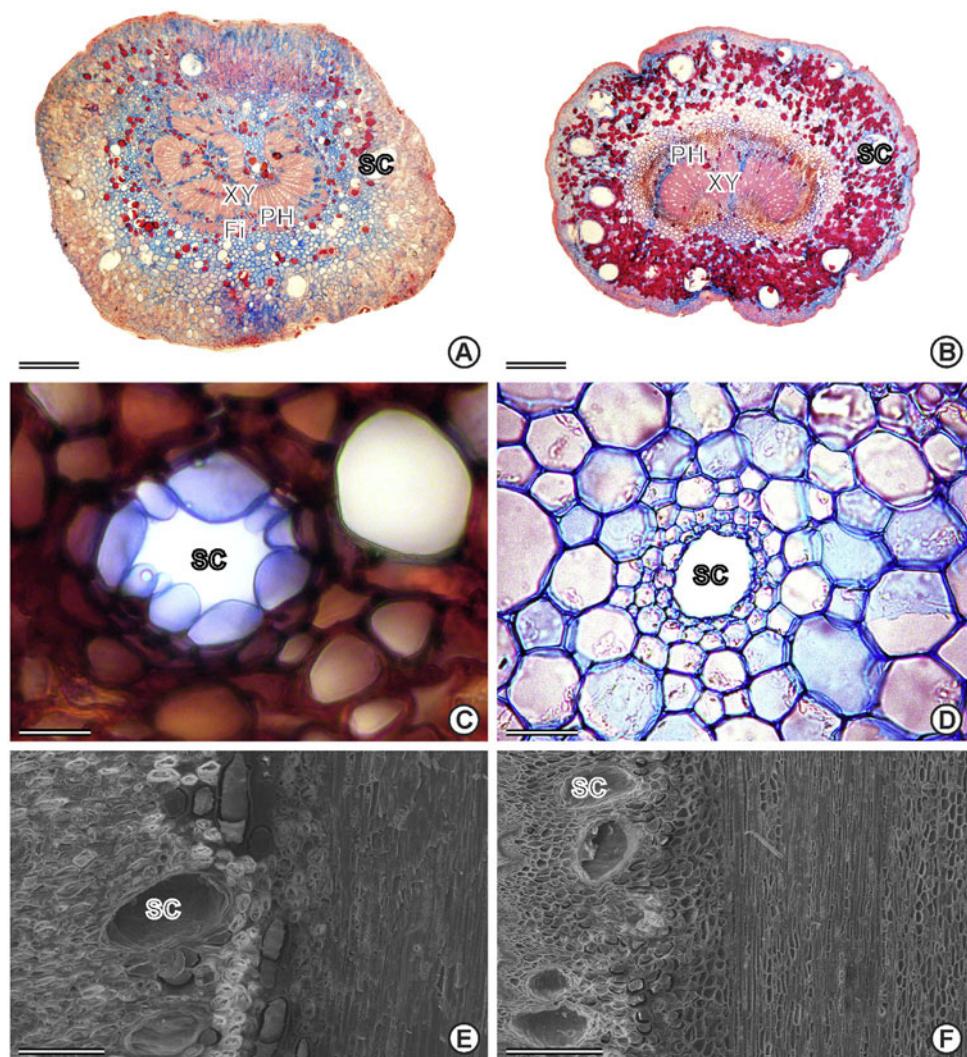


Fig. 3. Petiole anatomy of *Dipteryx odorata* (A, C and E) and *Taralea oppositifolia* (B, D and F) under light (A–D) and scanning electron (E and F) microscopy. A and B. overall view of petiole; C and D. detail of secretory cavity (SC); E and F. longitudinal section of secretory cavities. Legend: (Xy) xylem; (Ph) phloem; (Fi) fibers. Bars: 300 m (A and B); 50 m (C and D); 130 m (E); 230 m (F).

Table 2
Histochemistry of the idioblasts and secretory cavities of leaves of *Dipteryx odorata* and *Taralea oppositifolia*.

Group of compounds	Test	Color	<i>Dipteryx odorata</i>			<i>Taralea oppositifolia</i>		
			Idioblasts	Secretory cavities		Idioblasts	Secretory cavities	
				Epithelium	Lumen		Epithelium	Lumen
Polysaccharides	Periodic acid/Schiff reagent (PAS)	Pink	+	–	–	+	–	–
	Ruthenium red	Pink	+	–	–	+	–	–
	Tannic acid/ferric chloride 3%	Black	–	–	–	–	–	–
	Lugol Reagent	Purple or brown	+	–	–	–	–	–
Lipids	Sudan black B	Black	+	+	+	+	+	+
	Nile blue sulphate	Pink or blue	–	+	+	+	–	–
Terpenoids	NADI Reagent	Red	–	–	–	–	–	–
Alkaloids	Dragendorff's Reagent	Reddish-brown	+	–	–	+	–	–
	Wagner's Reagent	Reddish-brown	+	–	–	+	–	–
Phenolic compounds	Ferric chloride	Black	+	–	–	+	–	–
	Fixative (SFF)	Black	+	–	–	+	–	–

(Raddi) J.F. Macbr. (Duarte and Debur, 2003), *Hymenaea stilbo-carpa* Hayne (Moreira-Coneglian and Oliveira, 2006), *Glycine max* (L.) Merr. (Leal-Costa et al., 2008), and *Senna alata* (L.) Roxb. (Rodrigues et al., 2009), as well as for various species of *Chamaecrista* (L.) Moench (Coutinho et al., 2016). Sinuosity of cell walls,

however, may be related to the environment where a plant occurs (Esau, 1997), as observed by Araújo and Mendonça (1998) for the walls of the epidermal cells of *Aldina heterophylla*, which can be strait in the majority of sun leaves and sinuous in shade leaves.

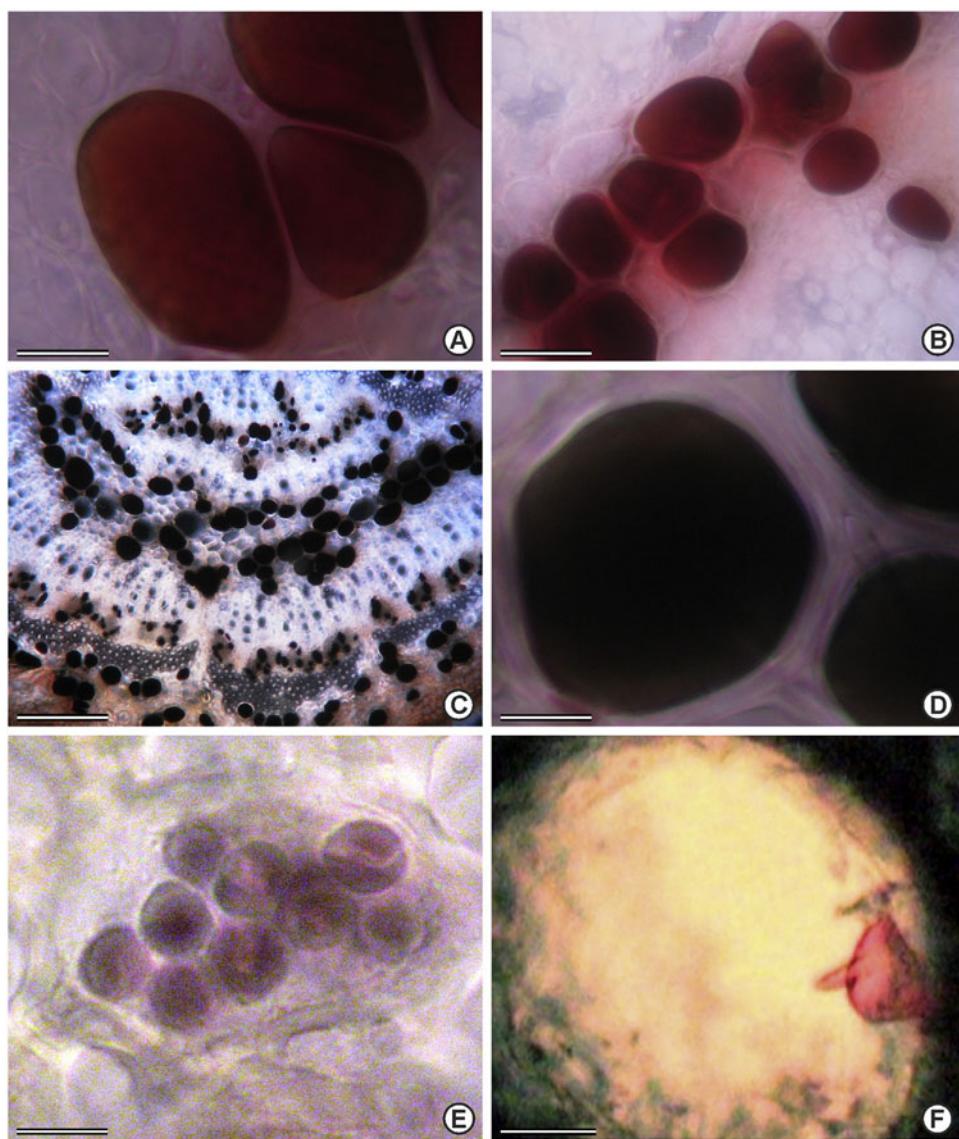


Fig. 4. Leaf histochemistry of *Dipteryx odorata* (A–F). A–E, idioblasts; F, cavities; A and B, Wagner and Dragendorff reaction for “alkaloids”; C and D, ferric chloride reaction for “tannins”; E, Lugol reaction for “starch”; F, Nile blue sulphate reaction for “acidic and neutral lipids”. Bars: 50 m (A–F).

Table 1
Leaf anatomy of *Dipteryx odorata* and *Taralea oppositifolia*.

Characteristics	<i>Dipteryx odorata</i>	<i>Taralea oppositifolia</i>
Amphistomatic leaf	x	–
Hypoestomate leaf	–	x
Anomocytic stomata	x	–
Paracytic stomata	x	x
Simple non-glandular trichomes	–	x
Stellate non-glandular trichomes	x	–
Sinuous epidermis on both faces	–	x
Leaf with collateral vascular bundle	x	x
Presence of secretory cavity	x	x
Presence of secretory idioblast	x	x
Curved downward leaf margin	x	–
Fibrous leaf margin	–	x
Petiole with an “open arch”- shaped vascular bundle	–	x

The amphistomatic pattern with paracytic stomata observed here for leaves of *D. odorata*, as was also observed by Silva et al. (2018), in *Senna alata* (L.) Roxb. by Rodrigues et al. (2009), and in species of *Bauhinia* L. by Pereira et al. (2018). Hypostomate leaves,

as observed in *T. oppositifolia*, are more common in the subfamily Mimosoideae, as reported by Metcalfe and Chalk (1950). Amphistomatic leaves have also been observed in *Schnella Raddi* (Pereira et al., 2018), as well as in the genera *Abarema* Pittier and *Inga* Mill. (Silva et al., 2012).

The occurrence of different types of stomata in the same species, as observed for *D. odorata* and *T. oppositifolia*, corroborate that, in general, species of Fabaceae do not have a species-specific stomatal type, as previously reported by Silva et al. (2018). The occurrence of anomocytic and paracytic stomata in Fabaceae is common, with the latter being most frequent in the subfamilies Caesalpinoideae and Papilionoideae, as mentioned by Solereder (1908); Metcalfe and Chalk (1979) and Araújo and Mendonça (1998).

The patterns of the epidermis and stomata observed in the present study have also been observed in other species of Fabaceae, such as species of *Albizia* Durazz. (Simões et al., 2003), and *Bauhinia forficata* Link and *B. variegata* Linn. (Lusa and Bona, 2009), and *Indigofera microcarpa* Desv. (Lima et al., 2003), as well as among species of the Diptergeae clade (Silva et al., 2018).

Different from the results of the present study, the fine cuticle observed in *D. odorata* by Silva et al. (2018) was probably due to the

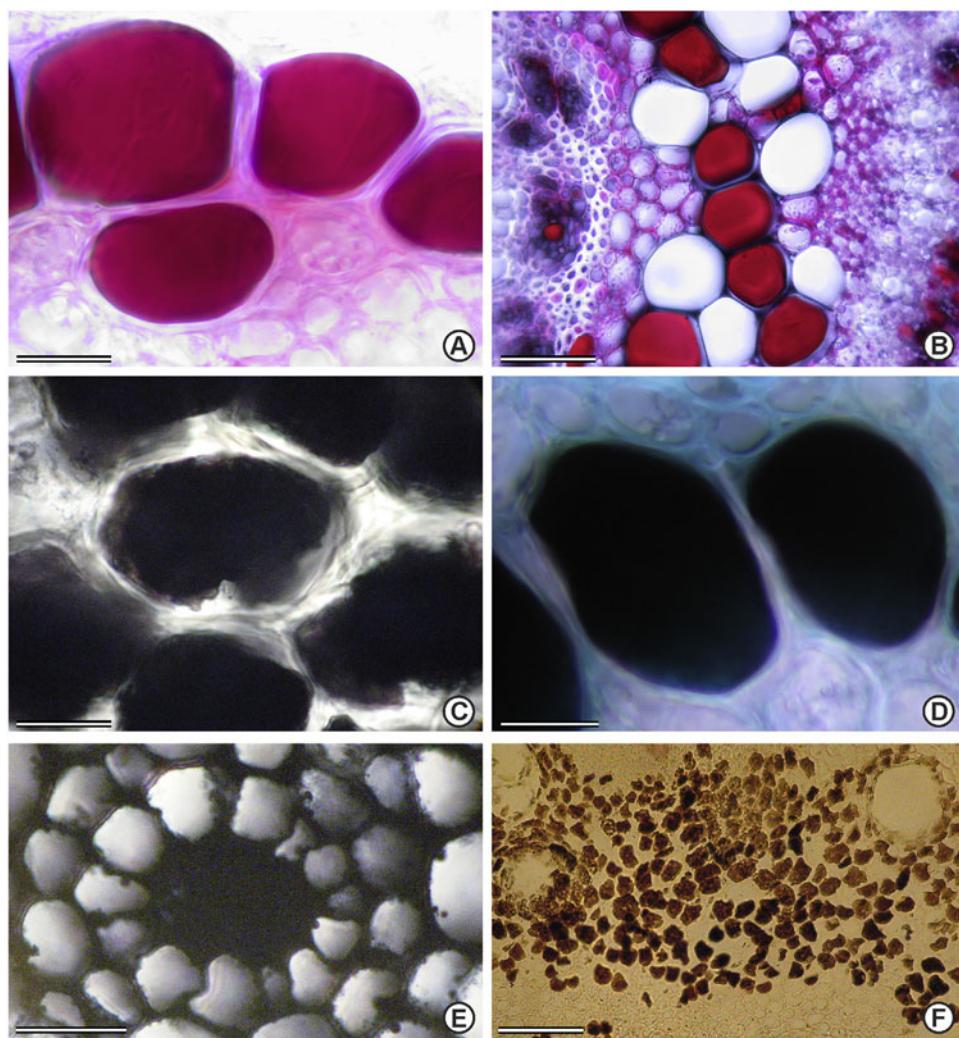


Fig. 5. Leaf histochemistry of *Taralea oppositifolia* (A–F). A–D. idioblasts; E and F. cavities; A. PAS reaction for “polysaccharides”; B. Ruthenium red reaction for “mucilage”; C and E. Sudan black B reaction for “total lipids”; D. Nile blue sulphate reaction for “acidic and neutral lipids”; F. iron sulphate in formalin reaction for “phenolic compounds”. Bars: 50 µm (A–E); 500 m (F).

use of herborized material from different collection sites. The thick cuticle observed in the present study for *T. oppositifolia* was also observed by these authors, as well as in other species of *Taralea*.

Non-glandular trichomes have been frequently reported among representatives of Papilionoideae and Caesalpinoideae (Metcalfe and Chalk, 1950). This trichome type has been observed in several tribes of Leguminosae, such as Dalbergieae, Hedysareae, Phaseoleae, Sophoreae and Swartzeae (Solereder, 1908), including the Dipterygeae clade (Silva et al., 2018). The taxonomic importance of trichomes in Leguminosae has also been discussed for other angiosperm families (Cowan, 1950; Carlquist, 1961; Tomlinson, 1969). In addition, analyses of the significance of trichomes for the taxonomy of Phaseoleae proved essential for delimiting groups (Lackey, 1978). In contrast to the findings reported here, Silva et al. (2018) found glabrous leaves in *T. oppositifolia*, which was probably due to the different origins of the plant material used and the methodologies employed.

The dorsiventral mesophyll recorded in the two species studied here conforms with what has been reported for other genera of Fabaceae by Metcalfe and Chalk (1950) and Silva et al. (2012), and who reported the same characteristic in *Bauhinia microstachya* (Duarte and Debur, 2003). According to Silva et al. (2018), dorsiventral mesophyll prevails in the Dipterygeae clade, although these authors observed isolateral mesophyll in *D. odorata*, in contrast to

the findings reported here. In addition to the dorsiventral organization of the mesophyll, the occurrence of bistratified palisade parenchyma and developed spongy parenchyma have been widely reported among species of Fabaceae (Araújo and Mendonça, 1998; Duarte and Debur, 2003; Rodrigues et al., 2009).

Small-caliber vascular bundles surrounded by fibers forming a sheath have also been reported to occur in the Dipterygeae clade by Silva et al. (2018). According these authors, the shape of the leaflet margin is curved downward in *D. odorata* and *T. oppositifolia*, which was confirmed here only for the former.

Secretory structures, such as idioblasts and cavities, have been previously reported for species of Papilionoideae and Caesalpinoideae (Solereder, 1908; Metcalfe and Chalk, 1950; Fahn, 1985; Teixeira and Gabrielli, 2000; Lusa and Bona, 2009; Leite et al., 2014; Palermo et al., 2017), including the Dipterygeae clade (Silva et al., 2018). Although the present study did not find secretory canals in the cortical parenchyma of the main rib and petiole of *T. oppositifolia*, as previously reported by Palermo et al. (2017), their presence in this species would serve as a valuable taxonomic character in distinguishing it from *D. odorata*, for which there are no records of these structures. Except for starch and acidic and neutral lipids, the secretory systems of *D. odorata* and *T. oppositifolia* produced secretions of the same chemical nature. This similarity is probably due to the fact that these two species occur in simi-

lar phytogeographic domains, such as the Amazon. Histochemical tests performed by Palermo et al. (2017) on leaves of *T. oppositifolia* detected the presence of total lipids in the epithelial cells and/or lumen of secretory spaces, as was found in the present study. Idioblasts and secretory cavities are structures that are specialized in the secretion of compounds such as mucilage and/or gum, phenolic compounds, and lipophilic material, including heterogeneous secretions, among others (Fahn, 1985), which have deterrent and antimicrobial effects (Langenheim, 2003). The presence of phenolic compounds in the leaves of *D. odorata* and *T. oppositifolia*, as well as the absence of mucilage, observed in the present study, was similar to that reported by Silva et al. (2018). However, the results of this author diverged in regards to the other compounds (starch, lipids and pectins), probably due to the fact that they worked with herbaria material.

The categories of chemical compounds inventoried in the idioblasts and cavities in the two studied species represent an important series for plants in general. For example, polysaccharides are one way that plants store sugar and build structures, such as the cell wall. Starch, also detected in the present study, is a polysaccharide with great food value, and has also been reported to occur in large quantities in the cotyledons of *D. odorata*, from which coumarin, a substance with proven therapeutic action, is extracted (Bessa et al., 2001).

Mucilage comprises complex polymers of acidic or neutral polysaccharides of hydrophilic nature and with high molecular weights (Mastroberti and Mariath, 2008), conferring important roles in plant development, such as protection of structures and organs, water retention, carbohydrate reserve, transpiration reduction, protection from solar radiation and herbivory, root lubrication, insect trapping, seed dispersal and regulation of germination (Langenheim, 2003; Pimentel et al., 2011; Rocha et al., 2011; Alves et al., 2012).

Alkaloids, which were found in the present work, have also been found in other species of Dipterygaceae, such as *Dipteryx alata* Vogel and *Pterodon pubescens* (Benth.) Benth. (Palermo et al., 2017). They are nitrogen compounds of varying structure and are among the most toxic classes of compounds. Pyrrolizidine-like alkaloids are found in several plant families, but are most common in Asteraceae, Boraginaceae and Fabaceae. In general, they function in defense against predators and are known to cause intoxication in animals, including humans (Silva et al., 2006; Lucena et al., 2010; Matos et al., 2011).

Braga et al. (2007) attributed the biological activity of extracts of various medicinal plants with anti-leishmaniasis and anti-fungal actions to secondary metabolites, such as flavonoids, alkaloids and coumarin. In general, the uses of the oil that these species provide are widely known, both in folk medicine and in cosmetics, which has resulted in great commercial value and reinforces the importance of studies characterizing species that have these substances to enhance their economic potential.

Although *D. odorata* and *T. oppositifolia* produce secretions with similar classes of chemical compounds, the anatomical structure of their leaves can aid in distinguishing them (the species are morphologically similar, which makes distinguishing them in the field difficult when fruit is absent), and support their inclusion in the Dipterygaceae clade.

In conclusion, the occurrence and chemical composition of idioblasts and secretory cavities in leaves, the location of stomata, the types of trichomes, the curvature and lignification of the leaf margin and the shape of the vascular bundle of the petiole are all anatomical characters with potential diagnostic value for the Dipterygaceae clade, and especially for the species *D. odorata* and *T. oppositifolia*.

Conflict of interest

The authors declare no conflicts of interest.

Authors' contributions

PMFS contributed in collecting plant samples, and conducted the laboratory work. PMFS, EOS and AISS wrote the text. MSCR and LMRC contributed to critical reading of the manuscript. All of the authors have read the final manuscript and approved its submission.

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